Certificate of Analysis - Amended

Product Description	WA01 Master Cell Bank	
Cell Line Provider	WiCell Research Institute	
MCB Lot Number	WA01-MCB-01	
Date Vialed	19-November-2006	
Passage Number	P20	
Culture Platform	Feeder dependent – MEFs	
	Media: hES Medium	Matrix: MEFs

The following testing specifications have been met for the specified product lot:

Test Description	Test Provider	Test Method	Test Specification	Result
Post-Thaw Viable Cell Recovery	WiCell Research Institute	SOP-CH-305	≥ 15 Undifferentiated Colonies, ≤ 30% Differentiation	Pass
Identity by STR	UW Molecular Diagnostics Laboratory	PowerPlex 1.2 System by Promega	Positive identity	Pass
HLA profile	UW Molecular Diagnostics Laboratory	AlleleSEQR Kits by Abbott	Positive identity	Pass
Sterility - Direct Transfer Method	WuXi Apptec	30774	No contamination detected	Pass
Bacteriastasis & Fungistasis	WuXi Apptec	30736	Pass	Pass
Mycoplasma - FDA PTC method	WuXi Apptec	31216	No contamination detected	Pass
Karyotype by G-banding	WiCell Research Institute	SOP-CH-003	Normal karyotype	Pass
Bovine pathogens	BioReliance	032901	No contamination detected	Pass
Porcine pathogens	BioReliance	033901	No contamination detected	Pass
Murine Antibody Production (MAP)	BioReliance	004000	No contamination detected	Pass
In vitro adventitious virus	Covance	Not Available	No contamination detected	Pass
In vivo adventitious virus	BioReliance	005002	No contamination detected	Pass
Retrovirus by thin section EM	WuXi Apptec	30610	No contamination detected when cultured without MEFs	Pass
Co-cultivation with Mus Dunni Cells and PG4 S+L- assay	WuXi Apptec	30201	No contamination detected	Pass
HIV 1&2 by PCR	Covance	Not Available	No contamination detected	Pass
HTLV 1 PCR HTLV 1&2 by PCR	Covance BioReliance	Not Available 105013	No contamination detected	Pass
HBV by PCR	Covance	Not Available	No contamination detected	Pass
HCV by PCR	Covance	Not Available	No contamination detected	Pass
CMV by PCR	BioReliance	105012	No contamination detected	Pass

Certificate of Analysis - Amended

EBV by PCR	Covance	Not Available	No contamination detected	Pass
HHV-6 by PCR	BioReliance	105020	No contamination detected	Pass
HHV-7 by PCR	Covance	Not Available	No contamination detected	Pass
HHV-8 by PCR	Covance	Not Available	No contamination detected	Pass
HP B19 by PCR	Covance	Not Available	No contamination detected	Pass
Comparative Genome Hybridization	WiCell Research Institute	SOP-CH-308 SOP-CH-309 SOP-CH-310	Report - no specification	See report
Flow Cytometry for ESC Marker Expression	UW Flow Cytometry Laboratory	SOP-CH-101 SOP-CH-102 SOP-CH-103 SOP-CH-105	Report - no specification	See report
Gene Expression Profile	UW Gene Expression Center	SOP-CH-321 SOP-CH-322 SOP-CH-333 SOP-CH-311	Report - no specification	See report
ABO and rH typing	American Red Cross	ABO/rH System	Report Blood type	O+

Master Cell Bank (MCB) cells are not available for distribution. WiCell produced and tested these cells with the intended purpose of having high quality starting material for distribution lots.

Appropriate biosafety precautions should be followed when working with these cells. The end user is responsible for ensuring that the cells are handled and stored in an appropriate manner. WiCell is not responsible for damages or injuries that may result from the use of these cells.

Please contact technical service via the website to request test methods and other assistance with your cells. The knowledgeable technical support staff can assist with cell culture concerns, training, and any other customer service concerns.

Date of Lot Release	Quality Assurance Approval
30-November-2007	1/4/2024 X JKG Jenna Gay Quality Assurance Signed by: Gay, Jenna

UW Health
University of Wisconsin
Hospital and Clinics

Histocompatibility/Molecular Diagnostics Laboratory

Short Tandem Repeat Analysis*

Sample Report: NSCB#9592 UW HLA#: 57101 Sample Date: 09/18/07
Lab Received 09/18/07

Requestor: WiCell Research Institute
Test Date: 09/21/07 File Name: 070921, 071009 Report Date: 10/10/07

Sample Name: (label on tube) NSCB#9592

Description: WI Cell Cytogenetics provided genomic DNA of NSCB#9592
220ug/mL 260/280=1.9

Locus	Repeat #	STR Genotype
D16S539	5, 8-15	9,13
D7S820	6-14	8,12
D13S317	7-15	8,11
D5S818	7-15	9,11
CSF1PO	6-15	12,13
TPOX	6-13	8,11
Amelogenin	NA	X,Y
TH01	5-11	9.3,9.3
vWA	11, 13-21	15,17

Comments: Based on the NSCB#9592 DNA submitted by WI Cell dated 09/18/07 and received on 09/18/07, this sample (UW HLA# 57101) matches exactly the STR profile of the human stem cell line H1 comprising 15 allelic polymorphisms across the 8 STR loci analyzed. No STR polymorphisms other than those corresponding to the human H1 stem cell line were detected and the concentration of DNA required to achieve an acceptable STR genotype (signal/ noise) was equivalent to that required for the standard procedure (~1 ng/amplification reaction) from human genomic DNA. This result suggest that the DNA sample submitted corresponds to the H1 stem cell line and was not contaminated with any other human stem cells or a significant amount of mouse feeder layer cells. Sensitivity limits for detection of STR polymorphisms unique to either this or other human stem cell lines is ~5%. A preliminary copy of this report was issued via electronic mail to and of WI Cell Research Institute on Wednesday, October 10, 2007.

HLA/Molecular Diagnostics Laboratory HLA/Molecular Diagnostics Laboratory

* Testing to assess engraftment following bone marrow transplantation was accomplished by analysis of human genetic polymorphisms at STR loci. This methodology has not yet been approved by the FDA and is for investigational use only.

UW Health
University of Wisconsin Hospital and Clinics

Histocompatibility/Molecular Diagnostics Laboratory

Date: 10/12/2007 13:23:05

To: Cytogenetics, WiCell Research Institute

Re: High-resolution HLA results

Patient

Name HLA / MR# received	Method / Test date		HLA DNA-based typing* Method: PCR-SSP			Direct Sequencing				PCR-SSP
			A*	B*	C*	DRB1*	DRB3*	DRB4*	DRB5*	DQB1*
WICELL, NSCB#9592	DQB SSP		0201	0801	0401/09N	0101				
57101 /	A,B,C Seq	10/04/2007	0301	3501	0701/6/18	0301				
10/04/2007	DRB Seq	10/04/2007	Class I comment: The following allele combinations, where either or both are listed by the NMDP as "rare" or with a zero frequency in the registry, cannot be excluded: A*0224, *0317 A*0226, *0307 B*0801, *3542 B*0823, *3529 B*0832, *3549							

HLA/Molecular Diagnostics Laboratory

Date

HLA/Molecular Diagnostics Laboratory

Date

WiCell Research Institute

April 24, 2007
P.O. #:

STERILITY TEST REPORT

Sample Information: Cryopreserved Human embryonic stem cell 4: line H1, H1-MCB.1

Date Received: April 05, 2007
Date in Test: April 10, 2007
Date Completed: April 24, 2007

Test Information: Test Codes: 30744, 30744A
Immersion, USP / 21 CFR 610.12
Procedure #: BS210WCR.01

TEST PARAMETERS	PRODUCT	
Approximate Volume Tested	0.5 mL	0.5 mL
Number Tested	2	2
Type of Media	SCD	FTM
Media Volume	200 mL	200 mL
Incubation Period	14 Days	14 Days
Incubation Temperature	20 °C to 25 °C	30 °C to 35 °C
RESULTS	2 NEGATIVE	2 NEGATIVE

QA Reviewed: Page 1 Signed

Reviewed: Page 1 Signed

Testing conducted in accordance with current Good Manufacturing Practices

Test Facility:

AppTec

WiCell Research Institute

April 24, 2007
P.O. #:

STERILITY TEST REPORT

Sample Information: Cryopreserved Human embryonic stem cell
1: line H1, WCDFR002A-H1-1 Sterility

2: line H9, WCDFR002A-H9-1

3: line H9, H9-MCB.1

4: line H1, H1-MCB.1

Date Received: April 05, 2007
Date in Test: April 10, 2007
Date Completed: April 24, 2007

Test Information: Test Codes: 30744, 30744A
Immersion, USP / 21 CFR 610.12
Procedure #: BS210WCR.01

QA Reviewed: [redacted] **Reviewed** [redacted]

Testing conducted in accordance with current Good Manufacturing Practices

Test Facility:

AppTec

Report Number
744091
Page 1 of 1

WiCell Research Institute

April 19, 2007
P.O. #:

STERILITY TEST VALIDATION (B/F) REPORT

Sample Information: Cryopreserved Human embryonic stem cell line H1, H1-MCB.1

Date Received: April 05, 2007
Date in Test: April 12, 2007
Date Completed: April 16, 2007

Test Information:
Test Code: 30736
Immersion, USP / 21 CFR 610.12
Procedure #: BS210WCR.01
Media Volume: 20 mL
Volume Tested: 50 µL

SCD	*B. subtilis* ATCC 6633	*C. albicans* ATCC 10231	*A. niger* ATCC 16404
Test Sample	Positive	Positive	Positive
Inoculated Control	Positive	Positive	Positive
Inoculum Level (CFU)	37	53	36
RESULTS	PASS	PASS	PASS

FTM	*B. subtilis* ATCC 6633	*P. aeruginosa* ATCC 9027	*C. sporogenes* ATCC 11437
Test Sample	Positive	Positive	Positive
Inoculated Control	Positive	Positive	Positive
Inoculum Level (CFU)	38	26	55
RESULTS	PASS	PASS	PASS

Conclusion: The above test parameters do not demonstrate bacteriostatic/fungistatic activity.

Note: Product volume to media volume ratio is equivalent to test ratio employed for sterility testing.
Reference Sterility Test Report(s): 744081

QA Reviewed: [redacted]

Reviewed: [redacted]

Testing conducted in accordance with current Good Manufacturing Practices.

St. Paul

Report

FINAL STUDY REPORT

STUDY TITLE:	**MYCOPLASMA DETECTION: "Points to Consider" with Mycoplasmastasis**
PROTOCOL NUMBER:	31216A
TEST ARTICLE IDENTIFICATION:	H1-MCB.1
SPONSOR:	WiCell Research Institute
PERFORMING LABORATORY:	AppTec Laboratory Services
STUDY NUMBER:	57451
RESULT SUMMARY:	Considered **negative** for mycoplasma contamination and **non-inhibitory** for the detection of mycoplasma

Reference PO # I

WCR01

57451

QUALITY ASSURANCE UNIT SUMMARY

STUDY: Mycoplasma Detection: "Points to Consider" with Mycoplasmastasis

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. This study has been performed under Good Laboratory Practices regulations (FDA, 21 CFR, Part 58 - Good Laboratory Practice for Nonclinical Laboratory Studies) and in accordance to standard operating procedures and a standard protocol. The Quality Assurance Unit maintains copies of study protocols and standard operating procedures and has inspected this study on the dates listed below. Studies are inspected at time intervals to assure the quality and integrity of the study.

Critical Phase	Date	Study Director	Management
Inoculation of Plates and Broth	05/01/07	05/01/07	06/06/07
Final Report	06/05/07	06/05/07	06/06/07

The findings of these inspections have been reported to management and the Study Director.

Quality Assurance Auditor:_____ Date: 6/6/07

GOOD LABORATORY PRACTICES STATEMENT

The study referenced in this report was conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice (GLP) regulations set forth in 21 CFR part 58.

The studies not performed by or under the direction of AppTec Laboratory Services, are exempt from this Good Laboratory Practice Statement and include characterization and stability of the test compound(s)/test article.

Study Director: _____ Date: 6/6/07

Professional Personnel Involved:

1.0 PURPOSE

To demonstrate that a test article consisting of a cell bank, production or seed lots, or raw materials is free of mycoplasmal contamination, according to "Points to Consider" criteria with the addition of a mycoplasmastasis (test article inhibition) assay to evaluate for the presence of test article (product) specific inhibition.

2.0 SPONSOR: WiCell Research Institute

3.0 TEST FACILITY: AppTec Laboratory Services, Inc.

4.0 SCHEDULING

DATE SAMPLE RECEIVED: 04/20/07
STUDY INITIATION DATE: 04/25/07
STUDY COMPLETION DATE: 06/06/07

5.0 TEST ARTICLE IDENTIFICATION: WiCell Research Institute; H1-MCB.1

6.0 TEST ARTICLE CHARACTERIZATION

The Sponsor was responsible for all test article characterization data as specified in the GLP regulations. The identity, strength, stability, purity, and chemical composition of the test article were solely the responsibility of the Sponsor. The Sponsor was responsible for supplying to the testing laboratory results of these determinations and any others that may have directly impacted the testing performed by the testing laboratory, prior to initiation of testing. Furthermore, it was the responsibility of the Sponsor to ensure that the test article submitted for testing was representative of the final product that was subjected to materials characterization. Any special requirements for handling or storage were arranged in advance of receipt and the test article was received in good condition.

The test article was maintained according to the Sponsor's instructions. The Vero cells were maintained by AppTec's Cell Production Laboratory.

7.0 EXPERIMENTAL DESIGN

7.1 OVERVIEW

Whereas no single test is capable of detecting all mycoplasmal strains, freedom from mycoplasmal contamination may be demonstrated by the use of both an indirect and direct procedure.

7.2 JUSTIFICATION FOR SELECTION OF THE TEST SYSTEM

Contamination of cell cultures by mycoplasma is a common occurrence and is capable of altering normal cell structure and function. Among other things, mycoplasma may affect cell antigenicity, interfere with virus replication, and mimic viral actions. Testing for the presence of mycoplasma for cell lines used to produce biologicals is recommended by the FDA, Center for Biologics Evaluation and Research (CBER) under "Points to Consider."

8.0 EXPERIMENTAL SUMMARY

The indirect method of detection allows visualization of mycoplasma, particularly non-cultivable strains, by growing the mycoplasma on an indicator cell line and then staining using a DNA-binding fluorochrome (Hoechst) stain. The indicator cell line should be easy to grow, have a large cytoplasmic to nuclear area ratio and support the growth of a broad spectrum of mycoplasma species. The African green monkey kidney cell line, Vero, fits this description and was used in this assay. The assay was performed with negative and positive controls. Both a strongly cyto-adsorbing (*M. hyorhinis*) and a poorly cyto-adsorbing (*M. orale*) mycoplasma species were used as positive controls. Poorly cyto-adsorbing mycoplasma species may not give reliable positive results when inoculated in low numbers. A second dilution of *M. orale* was used to ensure cyto-adsorption. Staining the cultures with DNA binding fluorochrome allows for the detection of mycoplasma based on the staining pattern observed. Only the cell nuclei demonstrate fluorescence in the negative cultures but nuclear and extra-nuclear fluorescence is observed in positive cultures.

Direct cultivation is a sensitive and specific method for the detection of mycoplasma. The agar and broth media employed supply nutrients necessary for the growth of cultivable mycoplasmas. These media also supply a source of carbon and energy, and favorable growth conditions. The direct assay was performed with both negative and positive controls. A fermentative mycoplasma (*M. pneumoniae)* and a non-fermentative mycoplasma *(M. orale)* were used as positive controls.

A mycoplasmastasis assay was performed to evaluate for the presence of product-specific inhibitory substances. In this assay the test article was spiked with known concentrations of the positive control organisms and tested in both the direct and indirect assays. A comparison of the spiked test article result to the positive control result was used to determine the presence or absence of inhibitory substances. The procedure employed in this study is based on the protocol described in the 1993 Attachment # 2 to the "Points To Consider" document, as recommended by the FDA, Center for Biologics Evaluation and Research (CBER) and portions of the European Pharmacopoeia (2007).

9.0 TEST MATERIAL AND PREPARATION

9.1 TEST ARTICLE IDENTIFICATION:

Test Article Name:	H1-MCB.1
Stability (Expiration):	Not Applicable
Storage Conditions:	Ultracold (≤ -60°C)
Safety Precautions:	BSL-1
Intended Use/Application:	Master cell bank cells scaled up for distribution

9.2 TEST SAMPLE PREPARATION

The test article was thawed in a water bath at 37 ± 2°C and 1:5 and 1:10 dilutions were prepared in sterile phosphate buffered saline (PBS). 1 mL of the undiluted sample, the 1:5 and 1:10 dilutions were then inoculated onto each of two (2) coverslips (per sample/dilution) containing previously incubated Vero cells. The coverslips were incubated in incubator E770 for 1-2 hours at 37 ± 1°C / 5 ± 2% CO_2 and then 2 mL of EMEM, 8% Fetal Bovine Serum (FBS) was added to each coverslip. The coverslips were returned to incubator E770 at 37 ± 1°C / 5 ± 2% CO_2. After three days of incubation, the coverslips were fixed, stained, and then read using an epifluorescent microscope.

0.2 mL of the undiluted test article was then inoculated onto each of three (3) SP-4 agar plates, and 10 mL was inoculated into a 75 cm^2 flask containing 50 mL of SP-4 broth. The plates were placed in an anaerobic GasPak system and incubated at 36 ± 1°C for a minimum of 14 days.

The broth culture flask was incubated aerobically at 36 ± 1°C, and subcultured onto each of two (2) SP-4 agar plates (0.2 mL/plate) on Days 3, 7, and 14. These subculture plates were placed in an anaerobic GasPak system and incubated at 36 ± 1°C for a minimum of 14 days. The broth culture flask was read each working day for 14 days. The SP-4 agar plates (Day 0) were read after 14 days of incubation. The SP-4 broth subculture plates (Days 3, 7, and 14) were read after 14 days incubation.

9.3 PREPARATION OF SPIKED TEST ARTICLES

9.3.1 1.8 mL of the test article was spiked with 0.2 mL of 940 CFU/mL of *M. hyorhinis* for a final concentration of 94 CFU/mL.

9.3.2 1.8 mL of the test article was spiked with 0.2 mL of $2x10^5$ CFU/mL of *M. orale* for a final concentration of 2×10^4 CFU/mL.

9.3.3 0.6 mL of the test article was spiked with 0.3 mL of 500 CFU/mL of *M. orale* for a final concentration of 167 CFU/mL.

9.3.4 1.6 mL of the test article was spiked with 0.4 mL of 500 CFU/mL of *M. orale* for a final concentration of 100 CFU/mL.

9.3.5 9.0 mL of the test article was spiked with 1.0 mL of 98 CFU/mL of *M. orale* for a final concentration of 9.8 CFU/mL.

9.3.6 0.4 mL of the test article was spiked with 0.4 mL of 500 CFU/mL of *M. pneumoniae* for a final concentration of 250 CFU/mL.

9.3.7 9.0 mL of the test article was spiked with 1.0 mL of 96 CFU/mL of *M. pneumoniae* for a final concentration of 9.6 CFU/mL.

9.3.8 Spiked test articles were inoculated in the same manner and in the same concentrations as the positive controls.

9.4 CONTROLS AND REFERENCE MATERIALS

9.4.1 Sterile SP-4 broth served as the negative control for the indirect and direct assays.

9.4.2 Positive Controls

a. Indirect Assay

a.1 Strongly cyto-adsorbing species - *M. hyorhinis* GDL (ATCC #23839) at 100 or fewer colony forming units (CFU) per inoculum.

a.2 Poorly cyto-adsorbing species - *M. orale* (ATCC #23714) at 100 or fewer CFU per inoculum and at approximately 100 ID_{50}.

b. Direct Assay

b.1 Nonfermentative mycoplasma species - *M. orale* (ATCC #23714) at 100 or fewer CFU per inoculum.

b.2 Fermentative mycoplasma species - *M. pneumoniae* FH (ATCC #15531) at 100 or fewer CFU per inoculum.

9.4.3 Control Preparation

a. Negative Controls

a.1 1 mL of SP-4 broth was inoculated onto each of two (2) coverslips containing previously incubated Vero cells to serve as the negative control in the indirect assay.

a.2 0.2 mL of SP-4 broth was inoculated onto each of three (3) SP-4 agar plates to serve as the negative control in the direct assay. 10 mL of SP-4 broth was inoculated into a 75 cm^2 flask containing 50 mL of SP-4 broth to serve as the negative control in the direct assay.

b. Positive Controls

b.1 *M. hyorhinis, M. orale,* and *M. pneumoniae* were diluted to less than 100 CFU / inoculum in SP-4 broth. 1 mL of *M. hyorhinis* and *M. orale* at less than 100 CFU / inoculum was inoculated onto each of two (2) coverslips containing previously incubated Vero cells. 1 mL of *M. orale* at 100 ID_{50} (approximately 2 x 10^4 CFU/mL) was also inoculated onto each of two (2) coverslips containing Vero cells. These coverslips served as the positive controls in the indirect assay.

b.2 The coverslips were incubated in incubator E770 for 1-2 hours at 37 ± 1°C / 5 ± 2% CO_2 and then 2 mL of EMEM, 8% Fetal Bovine Serum (FBS) was added to each coverslip. The coverslips were returned to incubator E770 at 37 ± 1°C / 5 ± 2% CO_2. After three days of incubation, the coverslips were fixed, stained, and then read using an epifluorescent microscope.

b.3 0.2 mL of *M. orale* and *M. pneumoniae* at less than 100 CFU/plate were inoculated onto each of three (3) SP-4 agar plates. 10 mL of *M. orale* and *M. pneumoniae* at less than 10 CFU/mL (≤ 100 CFU / inoculum) were each inoculated into a 75 cm^2 flask containing 50 mL of SP-4 broth.

b.4 The agar plates were placed in an anaerobic GasPak system and incubated at 36 ± 1°C for 14 days. The broth culture flasks were incubated aerobically at 36 ± 1°C for a minimum of 14 days and were read each working day. On Days 3, 7, and 14, 0.2 mL from each broth culture flask was subcultured onto each of two (2) SP-4 agar plates. These subculture plates were placed in an anaerobic GasPak system and incubated at 36 ± 1°C for a minimum of 14 days. The subculture plates were observed microscopically after a minimum of 14 days incubation.

c. See Section 14.0, Results, for the results of these controls.

10.0 DATA ANALYSIS

The results of this study were based on visual observations, therefore, no data analysis was required.

11.0 STATISTICAL METHODS

The results of this study were qualitative, therefore, no statistical analysis was required.

12.0 EVALUATION CRITERIA

Final evaluation of the validity of the assay and test article results was based upon the criteria listed below and scientific judgment.

12.1 Indirect Assay

DETECTION OF MYCOPLASMA CONTAMINATION BY INDIRECT ASSAY

CONTROLS	MYCOPLASMA FLUORESCENCE OBSERVED (AT LEAST ONE COVERSLIP REQUIRED FOR THE EVALUATION)
Negative Control	-
M. hyorhinis	+
M. orale (≤100 CFU)	+/-
M. orale (100 ID_{50})	+

12.1.1 Mycoplasma fluorescence must be observed for the strongly cyto-adsorbing mycoplasma species (*M. hyorhinis)* and for at least one dilution of the poorly cyto-adsorbing mycoplasma species *(M. orale).*

12.1.2 Mycoplasmal fluorescence must not be observed for the negative controls.

12.2 Direct Assay

DETECTION OF MYCOPLASMA CONTAMINATION BY DIRECT ASSAY

	NEGATIVE CONTROL	*M. PNEUMONIAE*	*M. ORALE*
Broth (Color change or turbidity change)	-	+ / -	+ / -
Agar Day 0 (at least one plate)	-	+	+
Agar Day 3, 7, 14 (at least one plate on one day)	-	+	+
Results	-	+	+

12.2.1 Mycoplasmal growth must be observed on the agar plates for both positive controls; *M. orale* and *M. pneumoniae*.

12.2.2 The mycoplasmal growth must not be observed on agar plates for the negative controls.

13.0 TEST EVALUATION

13.1 Indirect Assay

Hoechst stain will bind to most DNA containing organisms and organelles present in the culture; this includes indicator cell nuclei, prokaryotes including mycoplasma and cellular debris. The source of DNA is determined by the staining pattern. The indicator cell nuclei fluoresce brightly and are generally 10-20 μm in diameter. Mycoplasma fluorescence is less intense, is extra-nuclear and typically appears as small round bodies approximately 0.3 μm in diameter.

13.2 Direct Assay

Change in color or turbidity of broth culture can be an indicator of the presence of mycoplasma growth. Fermentative mycoplasma produce acid from the carbohydrates in the medium causing the pH of the medium to drop and the broth to turn yellow in color. Nonfermentative mycoplasma produce ammonia by arginine hydrolysis causing the pH to rise and the broth to turn red. In general, growth of mycoplasma can cause the broth to become turbid. Changes in the appearance of the broth culture must be confirmed by agar plate subculture or DNA-staining since these changes can also be caused by the properties of the inoculum.

Mycoplasma colonies grow down into the agar causing the center of the colony to appear opaque and the peripheral surface growth to appear translucent. These "fried-egg" colonies vary in size, 10-500 μm, and can be readily observed unstained using a light microscope.

13.3 Indirect Assay and Direct Assay Results Interpretation

IF:	TEST ARTICLE				
Mycoplasmal fluorescence	-	+	+/-	+/-	-
Broth (Color change or turbidity change)	-	+/-	+/-	+/-	+*
Agar - Day 0 (at least one plate)	-	+/-	+/-	+	-
Agar - Day 3, 7, 14 (at least one plate on one day)	-	+/-	+	+/-	-
THEN: OVERALL FINAL RESULT	-	+	+	+	-

* A change in the appearance of the broth culture must be confirmed by positive subculture plate(s).

13.4 Negative Results

The test article is considered as negative if both the direct assay (agar and broth media procedure) and the indirect assay (indicator cell culture procedure) show no evidence of mycoplasma contamination and resemble the negative control for each procedure.

13.5 Positive Results

The test article is considered positive if the direct assay (agar and / or broth media procedure) or the indirect assay (indicator cell culture procedure) show evidence of mycoplasma contamination and resemble the positive controls for each procedure.

13.6 Mycoplasmastasis (Test Article Inhibition) Results Interpretation

IF:			
TEST ARTICLE SPIKED WITH	Mycoplasma fluorescence	Agar Plates - anaerobic	Broth Culture - aerobic
M. hyorhinis ≤ 100 CFU	+	NA	NA
M. orale ≤ 100 ID_{50}	+	NA	NA
M. orale ≤ 100 CFU	+/-*	+**	+
M. pneumoniae ≤ 100 CFU	NA	+**	+
THEN: Overall Inhibitory Result	Non-Inhibitory	Non-Inhibitory	Non-Inhibitory

* Must be positive in at least one dilution of the poorly cyto-adsorbing *M. orale*.
**See section 13.6.1 for additional criteria.

13.6.1 Direct Assay

A test article is considered inhibitory if growth of the control organism (positive control) is observed more than 1 subculture sooner than in the corresponding spiked test article.

A test article is also considered inhibitory if plates directly inoculated (Day 0 agar plates) with the spiked test article have less than 1/5 the number of colonies of the corresponding day 0 positive controls. This ratio will be based on the average CFU / plate calculated for each spiked test article and each positive control (European Pharmacopoeia).

13.6.2 Indirect Assay

A test article is considered inhibitory if growth of the control organism is observed in the positive control, but not in the corresponding spiked test article.

13.6.3 Repeat Testing for Products Containing Inhibitory Substances

If a test article is found to cause inhibition, the inhibitory substances must be neutralized or their effect otherwise countered. For example, by passage in substrates not containing inhibitors or dilution in a larger volume of medium prior to testing. If dilution is used, larger media volumes may be used or the inoculum volume may be divided among several 100 mL flasks. The effectiveness of the neutralization or other process is confirmed by repeating the assay for inhibitory substances (European Pharmacopoeia).

14.0 RESULTS

14.1 Mycoplasmastasis (Test Article Inhibition)

14.1.1 Indirect assay

For the indirect assay, the test article spiked with *M. hyorhinis* at ≤ 100 CFU per inoculum as well as those spiked with *M. orale* at ≤ 100 CFU per inoculum were positive and resembled the corresponding positive controls. No growth inhibition was observed.

14.1.2 Direct assay – Day 0 Agar Plates

$$\text{Average CFU / Plate} = \frac{(\text{Plate 1 CFU + Plate 2 CFU + Plate 3 CFU})}{3}$$

$$\text{TA Spike Ratio} = \frac{\text{Spiked test article average CFU / plate}}{\text{Positive control average CFU / Plate}}$$

If TA Spike Ratio:

Was ≥ 0.2 (or 1/5) then growth inhibition has not occurred

Was < 0.2 (or 1/5) then growth inhibition has occurred

TABLE 2 – DAY 0 AGAR PLATES – POSITIVE CONTROLS

POSITIVE CONTROL	AVE. CFU / PLATE
M. orale	**37.7**
M.pneumoniae	**42.3**

TABLE 3 – DAY 0 AGAR PLATES – SPIKED TEST ARTICLES

		AVE. CFU / PLATE	TA SPIKE RATIO	INHIBITORY / NON-INHIBITORY
Test Article: H1-MCB.1	*M. orale* spike	**38.7**	**1.0**	Non-Inhibitory
	M. pneumoniae spike	**42.0**	**1.0**	Non-Inhibitory

14.1.3 Direct assay – Subculture Plates

The subculture plates for the test article spiked with *M. orale* at ≤ 100 CFU per inoculum and those spiked with *M. pneumoniae* at ≤ 100 CFU per inoculum yielded a positive result no more than one subculture day later than the corresponding positive control plates. No growth inhibition was observed.

14.2 Overall Result

Indirect Assay and Direct Assay Results

	Indirect	Direct		Overall
		Broth Flasks	Agar Plates	
Test Article: H1-MCB.1	Negative	Negative	Negative	Negative
H1-MCB.1 Spiked with *M. orale*	Non-inhibitory Positive	Non-inhibitory Positive	Non-inhibitory Positive	Non-inhibitory Positive
H1-MCB.1 Spiked with *M. hyorhinis*	Non-inhibitory Positive			Non-inhibitory Positive
H1-MCB.1 Spiked with *M. pneumoniae*		Non-inhibitory Positive	Non-inhibitory Positive	Non-inhibitory Positive
Negative Control	Negative	Negative	Negative	Negative
M. hyorhinis	Positive			Positive
M. orale	Positive	Positive	Positive	Positive
M. pneumoniae		Positive	Positive	Positive

For the indirect assay, the coverslips for the undiluted test article were read and determined negative.

15.0 ANALYSIS AND CONCLUSION

15.1 The results of the negative and positive controls indicate the validity of this test.

15.2 These findings indicate that the test article, H1-MCB.1, is considered negative for the presence of mycoplasma contamination and non-inhibitory to the detection of mycoplasma.

16.0 DEVIATIONS: None.

17.0 AMENDMENT #1: The protocol was amended to reflect a change in positive control strains. *M. orale* (ATCC #29802) was changed to *M. orale* (ATCC #23714). Strain ATCC #23714 has been determined equivalent to ATCC #29802 and meets regulatory guidelines.

18.0 RECORD RETENTION

An exact copy of the original final report and all raw data pertinent to this study will be stored at AppTec Laboratory Services, 2540 Executive Drive, St. Paul, MN 55120. It is the responsibility of the Sponsor to retain a sample of the test article.

19.0 TECHNICAL REFERENCES

19.1 Barile, Michael F. and McGarrity, Gerard J. (1983). "Isolation of Mycoplasmas from Cell Culture by Agar and Broth Techniques." Methods in Mycoplasmology, Vol II, ed. J.G. Tully and S. Razin. (New York: Academic Press) pp. 159-165.

19.2 Del Giudice, Richard A. and Joseph G. Tully. 1996. "Isolation of Mycoplasma from Cell Cultures by Axenic Cultivation Techniques," ed. J.G. Tully and S. Razin, Molecular and Diagnostic Procedures in Mycoplasmology, Vol. II (New York: Academic Press).

19.3 European Pharmacopoeia, Edition 5.6. Section 2.6.7. Mycoplasmas. 01/2007.

19.4 McGarrity, Gerard J. and Barile, Michael F. 1983. "Use of Indicator Cell Lines for Recovery and Identification of Cell Culture Mycoplasmas," ed. J.G. Tully and S. Razin, Methods in Mycoplasmology, Vol. II (New York: Academic Press).

19.5 Masover, Gerald and Frances Becker. 1996. "Detection of Mycoplasma by DNA Staining and Fluorescent Antibody Methodology," ed. J.G. Tully and S. Razin, Molecular and Diagnostic Procedures in Mycoplasmology, Vol. II (New York: Academic Press).

19.6 Schmidt, Nathalie J. and Emmons, Richard W. 1989. "Cell Culture Procedures for Diagnostic Virology," ed. Nathalie J. Schmidt and Richard W. Emmons, 6th ed., Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections (Washington: American Public Health Association).

19.7 U.S. Food and Drug Administration (FDA) Center for Biologics Evaluation and Research (CBER). 1993. "Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals."

WiCell Cytogenetics Report: NSCB #9592

Report Date: September 14, 2007

Case Details:

Cell Line: *H1 (N)*

Passage #: *37*

Date Completed: *9/14/2007*

Cell Line Gender: *male*

Investigator: *National Stem Cell Bank*

Specimen: *hESC on MEF feeder*

Date of Sample: *9/12/2007*

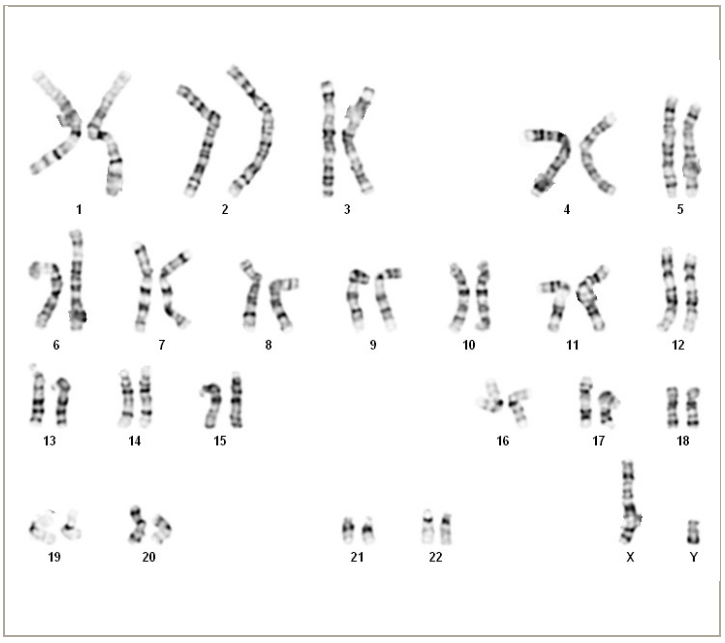
Tests,Reason for: *Confirm normal karyotype, NSCB #9592*

Results: *46,XY*

Completed by , CLSp(CG), on 9/14/2007

Reviewed and interpreted by PhD, FACMG, on 9/14/2007

Interpretation: *No abnormalities were detected at the stated level of resolution.*



Cell: *S01-02*

Slide: *A*

Slide Type: *Karyotyping*

Cell Results: *Karyotype: 46,XY*

# of Cells Counted: *20*

# of Cells Karyotyped: *3*

# of Cells Analyzed: *7*

Band Level: *450-475*

Results Transmitted by Fax / Email / Post **Date:**_____

Sent By:_____ **Sent To:**_____

Final Report

IN VITRO ASSAY FOR THE PRESENCE OF BOVINE VIRUSES ACCORDING TO 9 CFR REQUIREMENTS–NINE VIRUS ASSAY

Study Number: AC01UC.032901.BSV

Test Article ID: H1-MCB.1

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

Bovine viruses were not detected when the test article, H1-MCB.1, was examined for the presence of nine specific bovine viruses using IFA. In addition, hemadsorption and CPE were not observed in the test article inoculated cultures.

BioReliance®

STUDY INFORMATION

Test Article: H1-MCB.1 was received by BioReliance on 05-Apr-2007. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor. Retention of reserve sample from each batch of test article is the responsibility of the sponsor.

Testing Facility: BioReliance

Schedule:

Study Initiation: 11-Apr-2007

Lab Initiation: 13-Apr-2007

Lab Completion: 08-May-2007

Study Completion: See Study Director's signature date in "Approval" Section.

Study Director:

Archives: All raw data, the protocol, and a copy of the final report will be maintained according to Standard Operating Procedure OPQP3040 by the BioReliance Quality Assurance unit headquartered at:

BioReliance

Positive Controls:

Bovine viral diarrhea virus (BVDV), ATCC VR-534
Source: American Type Culture Collection (ATCC)
Manassas, VA

Bovine adenovirus type 5 (BAV5) or Type 3 (BAV3)
ATCC VR-641 or ATCC VR-639
Source: ATCC

Bovine parvovirus (BPV), ATCC VR-767
Source: ATCC

Bluetongue virus (BTV), Strain BT-2
Source: National Veterinary Services Laboratories (NVSL)
Ames, IA

Bovine respiratory syncytial virus (BRSV), ATCC VR-1339
Source: ATCC

Reovirus type 3 (REO-3), Strain Abney, ATCC VR-232
Source: ATCC

Infectious bovine rhinotracheitis virus (IBR)
(Bovine herpesvirus 1), ATCC VR-188
Source: ATCC

Bovine parainfluenza virus type 3 (PI3)
(Shipping fever virus), ATCC VR-281
Source: ATCC

Rabies virus (positive control slides)
Source: NVSL

Negative Control:

Dulbecco's Modified Eagle's Medium/Ham's F-12 (1:1 mix) + 15% foal serum, 1% L-glutamine, 0.1% Amphotericin B, and 0.1% Gentamicin (BT)

Dulbecco's Modified Eagle's Medium/Ham's F-12 (1:1 mix) + 15% irradiated FBS or foal serum, 1% L-glutamine, 0.1% Amphotericin B, and 0.1% Gentamicin (Vero)

Source: BioReliance

Test System:

Bovine turbinate (BT) cells, ATCC CRL 1390 or equivalent
Source: ATCC
Indicator cells for BVDV, BAV5, BPV, BTV, BRSV, IBR, PI3 and Rabies

Vero (African green monkey kidney) cells, ATCC CCL 81
Source: ATCC
Indicator cells for REO-3, PI3 and Rabies

Erythrocytes:
Chicken erythrocytes
Source: Cambrex Biosciences Walkersville
Walkersville, MD

Guinea-pig erythrocytes
Source: BioReliance

Antibodies:
FITC-conjugated virus-specific immunoglobulins
Source: VMRD Inc. Pullman, Washington
NVSL Ames, Iowa

OBJECTIVE

The study objective is to determine if the test article contains bovine viruses that can be detected by culture with BT and Vero cells. The detection of these viruses is based upon microscopic observation of viral cytopathology in indicator cells, immunofluorescent staining with virus-specific antibodies, a hemadsorption assay, and a cytological staining procedure.

PROCEDURES

Sample Preparation

The test article provided by the sponsor was frozen and thawed three times and the resulting lysate was clarified by low speed centrifugation prior to inoculation onto indicator cells.

Methods

The assay was performed according to SOP OPBT0834. The test article was prepared as described above and was used to inoculate subconfluent monolayers of BT and Vero indicator cells seeded at an appropriate passage level for each cell line. After adsorption for 90 ± 9 minutes at 36 ± 2° C, the test article was aspirated and the cells were refed with negative control medium. The cells were observed for viral cytopathology throughout the assay. Negative control and test article cells were first subcultured on day 7 post inoculation. At the time of the second subculture, negative

control and test article cells were subcultured into 75cm^2 flasks and 6-well plates.

One day prior to the second subculture, negative control cells from each indicator line were subcultured to 25-cm^2 flasks and 6-well plates for the positive control inoculation. At the time of the second subculture, flasks of Vero cells were inoculated with REO-3 and flasks of BT cells inoculated with BVDV, BAV5, BPV, BTV, IBR, PI3 and BRSV at 100-300 $FAID_{50}$. The cells were fixed for immunofluorescent staining when the monolayers exhibited ≥ 10% CPE and slides were stored at ≤-60°C. One flask each of BT and Vero negative control and test article cells were harvested the same day their respective positive control flasks were harvested and fixed for IFA testing. Additional flasks of test article and negative control cells were maintained in the lab until assay completion, at which time they were fixed for IFA testing. All fixed cells were stained for IFA at the completion of the assay.

Also at the second subculture, 6-well plates seeded with BT cells were inoculated with PI3 and BVDV, positive controls for hemadsorption and cytological staining, respectively. The Vero cell 6-well plates were inoculated with PI3 for both hemadsorption and cytological staining. The hemadsorption assay and the cytological staining procedure were performed on all conditions at the completion of the assay or when CPE became apparent.

Immunofluorescent Staining

Fixed indicator cells were evaluated for the presence of BVDV, BAV5, BPV, BTV, BRSV, REO-3, IBR, PI3, and Rabies by immunofluorescent staining according to SOP BPBT0829. FITC-conjugated antibodies were incubated with the fixed cells for approximately 60 minutes at 36 ± 2°C. Following incubation, cells were washed with PBS, counterstained with Evans Blue, washed with PBS, and examined by fluorescent microscopy.

Hemadsorption Assay

The negative control, test article and positive control inoculated cultures in 6-well plates were tested by hemadsorption according to SOP OPBT0608. Guinea pig and chicken erythrocytes were inoculated onto the plates and incubated at 2 - 8°C for approximately 30 minutes. The 6-well plates were then incubated at 20 - 25°C for an additional 30 minutes. Cultures were examined microscopically for areas of adherent erythrocytes after each incubation.

Cytological Staining

The negative control, test article and positive control inoculated cultures in 6-well plates were observed for CPE according to SOP OPBT1223. The plates were fixed with a methanol solution and stained with Giemsa, washed with PBS and then examined for CPE.

RESULTS

Bovine viruses were not detected in the test article, H1-MCB.1. Cytopathic effects were not observed in the test article-inoculated BT or Vero cells cultured for 21 days (Table 1). Additionally, CPE was not observed in the test article inoculated BT or Vero cells using cytological staining (Table 2). The test article-inoculated cultures did not hemadsorb with either erythrocyte at either temperature (Table 3). The acetone-fixed indicator cell suspensions did not exhibit fluorescence when reacted with antisera specific for the nine bovine viruses used in this assay (Table 4). All assay controls met the criteria for a valid assay.

APPROVAL

I accept responsibility for the conduct of this study which was performed in compliance with the U.S. FDA Good Laboratory Practice regulations (21 CFR 58), the UK GLP Regulations, the Japanese GLP Standard and the Organization for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice.

18 MAY 07
Date

Study Director

TABLE 1

Observations for Cytopathic Effects in Cultures of BT and Vero Cells Inoculated with H1-MCB.1

Sample	Results
Negative control[a]	-
Test article[a]	-
Negative control[b]	-
Test article[b]	-
Positive Control Cultures:	
BAV5[a]	+
BPV[a]	+
BRSV[a]	+
BTV[a]	+
BVDV[a]	+
REO-3[b]	+
IBR[a]	+
PI3[a]	+

[a] Inoculated onto BT cells
[b] Inoculated onto Vero cells
- CPE not observed
+ CPE observed

TABLE 2

Observations for CPE using Cytological Staining on BT and Vero Cultures Inoculated with H1-MCB.1

Cytological Staining Results	
Day 21 BT Cells	
Negative Control	-
Test Article	-
Positive Control BVDV [a]	+
Day 21 Vero Cells	
Negative Control	-
Test Article	-
Positive Control PI3 [a]	+

[a] Positive control tested on day 17
- CPE not observed
+ CPE observed

Table 3

Observations for Hemadsorption in BT and Vero Cultures Inoculated with H1-MCB.1

	Hemadsorption Results[b]			
	2-8°C		20-25°C	
	C[a]	G	C	G
Day 21 BT Cells				
Negative Control	-	-	-	-
Test Article	-	-	-	-
Positive Control PI3[b]	+	+	+	+
Day 21 Vero Cells				
Negative Control	-	-	-	-
Test Article	-	-	-	-
Positive Control PI3[b]	+	+	+	+

[a] Erythrocytes used in these assays: C = chicken, G = guinea pig
[b] Positive control tested on day 17
- Hemadsorption not observed
+ Hemadsorption observed

TABLE 4

Immunofluorescent Staining Results for BT and Vero Cultures Inoculated with H1-MCB.1

	Antisera									
	PBS [a]	αBAV5 [a]	αBPV [a]	αBRSV [a]	αBTV [a]	αBVDV [a]	αREO3 [b]	αRabies [a, b]	αIBR [a]	αPI3 [a]
Slides Prepared Day 17										
Negative Control	-	NA	NA	NA	-	-	NA	NA	-	NA
Test Article	-	NA	NA	NA	-	-	NA	NA	-	NA
Slides Prepared Day 19										
Negative Control	-	-	NA	-	NA	NA	NA	NA	NA	-
Test Article	-	-	NA	-	NA	NA	NA	NA	NA	-
Slides Prepared Day 21	PBS [a, b]	αBAV5 [a]	αBPV [a]	αBRSV [a]	αBTV [a]	αBVDV [a]	αREO3 [a, b]	αRabies [a, b]	αIBR [a]	αPI3 [a]
Negative Control	-	-	-	-	-	-	-	-	-	-
Test Article	-	-	-	-	-	-	-	-	-	-
	PBS [a, b]	αBAV5 [a]	αBPV [a]	αBRSV [a]	αBTV [a]	αBVDV [a]	αREO3 [b]	αRabies [c]	αIBR [a]	αPI3 [a]
Positive Control	-	+ [d]	+	+ [d]	+ [d]	+ [d]	+	+	+ [d]	+ [d]

[a] Tested in BT indicator cells

[b] Tested in Vero indicator cells

[c] Tested on Rabies infected Vero positive control slide

[d] Data reflects results of positive control slides that were prepared on day 17 or 19

NA = Not Applicable

- Immunofluorescence not observed

+ Immunofluorescence observed

Quality Assurance Statement

Study Title: *IN VITRO* ASSAY FOR THE PRESENCE OF BOVINE VIRUSES ACCORDING TO 9 CFR REQUIREMENTS - NINE VIRUS ASSAY

Study Number: AC01UC.032901.BSV

Study Director:

Quality Assurance performed the inspections listed below for this study. Verification of the study protocol was also performed and documented by QA. Procedures, documentation, equipment records, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the UK GLP Regulations, the Japanese GLP Standard, and the OECD Principles of Good Laboratory Practice and to assure that the study is conducted according to the protocol and relevant Standard Operating Procedures.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

**	Inspect On	17-May-07 - 17-May-07 To Study Dir 17-May-07 To Mgmt 18-May-07
	Phase	Final Report and data audit
*	Inspect On	28-Mar-07 - 04-Apr-07 To Study Dir 04-Apr-07 To Mgmt 04-Apr-07
	Phase	Systems Inspection - Administration of Test Substance to Test System
*	Inspect On	28-Mar-07 - 04-Apr-07 To Study Dir 04-Apr-07 To Mgmt 04-Apr-07
	Phase	Systems Inspection - Manipulation of Test System
*	Inspect On	28-Mar-07 - 04-Apr-07 To Study Dir 04-Apr-07 To Mgmt 04-Apr-07
	Phase	Systems Inspection - Observation of Test System/Data Collection and/or Analysis
*	Inspect On	28-Mar-07 - 04-Apr-07 To Study Dir 04-Apr-07 To Mgmt 04-Apr-07
	Phase	Systems Inspection - Test System Preparation

** Inspection specific for this study
* Systems Inspection

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

18 May 07

DATE

QUALITY ASSURANCE

Final Report

In Vitro Assay for the Presence of Porcine Viruses According to Modified 9 CFR Requirements. PT-1 Indicator Cells Only

Study Number: AC01UC.033901.BSV

Test Article ID: H1-MCB.1

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

Porcine viruses, BVDV, reovirus and Rabies were not detected when the test article H1-MCB.1 was examined for the presence of porcine viruses using IFA. In addition, hemadsorption and CPE were not observed in the test article inoculated cultures.

BioReliance®

STUDY INFORMATION

Test Article: H1-MCB.1 was received by BioReliance on 05-Apr-2007. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor. Retention of reserve sample from each batch of test article is the responsibility of the sponsor.

Testing Facility: BioReliance

Schedule:

Study Initiation: 11-Apr-2007

Lab Initiation: 13-Apr-2007

Lab Completion: 08-May-2007

Study Completion: See Study Director's signature date in "Approval" Section.

Study Director:

Archives: All raw data, the protocol, and a copy of the final report will be maintained according to Standard Operating Procedure OPQP3040 by the BioReliance Quality Assurance unit headquartered at:

BioReliance,

Positive Controls:

Porcine Parvovirus (PPV), ATCC VR-742
Source: American Type Culture Collection (ATCC)
Manassas, Virginia

Transmissible Gastroenteritis Virus (TGE)
Source: National Veterinary Services Laboratories (NVSL)
Ames, Iowa

Porcine Adenovirus (PAV)
Source: NVSL

Bovine parainfluenza virus type 3 (PI3)
(Shipping fever virus), ATCC VR-281
Source: ATCC

Rabies virus (positive control slide)
Source: NVSL

Negative Control:

Eagle's Minimum Essential Medium + 10% fetal bovine serum, 1% L-glutamine, 0.1% Amphotericin B, 0.1% Gentamicin
Source: BioReliance

Test System:

Porcine testicle (PT-1) cells
Source: American BioResearch,
Seymour, Tennessee
Indicator cells for PAV, PPV and TGE

Erythrocytes:
Chicken erythrocytes
Source: Cambrex Bioscience Walkersville
Walkersville, MD

Guinea-pig erythrocytes
Source: BioReliance

Antibodies:
FITC-conjugated virus-specific immunoglobulins
Source: VMRD Inc.
Pullman, Washington

OBJECTIVE

The study objective is to determine if the test article contains porcine viruses that can be detected by culture with PT-1 cells. The detection of these viruses is based upon microscopic observation of viral cytopathology in indicator cells, immunofluorescent staining with virus-specific antibodies and a hemadsorption assay.

PROCEDURES

Sample Preparation

The test article provided by the sponsor was frozen and thawed three times and the resulting lysate was clarified by low speed centrifugation prior to inoculation onto PT-1 indicator cells.

Methods

The assay was performed according to SOP OPBT0874. The test article was prepared as described above and was used to inoculate subconfluent monolayers of PT-1 indicator cells. After adsorption for 90 ± 9 minutes at 36± 2°C, the test article was aspirated and cells were refed with negative control medium. The cultures were observed for viral cytopathology throughout the assay. Negative control and test article cells were first subcultured on day 7 post-inoculation. At the time of the second subculture, negative control and test article cells were subcultured into 75 cm^2 flasks and 6-well plates.

One day prior to the second subculture, negative control PT-1 cells were subcultured to 25 cm^2 flasks and a 6-well plate for the positive control inoculation. At the time of the second subculture, flasks of PT-1 cells were inoculated with PAV, PPV and TGE. Flasks of test article and control cells were harvested and fixed for IFA staining and the slides were stored at ≤-60°C. The fixed cells were stained for IFA at the completion of the assay.

Also at the second subculture, 6-well plates seeded with PT-1 cells were inoculated with PI3 as positive controls for hemadsorption. The hemadsorption assay was performed on all conditions at the completion of the assay or when CPE became apparent.

Immunofluorescent Staining

Fixed indicator cells were evaluated for the presence of PAV, PPV, TGE, BVDV, REO-3 and Rabies by immunofluorescent staining according to SOP BPBT0829. FITC-conjugated antibodies were incubated with the fixed cells for approximately 60 minutes at 36 ± 2°C. Following incubation, cells were washed with PBS, counterstained with Evans Blue, washed with PBS, and examined by fluorescent microscopy.

Hemadsorption Assay

The negative control, test article and positive control inoculated cultures in 6-well plates were

tested by hemadsorption according to SOP OPBT0608. Guinea pig and chicken erythrocytes were inoculated onto the plates and incubated at 2 - 8°C for approximately 30 minutes. The 6-well plates were then incubated at 20 - 25°C for an additional 30 minutes. Cultures were examined microscopically for areas of adherent erythrocytes after each incubation.

RESULTS

Porcine viruses were not detected in the test article H1-MCB.1. Cytopathic effects were not observed in the test article inoculated PT-1 indicator cells cultured for 21 days (Table 1). The test article-inoculated cultures did not hemadsorb with either erythrocyte at either temperature (Table 2). The acetone-fixed indicator cell suspensions did not exhibit fluorescence when reacted with antisera specific for the porcine and bovine viruses used in this assay (Table 3). All assay controls met the criteria for a valid assay.

APPROVAL

I accept responsibility for the conduct of this study which was performed in compliance with the U.S. FDA Good Laboratory Practice regulations (21 CFR 58), the UK GLP Regulations, the Japanese GLP Standard and the Organization for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice.

18 May 07
Date

Study Director

TABLE 1

Observations for Cytopathic Effects in Cultures of PT-1 Cells Inoculated with H1-MCB.1

Sample	Results
Negative control	-
Test article	-
Positive Control Cultures	
PAV	+
PPV	+
TGE	+

- CPE not observed
+ CPE observed

TABLE 2

Observations for Hemadsorption in Monolayers of PT-1 Cells Inoculated With H1-MCB.1

	Hemadsorption Results			
	2-8°C		20-25°C	
	C [a]	G	C	G
Day 21 PT-1 cells				
Negative Control	-	-	-	-
Test Article	-	-	-	-
Positive Control PI3 [b]	+	+	+	+

[a] Erythrocytes used in these assays: C = chicken, G = Guinea pig
[b] Positive control tested on day 17
- Hemadsorption not observed
+ Hemadsorption observed

TABLE 3

Immunofluorescent Staining Results for PT-1 cells Inoculated With H1-MCB.1

	PBS	αPAV	αPPV	αTGE	αBVDV	αREO-3	αRabies
Slides Prepared Day 21							
Test Article	-	-	-	-	-	-	-
Negative Control	-	-	-	-	-	-	-
	PBS	**αPAV**	**αPPV**	**αTGE**	**αBVDV [b]**	**αREO-3 [b]**	**αRabies [a]**
Positive Control	-	+	+	+	+	+	+

- = immunofluorescence not observed

+ = immunofluorescence observed

[a] Tested on Rabies infected Vero positive control slide

[b] Slides from corresponding bovine study.

Quality Assurance Statement

Study Title: IN VITRO ASSAY FOR THE PRESENCE OF PORCINE VIRUSES ACCORDING TO MODIFIED 9 CFR REQUIREMENTS. PT-1 INDICATOR CELLS ONLY.

Study Number: AC01UC.033901.BSV

Study Director:

Quality Assurance performed the inspections listed below for this study. Verification of the study protocol was also performed and documented by QA. Procedures, documentation, equipment records, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the UK GLP Regulations, the Japanese GLP Standard, and the OECD Principles of Good Laboratory Practice and to assure that the study is conducted according to the protocol and relevant Standard Operating Procedures.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

**	Inspect On	18-May-07 - 18-May-07 To Study Dir 18-May-07 To Mgmt 18-May-07
	Phase	Final Report and data audit
*	Inspect On	28-Mar-07 - 04-Apr-07 To Study Dir 04-Apr-07 To Mgmt 04-Apr-07
	Phase	Systems Inspection - Administration of Test Substance to Test System
*	Inspect On	28-Mar-07 - 04-Apr-07 To Study Dir 04-Apr-07 To Mgmt 04-Apr-07
	Phase	Systems Inspection - Manipulation of Test System
*	Inspect On	28-Mar-07 - 04-Apr-07 To Study Dir 04-Apr-07 To Mgmt 04-Apr-07
	Phase	Systems Inspection - Observation of Test System/Data Collection and/or Analysis
*	Inspect On	28-Mar-07 - 04-Apr-07 To Study Dir 04-Apr-07 To Mgmt 04-Apr-07
	Phase	Systems Inspection - Test System Preparation

** Inspection specific for this study
* Systems Inspection

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

18 May 07

DATE

QUALITY ASSURANCE

Final Report

MOUSE ANTIBODY PRODUCTION (MAP) TEST

Study Number: AC01UC.004000.BSV

Test Article ID: H1-MCB.1

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

Based on the data obtained in the assays performed, the test article, H1-MCB.1, has been shown to be free of all of the seventeen murine viruses for which it was examined.

BioReliance®

STUDY INFORMATION

Test Article Receipt:	H1-MCB.1 was received at BioReliance on 04/05/2007. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor. Retention of samples of the test article is the sole responsibility of the sponsor.
Testing Facility:	BioReliance
Animal Facility:	BioReliance
Schedule:	
Study Initiation:	04/10/2007
Lab Initiation:	04/11/2007
Lab Completion:	05/14/2007
Study Completion:	See Study Director's signature date in the "Approval" Section.
Study Director:	
Technical Support Staff:	Laboratory Manager, Serology
Archives:	All raw data, records, the protocol and a copy of the final report will be maintained according to Standard Operating Procedure OPQP3040 by the BioReliance Quality & Regulatory Affairs Unit headquartered at: BioReliance

Negative Control:	Eagle's Minimum Essential Medium with Penicillin/Streptomycin
LCM Challenge Virus:	Lymphocytic Choriomeningitis (CA1371 Strain)
Test System:	Mice, HSD:ICR twelve females, four to ten weeks old Source: Harlan Sprague Dawley Frederick, Maryland

JUSTIFICATION

The purpose of the Mouse Antibody Production (MAP) Test is to detect the presence of one or more specific murine viruses in a test article. For over three decades, the MAP test has been considered the foremost method for the detection of adventitious murine viruses in cell lines (Collins, 1972 and Rowe, 1959). This determination is made by the injection of the test article into viral antibody free mice and the subsequent testing of the sera, four weeks later, for antibodies to murine viruses.

Four routes of injection are used in the assay to provide optimum conditions for infection with a broad range of adventitious viruses. The per os route provides enteric viruses (MHV, GDVII) access to their most common receptor sites within the alimentary canal. The intranasal route exposes respiratory viruses (PVM, Sendai) to their most common receptor sites in the nasal mucosa. The intraperitoneal route assures that adventitious viral contaminants in the test article are exposed to the internal organs, but bypass the virucidal mucous membranes of the alimentary canal. The abrasion of the skin at the puncture site of the IP injection serves as an entry route for the ectromelia virus. The intracerebral injection monitors for LCM virus by permitting access to the meninges of the brain.

In order to detect avirulent strains of the LCM virus (LCMV), an In-Vivo challenge test is performed as part of the MAP assay. Mice injected intracerebrally with the test article are challenged with a known lethal dose of LCMV. The presence of LCMV in the test article will render these mice immune to challenge and they will survive. The absence of LCMV in the test article is indicated by the death of the animals challenged with the lethal dose of LCMV.

The presence of Lactate Dehydrogenase-Elevating Virus (LDV) in the test article is demonstrated by elevation of the level of lactate dehydrogenase (LDH) in the plasma, which is determined by a reduction (NAD) assay and measured spectrophotometrically.

PROCEDURES

Animal Husbandry

All animals were fed autoclavable diets *ad libitum*. Autoclaved water was supplied via water bottles. Corncob bedding was utilized.

The animal facilities are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International.

Methods

Healthy, adventitious viral antibody free mice were randomized according to SOP OPBT0213 into micro-isolator cages labelled with the various groups. The animals were not quarantined and were identified by group (cage) and animal number. All animal manipulations were carried out within a Class II biological safety cabinet.

The mice were injected with the test article or the negative control article as indicated in Table 1. No sooner than three, but no later than 10 days post injection, the plasma or serum of each of three test article mice (Group II), two negative control article mice (Group III), and two LDV positive control mice was examined for lactate dehydrogenase activity.

No sooner than 14 days post-injection, the mice in Group II, as well as three LCMV challenge control mice, were injected with a known lethal strain of LCM virus and observed for morbidity and mortality each working day for as long as three weeks.

No less than twenty-eight days post-injection, the remaining mice (Groups I and III) were bled and their serum assayed by ELISA, Hemagglutination Inhibition or Indirect Fluorescent Antibody assays for the presence of antibody to the murine viruses listed below. Questionable results are retested as necessary.

The following table lists each specific test which may have been performed on the mouse sera, to detect the presence of antibody to adventitious murine viruses.

Virus	Assay
Ectromelia	ELISA[1] or IFA[3]
GDVII	ELISA or IFA
Lactate Dehydrogenase Virus (LDV)	NAD Reduction[2]
Lymphocytic Choriomeningitis	ELISA or IFA and LCM virus challenge
Hantaan Virus	ELISA or IFA
Mouse Minute Virus (MMV)	ELISA, IFA, or HI[4]
Mouse Parvovirus (MPV)	ELISA or IFA
Mouse Adenovirus	ELISA or IFA
Mouse Hepatitis Virus (MHV)	ELISA or IFA
Pneumonia Virus of Mice (PVM)	ELISA, IFA, or HI
Polyoma	ELISA, IFA, or HI
Sendai	ELISA, IFA, or HI
Epizootic Diarrhea of Infant Mice (EDIM)	ELISA or IFA
Mouse Salivary Gland Virus (Mouse Cytomegalovirus) (MCMV)	IFA
Reovirus Type 3	ELISA, IFA, or HI
K	HI
Mouse Thymic Virus (MTV)	IFA

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[1] Enzyme Linked Immunosorbent Assay (OPDL0806)

[2] Testing performed using BioReliance SOP OPVM7009

[3] Indirect Fluorescent Antibody Test (OPDL0810)

[4] Hemagglutination Inhibition (OPDL0621)

CRITERIA FOR A VALID TEST

Serology Assays

Each serology test is considered valid, if sera from the negative control injected mice are negative for antibody to the virus, if the serology negative control sera are negative and if the serology positive control sera give appropriate virus-specific reactivity for the test which is being performed.

LDV Assay

If all of the test article injected mice have LDH levels less than 600 IU/L, and both of the negative control article injected mice have LDH levels less than 600 IU/L, the test is considered valid. If all of the test article injected mice have LDH values less than 600 IU/L and one or both of the negative control article injected mice have LDH levels greater than 600 IU/L, the test is considered valid.

If one or more of the test article injected mice and one or both of the negative control injected mice have LDH levels greater than or equal to 600 IU/L, the test is considered invalid.

If one or both of the positive control mice have LDH levels less than 600 IU/L, the test is considered invalid.

LCM Virus Assay

The LCM virus challenge test is considered valid if a minimum of 2 of the 3 LCM virus challenged control mice die within 21 days post-challenge due to evidence of LCM virus infection, and not due to injection trauma. Signs of injection trauma would occur within 48 hours post-challenge and would include morbidity, moribundity, lethargy, ruffled coat and/or neurological signs (tilted head, abnormal gait, and tremors).

In the repeat assay, if performed, the LCM virus challenge test is considered valid if 4 of the 6 LCM virus challenged control mice die within 21 days post-challenge due to evidence of LCM virus infection, and not due to injection trauma. Signs of injection trauma would occur within 48 hours post-challenge and would include morbidity, moribundity, lethargy, ruffled coat and/or neurological signs (tilted head, abnormal gait, and tremors).

EVALUATION OF TEST RESULTS

Serology Assays

Positive viral antibody titers are indicative of viral contamination of the test article. A minimum of 2 of the 4 mice injected with the test article must sero-convert for the test article to be considered positive. If only one of the 4 test article injected animals sero-converts, a repeat assay may be recommended.

LDV Assay

Elevated LDH levels ($\geq$600 IU/L) in the test article injected animals are indicative of the presence of LDV, if confirmed by the LDV passage procedure.

LCM Virus Assay

A test article is considered negative for LCM virus when a minimum of two of the three test article injected animals die due to evidence of LCM virus infection, and not due to injection trauma. Signs of injection trauma would occur within 48 hours post-challenge and would include morbidity, moribundity, lethargy, ruffled coat and/or neurological signs (tilted head, abnormal gait, and tremors).

If one or more test article injected animals survives the lethal challenge with LCM virus, a repeat LCM challenge is performed using twice the number of test article injected animals.

In the six mouse repeat assay, a test article is considered negative for LCM virus when a minimum of four of the six test article injected animals die within 21 days post-challenge due to evidence of LCM virus infection and not due to injection trauma. Signs of injection trauma would occur within 48 hours post-challenge and would include morbidity, moribundity, lethargy, ruffled coat and/or neurological signs (tilted head, abnormal gait, and tremors).

In the repeat assay, if one or more test article injected animals survives the lethal challenge of LCM virus, and the test is valid, the test article cannot be considered negative for the presence of LCM. Additional testing may be required.

RESULTS

All sera from animals injected with the test article or the negative control article (Eagle's Minimum Essential Medium) were negative for the presence of antibody to Ectromelia, GDVII, LCM, Hantaan, MMV, MPV, Mouse Adenovirus, MHV, PVM, Polyoma, Reovirus Type 3, EDIM, MCMV, K, MTV and Sendai viruses as determined by ELISA, IFA, or HI. See Table 2.

All plasma from animals tested for lactate dehydrogenase activity showed normal levels except for LDV injected control animals, which showed elevated levels of LDH activity. See Table 3.

All animals challenged with LCM virus died within ten days of being challenged, indicating that they were not protected by antibody to LCMV produced in response to the original test article material. LCM virus control animals from the same source and shipment as the test group exhibited a rate of mortality, after challenge, which confirmed the absence of LCM virus in the test article.

REFERENCES

Collins, M.J. Jr. and J.C. Parker. (1972) Murine Viral Contaminants of Leukemia Viruses and Transplantable Tumors. J. Nat. Cancer Inst. 49: 1139-1143.

Rowe, W.P., J.W. Hartley, and R.J. Huebner (1959). Studies of Mouse Polyoma Virus Infection. Procedures for Quantitation and Detection of Virus. J. Exp. Med. 109: 379-391.

APPROVAL

I accept responsibility for the conduct of this study which was performed in compliance with the U.S. Food and Drug Administration Good Laboratory Practice Regulations (21 CFR 58), the UK GLP Regulations, the Japanese GLP Standard and the Organization for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice.

15 Jun 07

Date

Study Director

Table 1

Group No.	No. of Mice	Test Material	Route of Injection[a]	Vol. of Test Material	Day of Injection(s)	Treatments Post-Injection
I	4	Test Article	p.o. i.n. i.p.	0.05 ml 0.05 ml 0.5 ml	0	Animals were exsanguinated no sooner than 28 days post-injection and the sera were tested for antibody to murine viruses.
Ia	3	Test Article (1:10)			See note below	
II	3	Test Article (1:10)	p.o. i.n. i.p. i.c.	0.05 ml 0.05 ml 0.5 ml 0.03 ml	0	Animals were bled 3 to 10 days post-injection and the plasma or serum was tested for LDH activity.
		A lethal dose of LCM virus, as determined by pool titration, no less than 100 LD_{50} of LCM	i.c.	0.03 ml	No sooner than 14	Animals were observed for death.
III	2	EMEM[b]	p.o. i.n. i.p.	0.05 ml 0.05 ml 0.5 ml	0	3 to 10 days post-injection animals were bled and the plasma or serum was tested for LDH activity. Animals were exsanguinated no sooner than 28 days post-injection and the sera was tested for antibody to murine viruses.
IV	3	A lethal dose of LCM virus, as determined by pool titration, no less than 100 LD_{50} of LCM	i.c.	0.03 ml	No sooner than 14	Animals were observed for death.

[a] p.o. = per os; i.n. = intranasal; i.p. = intraperitoneal; i.c. = intracerebral
[b] Eagle's Minimum Essential Medium with penicillin and streptomycin

NOTE: Group Ia was used only if the undiluted test article was toxic to the animals; therefore, group Ia was started, if necessary, later than the other cages.

Table 2

Serological Assays for H1-MCB.1

Serum from Animals injected with	PVM[2]	REO3[2]	SENDAI[2]	GDVII[2]	HANTAAN[2]	POLYOMA[2]	MMV[2]	MPV[2]	ADENO[2]	MHV[2]	LCM[2]	ECTROMELIA[2]	EDIM[2]	MCMV[3]	K[1]	MTV[3]
Test Article	0	0	0	0	0	.02	I[4]	.01	.03	0	0	0	.07	-	-	-
	.07	0	0	0	0	0	I[4]	.03	.07	0	.01	0	.10	-	-	-
	I[4]	0	0	0	0	0	I[4]	.03	I[4]	.01	0	0	.14	-	-	-
	.01	0	0	0	0	0	I[4]	.01	.02	0	0	0	.10	-	-	-
Negative Control	0	.01	.02	0	0	.01	0	.01	.01	.01	0	0	.03	-	-	-
	.04	0	0	.01	.01	0	.07	.01	.01	.01	0	0	.04	-	-	-
Serology Positive Control	1.14	1.03	1.15	1.12	1.10	1.15	1.13	1.14	.99	1.14	1.14	1.14	1.15	+	160	+

[1] Serum antibody titer less than 1:10 is negative (-) as measured by Hemagglutination Inhibition. A titer was reported for the serology positive control.
[2] These titers are measured by ELISA. A serum must have an absorbance value of greater than or equal to 0.17 to be considered positive.
[3] Serum antibody measured by Indirect Fluorescent Antibody. - = negative, + = positive
[4] I = Original results were inconclusive. Sample was retested using IFA. Sample was negative. Serology negative control was negative (-) and serology positive control was positive (+) for the IFA retest.

Table 3

LDV Assay for H1-MCB.1

Plasma from Animals Injected with	LDH Titer[a]
Test Article (1:10) (Group II)	197 359 265
Negative Control (Group III)	381 141
LDV Control	1019 802

[a] Plasma titers less than 600 IU/L are negative.

Quality Assurance Statement

Study Title: MOUSE ANTIBODY PRODUCTION (MAP) TEST

Study Number: AC01UC.004000.BSV

Study Director:

Quality Assurance performed the inspections listed below for this study. Verification of the study protocol was also performed and documented by QA. Procedures, documentation, equipment records, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the UK GLP Regulations, the Japanese GLP Standard, and the OECD Principles of Good Laboratory Practice and to assure that the study is conducted according to the protocol and relevant Standard Operating Procedures.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

** Inspect On	13-Jun-07 - 13-Jun-07 To Study Dir 13-Jun-07 To Mgmt 15-Jun-07
Phase	Final Report and data audit
* Inspect On	29-Mar-07 - 29-Mar-07 To Study Dir 29-Mar-07 To Mgmt 29-Mar-07
Phase	Systems Inspection - Administration of Test Substance to Test System
* Inspect On	01-May-07 - 01-May-07 To Study Dir 01-May-07 To Mgmt 01-May-07
Phase	Systems Inspection - Observation of Test System/Data Collection and/or Analysis
* Inspect On	22-May-07 - 22-May-07 To Study Dir 22-May-07 To Mgmt 22-May-07
Phase	Systems Inspection - Manipulation of Test System
* Inspect On	06-Jun-07 - 06-Jun-07 To Study Dir 06-Jun-07 To Mgmt 06-Jun-07
Phase	Systems Inspection - Test System Preparation

** Inspection specific for this study
* Systems Inspection

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

15 June 07
DATE

QUALITY ASSURANCE

Final Report

Study Title	*In Vitro* Evaluation of Adventitious Viruses in Cell Cultures – 28 day assay
Test Article	H1-MCB.1
Author	
Test Facility	Covance Laboratories Ltd
Sponsor Representative	
Sponsor	
Covance Study Number	2823/001
Covance Report Number	2823/001-D5141
Report Issued	October 2007
Page Number	1 of 28

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STUDY DIRECTOR AUTHENTICATION AND GLP COMPLIANCE STATEMENT

In Vitro Evaluation of Adventitious Viruses in Cell Cultures – 28 day assay

I, the undersigned, hereby declare that the work was performed by me or under my supervision and that the findings provide a true and accurate record of the results obtained.

The study was performed in accordance with the agreed Protocol and with Covance Laboratories Limited Standard Operating Procedures, unless otherwise stated, and the study objectives were achieved.

The study was conducted in compliance with:

The United Kingdom Statutory Instrument 1999 No.3106, the Good Laboratory Practice Regulations 1999, as amended by the Good Laboratory Practice (Codification Amendments Etc.) Regulations 2004.

The Organisation for Economic Co-operation and Development (OECD) Principles on Good Laboratory Practice (revised 1997, issued January 1998). ENV/MC/CHEM(98)17.

02/Oct/07
Date

Study Director

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QUALITY ASSURANCE STATEMENT

In Vitro Evaluation of Adventitious Viruses in Cell Cultures – 28 day assay

This study has been reviewed by the Quality Assurance Unit of Covance Laboratories Ltd. and the report accurately reflects the raw data. The following inspections were conducted and findings reported to the study director (SD) and associated management.

Critical procedures, which are performed routinely in an operational area, may be audited as part of a "process" inspection programme. This can be in addition to phases scheduled on an individual study basis. Selected process inspections conducted and considered applicable to this study are included below.

In addition to the inspection programmes detailed below, a facility inspection programme is also operated. Details of this programme, which covers all areas of the facility annually (at a minimum), are set out in standard operating procedures.

Inspection Dates		Phase	Date Reported to SD and SD Management
From	To		
11 Jun 2007	11 Jun 2007	Protocol Review	11 Jun 2007
10 Aug 2007	10 Aug 2007	Draft Report and Data Review	10 Aug 2007
02 Oct 2007	02 Oct 2007	Final Report Review	02 Oct 2007

Process

Inspection Dates		Phase	Date Reported to SD and SD Management
From	To		
04 Jul 2007	04 Jul 2007	Test Article Receipt	04 Jul 2007

2 Oct 07
Date

Quality Assurance Unit

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RESPONSIBLE PERSONNEL

In Vitro Evaluation of Adventitious Viruses in Cell Cultures – 28 day assay

The following personnel were responsible for key elements of the study:

Study Director:

Study Supervisor:

STUDY SCHEDULE

The study schedule was as follows:

Study initiation date:	6th June 2007 (Date Study Director signed Client Protocol).
Assay initiation date:	6th June 2007 (Date of the first study specific data capture).
Assay completion date:	10th July 2007 (Date of final data capture).
Study completion date:	Date Study Director signed Final Report.

ARCHIVE STATEMENT

In Vitro Evaluation of Adventitious Viruses in Cell Cultures – 28 day assay

All primary data, or authenticated copies thereof, specimens and the Final Report will be retained in the Covance Laboratories Limited archives for one year after issue of the Final Report. At this time, the Sponsor will be contacted to determine whether the data should be returned, retained or destroyed on their behalf. Sponsors will be notified of the financial implications of each of these options at that time.

Specimens or samples requiring frozen storage at Covance are specifically excluded from the above. These will be retained for as long as the material permits further evaluation, up to three months after issue of the Draft Report. At this time, the Sponsor will be contacted to determine whether samples should be returned, retained or destroyed on their behalf. Any financial implications of these options will also be notified at this time. Samples will not be destroyed without prior approval of the Study Director.

CONTENTS

SUMMARY

Four indicator cell lines (Vero, MRC-5, HeLa and NIH 3T3) were inoculated with either negative control (diluent), positive control virus or test article and incubated for 28 days. All positive controls turned positive for cytopathic effect (CPE). A fresh positive control for the MRC-5 cell line was set up on day 23 and this was positive for haemadsorption, as was the original MRC-5 positive control. All test article and negative control inoculated cells were negative for CPE and haemadsorption.

INTRODUCTION AND OBJECTIVE

The objective of this study was to determine the viral status of the test article. The assay was to detect the presence of viruses that induce CPE in culture cells; furthermore, the assay was to detect viruses capable of inducing haemadsorption.

MATERIALS

Protocol Adherence
The study described in this report was carried out according to the agreed Client Protocol, see Annex for details. Minor deviations, which are deemed not to have affected the study, are presented in the Appendix.

Test Article
The test article was received at Covance Laboratories Ltd on 29th March 2007 in two 15 ml centrifuge tubes each containing approximately 11 mls of a red/pink frozen material. The sample was received on dry ice and stored according to Sponsor instructions until required for the assay.

Identification:	H1-MCB.1
Source:	Sponsor.
Details on Test Article Vessel:	Covance 10 ml @ $1x10^6$ c/ml MCB.A.H1p30. 24 JAN07. DF

Appearance:	Red/pink frozen material.
Description:	Cell suspension.
Storage conditions:	< – 70°C.
Sterility check performed:	No.

This study to determine the presence of extraneous agents was conducted to define the purity of the test substance therefore, information regarding the purity of the test article was not available at study initiation. Stability of the test article was not considered relevant, as the objective of the study was to test for extraneous agents (adventitious viruses) that may be present in the test material.

Test Article Preparation

Prior to the assay starting, a cell lysate was prepared by freeze-thawing the test article three times in liquid nitrogen and a waterbath set at 37°C. The test article was then clarified by centrifugation.

TEST SYSTEM

Positive control virus:	*Parainfluenza 3* (PI3) strain SF-4 used at approximately $1x10^4$ $TCID_{50}$/ml (control for Vero, HeLa and MRC-5 cells). *Minute virus of mice* (MVM) used at approximately $1x10^4$ $TCID_{50}$/ml (control for CPE on NIH 3T3)
Source:	Maintained as laboratory stocks, original stocks supplied by ATCC.
Negative control (virus diluent):	Minimal essential medium + 5% tryptose phosphate broth.
Source:	Minimum essential medium. Tryptose phosphate broth. Gentamycin. Fungizone. Supplied by Invitrogen.

Indicator cell lines:	MRC-5. Vero. HeLa. NIH 3T3.
Source:	Maintained as laboratory stocks, original stocks supplied by ATCC or ECACC.
Growth medium:	Minimal essential medium containing Earles salts, non-essential amino acids plus 10% foetal calf serum. Minimum essential medium containing Earles Salts, non-essential amino acids plus 5% foetal calf serum for the re-feed.
Source:	Minimum essential medium. Non-essential amino acids. Gentamycin. Fungizone. Supplied by Invitrogen. Foetal calf serum. Supplied by PAA.

EXPERIMENTAL PROCEDURES AND DATA ANALYSIS

The experimental procedures were performed as outlined in the Client Protocol, see Annex for details. Minor deviations deemed not to have affected the study are presented in the Appendix.

The assay acceptance and evaluation criteria as detailed in the Client Protocol were achieved, see Annex for details.

RESULTS

Four indicator cell lines (Vero, MRC-5, HeLa and NIH 3T3) were inoculated with either negative control (diluent), positive control virus, test article or test article spiked with positive control and incubated for 28 days. MRC-5 cells that were negative for CPE were refed, and Vero, HeLa and NIH 3T3 cells that were negative for CPE were subcultured on day 7. On day 13 and on day 20 the NIH 3T3 cells were looking unhealthy so were refed to revive them. On day 14 and day 21 all cells that were negative for CPE were subcultured. They were observed for CPE and haemadsorption. Positive controls and spiked test article-inoculated cells for Vero and HeLa cells were positive for CPE by day 7. The positive control and spiked test article-inoculated cells for MRC-5 and NIH 3T3 cells were positive by day 28. A fresh positive control for the MRC-5 cell line was set up on day 23 and this was positive for haemadsorption with Guinea Pig, Human O and Adult Chicken blood at 7.6°C and 24.0°C. All test article and negative control-inoculated cells were negative for CPE and haemadsorption.

TABLES

Table 1: Day 14 and 28 Observation for Cytopathic Effect using Indicator Cells Inoculated with Test Article, Spike Test Article, Positive and Negative Controls

Sample	Indicator cell lines			
	Vero	MRC-5	HeLa	NIH 3T3
Indicator Assay First 14-Day Period Observations				
Negative Control	–	–	–	–*[1]
Test Article	–	–	–	–*[1]
Spiked Test Article	+	–*	+	–*[1]
Positive Control	+	–*	+	–*[1]
Indicator Assay Second 14-Day Period Observations				
Negative Control	–	–	–*[4]	–*[1]*[4]
Test Article	–	–*[2]	–*[4]	–*[1]*[4]
Spiked Test Article	N/A	+*[3]	N/A	+*[1]
Positive Control	N/A	+*[5]	N/A	+*[1]

\+ = Some or all flasks exhibited CPE.
– = Flasks did not exhibit CPE (normal morphology observed).
N/A = Not applicable as cells were discarded in first 14 days
* = Some vacuolation observed but not positive for CPE
*[1] = Cells were very overgrown and starting to die so were refed on day 13 and day 20.
*[2] = Some rounded cells observed on day 17 due to overgrowth and not CPE
*[3] = Early signs of CPE observed, which was confirmed as viral in haemadsorption assay (Table 2)
*[4] = Floating cells observed due to overgrowth
*[5] = Both original and fresh positive control (for haemadsorption assay) turned positive for CPE

Table 2: Day 28 Observation for Haemadsorption using Indicator Cells Inoculated with Test Article, Spiked Test Article, Positive and Negative Controls

Sample	Indicator cell lines			
	Vero	MRC-5	HeLa	NIH 3T3
		1-10°C Incubation		
Negative Control	–*[1]	–	–	–
Test Article	–*[1]	–	–	–
Spiked Test Article	N/A	+	N/A	N/A
Positive Control	N/A	+	N/A	N/A
Fresh Positive Control	N/A	+*	N/A	N/A
		37 ± 1°C Incubation		
Negative Control	–*[1]	–	–	–
Test Article	–*[1]	–	–	–
Spiked Test Article	N/A	+	N/A	N/A
Positive Control	N/A	+	N/A	N/A
Fresh Positive Control	N/A	+*	N/A	N/A

All Indicator cell lines were tested with a mixture of Adult Chicken blood, Guinea Pig blood and Human O blood.
+ = Haemadsorption observed.
– = No haemadsorption observed.
N/A = Not applicable.
* = Two fresh positive controls were set up, one inoculated with $1x10^4$ $TCID_{50}$/ml and one with $1x10^5$ $TCID_{50}$/ml, both were positive for haemadsorption.
*[1] = Some non-specific binding observed.

CONCLUSION

The test article (H1-MCB.1) was assessed for presence of adventitious viruses that are capable of causing cytopathic effects or haemadsorption within this system. The results show that no evidence of viral contamination was observed in the test article.

APPENDIX

Minor Deviations from the Protocol

1. DMEM was used to culture the 3T3 cell line during this study. The Protocol states MEM should be used, but DMEM is the preferred medium for this cell line. This deviation should improve the growth of the cells and would therefore not impact on the outcome of the study.

2. The NIH 3T3 cells were refed on day 13 and day 20 and then subcultured on day 14 and day 21. The refeed is in deviation to the Protocol but was necessary has cells had overgrown and the media had changed colour. This is a minor deviation to the Protocol that would not affect the outcome of the study.

3. The fresh positive control for the MRC-5 cell line was set up on day 23 instead of day 14 as stated in the Protocol. This is a minor deviation to the Protocol that did not affect the outcome of the study as the cells were positive for haemadsorption on day 28.

4. Medium containing 10% FCS was used for the subculture of cells on day 21. This is a deviation to the Protocol that states 5% FCS should be used following inoculation. This is considered a minor deviation to the Protocol that did not affect the outcome of the study as the cells were at a suitable concentration of the haemadsorption on day 28.

ANNEX

The Annex consists of 13 pages, including this one, and includes:

- Client Protocol (12 pages)

CLIENT PROTOCOL

Procedure Number	49001
Version Number	00
Supersedes	N/A
Study Title	In Vitro Evaluation of Adventitious Viruses in Cell Cultures – 28 day assay
Test Facility	Covance Laboratories Ltd.,

Protocol Produced on: 29 May 2007

Prepared at Covance Laboratories by 29/May/07
Date

..

INTRODUCTION

Rationale

The cell lines utilised in this assay are sensitive to a wide variety of viruses. The end-points utilised, cytopathic effect (CPE) and haemadsorption with three types of erythrocytes, provide a series of methods for evaluating the viral status of the test article cells [1, 2 & 3].

Objective

The objective of this assay is to evaluate the adventitious viral status of the test article.

TEST ARTICLE

The test article will be supplied by the Sponsor and all relevant information completed on the Test Article Safety & Pre-Study Questionnaire.

This study for the presence of extraneous agents will be conducted to define the purity of the test substance; therefore, information regarding the purity of the test article is not available at study initiation. Stability of the test article is not considered relevant, as the objective of this study is to test for extraneous agents (adventitious viruses) that may be present in the test material.

TEST SYSTEM

Positive control virus:	*Parainfluenza type 3* (PI3) strain SF-4 used at approximately $1x10^4$ $TCID_{50}$/ml (control for Vero, HeLa and MRC-5 cells).
	MVM virus used at approximately $1x10^4$ $TCID_{50}$/ml (control for CPE on NIH 3T3).
Source:	Maintained as laboratory stocks, original stocks supplied by ATCC.
Negative control:	Minimal essential medium + 5% tryptose

(virus diluent)	phosphate broth.
Source:	Minimum essential medium. Tryptose phosphate broth. Gentamycin. Fungizone. Supplied by Invitrogen.
Indicator cell lines:	MRC-5. Vero. HeLa NIH 3T3.
Source:	Maintained as laboratory stocks, original MRC-5, Vero, HeLa and NIH 3T3 stocks supplied by ATCC or ECACC.
Growth medium:	Minimal essential medium containing Earles salts, non-essential amino acids plus 10% foetal calf serum for culture establishment. Minimum essential medium containing Earles Salts, non-essential amino acids plus 5% foetal calf serum will be used for growth of cultures post inoculation.
Source:	Minimum essential medium. Non-essential amino acids. Gentamycin. Fungizone. Supplied by Invitrogen. Foetal calf serum. Supplied by PAA.

EXPERIMENTAL PROCEDURES

Preparation of Test Article

The test article will be prepared according to standard methods. Where needed the inoculation volume and surface of indicator cells, as described below, may be adapted. Any alterations will be noted in study records

Adventitious Virus Assay

For each indicator cell line, the following will be carried out:

Indicator cells will be seeded into T 75 flasks (4 x T 75 flasks). A flask will be labelled as the test article, a flask as the positive control, a flask as the test article interference control and a flask as the negative control. All flasks will be labelled with the cell type, the study number, and any other relevant information. The flasks will be incubated at 37 ± 1°C in a humidified 5% CO_2 in air incubator until required for assay.

For each indicator cell type, the medium will be aspirated from sub-confluent cultures and the cell sheets will be washed with a balanced salt solution. The final wash will be aspirated from the wells and 5ml of test article will be inoculated into a flask of each indicator cell line. To determine if the test article interferes with the assays ability to detect the relevant viruses, 5ml of test article spiked with the relevant positive control at an appropriate concentration will be inoculated into a flask of each indicator cell line. Appropriate positive and negative controls will be similarly inoculated into a flask of each indicator cell line. Flasks will be incubated at 37 ± 1°C for 60-90 minutes. The inoculum will be aspirated from the flasks and cells washed in a balanced salt solution, then refed with the appropriate growth medium. Flasks will be incubated and observed for cytopathic effect over 14 days. Each culture will be refed or subcultured on approximately day 7 with the appropriate growth medium.

If the test article inoculated indicator cells give a negative result on day 14, the test article and any other cultures negative for CPE will be split and seeded appropriately into fresh T75 flask. If positive controls turn positive they will be discarded. A fresh positive control will also be set up for the MRC-5 cell line and inoculated with the appropriate positive control virus. These flasks will then be incubated and observed for cytopathic effect over a further 14 days. Each culture will be refed or subcultured on approximately day 21 with the appropriate growth medium If the positive control shows CPE too early a fresh positive control may be set up for the MRC-5 cell line inoculated with the appropriate positive control virus. On day 28, a haemadsorption assay will be

carried out on the flasks inoculated with test article, the flasks inoculated with negative control and the MRC-5 flask inoculated with positive control.

For each indicator cell type, the haemadsorption assay will be carried out as follows:

The cell sheets will be washed with Dulbecco's Phosphate Buffered Saline and after aspirating the final wash, a mix of adult chicken, guinea pig and human type O blood will be added to each flask. Firstly, the flasks will be placed in the refrigerator for 30 ± 5 minutes and then incubated at room temperature for 30 ± 5 minutes. At the end of each incubation period the cell sheets will be observed for haemadsorption and the results recorded.

All culture vessels and plates will be discarded by the end of the experiment.

DATA ANALYSIS

Assay Acceptance Criteria

The assay will be considered acceptable if:

1. The positive control and test article spiked with positive control viruses cause CPE in all the indicator cells used.

2. The positive control inoculated cells cause haemadsorption in the indicator cells used.

3. The negative control inoculated indicator cells show normal morphology.

4. The negative inoculated indicator cells show no evidence of haemadsorption.

Evaluation Criteria

1. The assay will be considered as positive if any test article inoculated indicator cells or test article cells show a cytopathic effect or haemadsorption with any of the blood types assayed.

2. The test article will be considered to have interfered with the assay for the detection of adventitious viruses if CPE is not detected in all of the indicator cell lines inoculated with test article spiked with the appropriate positive control.

GLP COMPLIANCE

Following completion of the study a draft report will be issued. Client comments should be supplied for inclusion into a final document within six weeks of receipt of the draft document. If no client comments are received within six weeks of issue, the report will be finalised. Any further changes after this time will be addressed as an amended final report which may result in additional costs.

The study will be performed in compliance with:

United Kingdom Statutory Instrument 1999 No. 3106, The Good Laboratory Practice Regulations 1999, as amended by the Good Laboratory Practice (Codification Amendments Etc.) Regulations 2004.

OECD Principles on Good Laboratory Practice (revised 1997, Issued Jan 1998) ENV/MC/CHEM(98)17

All procedures will be performed in accordance with Covance Laboratories standard operating procedures (SOPs). The study will be subject to Quality Assurance evaluation by the Covance Laboratories Quality Assurance Department (QAD) in accordance with SOPs.

REFERENCES

[1] Jacobs J P, Magrath D I, Garrett A J and Schild G C (1981) Guidelines for the acceptability, management, and testing of serially propagated human diploid cells for the production of live virus vaccines for use in man. J Biol Standard *9*, 331-342.

[2] Schmidt N J (1979) Cell culture techniques for diagnostic virology. In: Diagnostic procedures for viral, rickettsial and chlamydial infections 5th edition. E H Lennette and N J Schmidt, editors. American Public Health Association, Washington, DC.

[3] Poiley JA (1990) Methods for the detection of adventitious viruses in cell culture used in the production of biotechnology products. In: Large-scale mammalian cell culture technology. (AS Lubiniecki, ed) Marcel Dekker Inc.

ARCHIVE STATEMENT

All primary data, or authenticated copies thereof, specimens and the Final Report will be retained in the Covance Laboratories Limited archives for one year after issue of the Final Report. At this time, the Sponsor will be contacted to determine whether the data should be returned, retained or destroyed on their behalf. Sponsors will be notified of the financial implications of each of these options at that time.

Specimens or samples requiring frozen storage at Covance are specifically excluded from the above. These will be retained for as long as the material permits further evaluation, up to three months after issue of the Draft Report. At this time, the Sponsor will be contacted to determine whether samples should be returned, retained or destroyed on their behalf. Any financial implications of these options will also be notified at this time. Samples will not be destroyed without prior approval of the Study Director.

APPENDIX

Study Records

The study records will be prepared to contain the following information:

Client Protocol, file note(s)*, study related correspondence*, Test Article Safety and Pre-Study Questionnaire, Test article receipt and utilisation, metrology#, records for reagents and stock solutions#, Test article cell culture records#, work sheets, indicator cell culture records#, positive control culture records#.

* Where appropriate.
# Some records held centrally.

Draft Report

The Draft Report will be prepared to contain but not be limited to the following information:

- The identity of the test substance as specified by the Sponsor.
- Any unforeseen circumstances which may have affected the quality or integrity of the study.

The following items will be presented in the Draft Report:

- Summary.
- Results.
- Conclusion.
- The name and address of the testing facility and the dates, on which the study was initiated, assay initiation and assay completion.

The Protocol will be attached as an Annex to the Report detailing procedures, assay acceptance criteria, evaluation criteria and references. Minor deviation(s) from the Protocol (if applicable) will be presented in the Report.

Final Report

The Final Report will be issued following Quality Assurance Department (QAD) evaluation of the Draft Report. The report will include all details described above and will include but not be limited to the following additions:

- Signed Quality Assurance statement.

- The signature of the Study Director for date of study completion and authentication of the Report.

PROTOCOL REVISION SUMMARY

Protocol Number: 49001

Protocol Title: In Vitro Evaluation of Adventitious Viruses in Cell Cultures – 28 day assay

Version Number	Revision Description	Authorisation Date
00	First issue	29 May 2007

TEST ARTICLE DETAILS AND STUDY AUTHORISATION

Test Article(s):
(As it should appear on all documentation) HI-MCB.1

Experimental Phase

Start Date: 6th June 2007

End Date: 10th August 2007

6th June 2007
Date

Study Director

6th Jun 2007
Date

Covance Biotechnology Management

SPONSOR ACCEPTANCE SHEET

Sponsor Name

Title

Sponsors Company

Sponsor Address

Sponsor Contact Details

Telephone

e-mail

5/29/07
Date

Sponsor Approval

6/4/07
Date

Sponsor QA

This form must be completed, signed and returned to Biotechnology Services Administration, Covance Laboratories by post.

A faxed copy sent to can be used for assay initiation.

TEST FOR THE PRESENCE OF INAPPARENT VIRUSES

Study No.: AC01UC.005002.BSV

Test Article: H1-MCB.1

Final Report
For

WiCell Research Institute

:s

By

BioReliance®

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Page

SUMMARY

The purpose of this assay is to detect viruses which do not cause a discernable effect in cell culture systems. The test article or the negative control article was injected into adult mice, guinea pigs and suckling mice. The suckling mouse portion of the assay included a subpassage of homogenized tissue after 14 days into a new group of suckling mice followed by an additional 14 day observation period. All animals were observed for signs of illness and any that became sick or showed any abnormalities were examined in an attempt to establish the cause of illness or death. Embryonated hens' eggs were injected with the test or the negative control article by the allantoic route followed by a subpassage of allantoic fluid via the same route. Allantoic fluid from the original and subpassage eggs was tested for hemagglutination at 4°C and room temperature using guinea pig, human O, and chick erythrocytes. A second group of embryonated hens' eggs was injected with the test article or the negative control article into the yolk sac, followed by a subpassage of the yolk sac material into a new set of eggs, via the yolk sac route. All embryos were examined for viability.

No evidence of contamination with adventitious viral agents was observed due to the test article, H1-MCB.1.

INTRODUCTION

The presence of latent or inapparent viruses in a cell line may not always be detected by injecting a battery of indicator cells and observing for cytopathic effect or other indications of viral infection. It is the purpose of this study to detect the presence of viruses that might be present in a cell line which do not cause cytopathogenic or other discernable effects in cell culture systems. The experimental design utilizes injections of adult and suckling mice, guinea pigs and embryonated hens' eggs as recommended by The Center for Biologics Evaluation and Research (CBER), United States Food and Drug Administration, in the 1993 "Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals".

Adult mice are included in the assay to detect possible contamination of the test article with neurotropic or other viruses such as lymphocytic choriomeningitis virus. Suckling mice are utilized to detect Coxsackie or other viruses which would cause the mice to become sick and die. Guinea pigs are sensitive to a variety of viral infections. The hens' eggs are used for detection of myxoviruses and other viruses.

All animals are observed for signs of illness and any that become sick or show any abnormalities are examined in an attempt to establish the cause of illness or death.

STUDY INFORMATION

Title:	Test for the Presence of Inapparent Viruses
Study Number:	AC01UC.005002.BSV
Test Article:	H1-MCB.1 was received by BioReliance on 04/05/2007. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor. Retention of samples of the test article is the responsibility of the sponsor.
Medium Test Article:	None
Positive Control:	None
Negative Control:	Hank's Balanced Salt Solution Lot No.: 16K2439 Source: Sigma St. Louis, Missouri

Vehicle Control:	None
Test System:	
Mice	Suckling litters (Primary Injection): HSD:ICR, four adult females, each with ten 1 day old suckling pups Source: Harlan Sprague Dawley Frederick, Maryland
	Suckling litters (Blind Passage): HSD:ICR, four adult females, each with ten 2 day old suckling pups Source: Harlan Sprague Dawley Frederick, Maryland
	Adult – HSD:ICR, ten males and ten females, 5 weeks old Source: Harlan Sprague Dawley Frederick, Maryland
Guinea Pigs	Hartley albino, five adult males and five adult females, 3 weeks old Source: Elm Hill Breeding Laboratories Chelmsford, Massachusetts
Hens' Eggs	Embryonated Hens' Eggs (allantoic route): forty, nine days old Source: Hy-Vac (BE Eggs) York Springs, Pennsylvania
	Embryonated Hens' Eggs (yolk sac route): forty, seven days old Source: Hy-Vac (BE Eggs) York Springs, Pennsylvania
Sponsor:	WiCell Research Institute

Authorized Representative:	
Testing Facility:	BioReliance
Animal Facility:	BioReliance
Study Director:	
Schedule:	
Study Initiation Date:	04/10/2007
Lab Initiation Date:	04/13/2007
Lab Completion Date:	05/18/2007
Study Completion Date:	See Study Director's signature date in the "Approval" section.
Archives:	All raw data, records, any specimens, the protocol and a copy of the final report will be maintained according to Standard Operating Procedure OPQP3040 by the BioReliance Regulatory Affairs/Quality Assurance Unit headquartered at: BioReliance

PROCEDURES

Objective

The study objective is to detect virus(es) that might be present in a cell line which do not cause any cytopathogenic or other discernable effects in cell culture systems.

Methods

Test System Identification and Randomization

Each animal cage was assigned a number and labelled as "test article" or "negative control". Guinea pigs were housed separately and were identified by ear tags. Adult mice were ear-tagged but housed in groups according to test material and sex. Suckling mice were not individually identified. Embryonated eggs were labelled individually in pencil.

Guinea pigs and adult mice were randomized according to SOP OPBT0213. Suckling litters were not individually randomized in order to decrease the likelihood of cannibalization.

Animal Injection with Test or Negative Control Articles

Adult mice and guinea pigs were injected according to Table 1. All adult mice and guinea pigs were then observed every working day, for 28 days, for clinical signs. The injection sites of the guinea pigs were observed for the development of lesions once each week of the testing period.

In the suckling mouse portion of the study, the animals were injected according to Table 1 and then observed every working day for 14 days for clinical signs. Fourteen days post-injection, all surviving suckling mice from each group were euthanized using cervical dislocation. Following euthanasia their skin and gastrointestine were removed, the carcasses cut into pieces and placed in a sterile pre-weighed bowl. After determining the weight of the entire group of mice from a cage, enough Hank's Balanced Salt Solution (HBSS) was added to make a 20% w/v suspension. The entire content of the bowl was then homogenized in a sterile blender, clarified by centrifugation, diluted 1:2 in HBSS, and subsequently injected into a new group of suckling mice by the same routes and in the same volumes as the original group. These newly injected mice were observed for a period of fourteen days.

Embryonated Eggs

Each of ten nine day old embryonated eggs was injected by the allantoic route with approximately 0.1 ml of each of the test or the negative control articles. Each egg was candled for viability at 24 hours post-injection. After three days incubation, eggs were examined for viability. Fluids were then collected and tested for hemagglutination at $4^{\circ}C \pm 2^{\circ}C$ and $25^{\circ}C \pm 2^{\circ}C$ using chicken, guinea pig, and human O erythrocytes.

The fluids from each group were pooled and these pooled allantoic fluids were then passaged to a new group of nine day old embryonated eggs. Each egg was candled for viability at 24 hours post-injection. After three days incubation eggs were examined for viability. Allantoic fluids were

harvested and tested for hemagglutination using chicken, guinea pig, and human O erythrocytes at 4°C ± 2°C and 25°C ± 2°C.

Each of ten seven day old embryonated eggs was injected by the yolk sac route with approximately 0.1 ml of the test or negative control articles. Each egg was candled for viability at 24 and 48 hours post-injection. After 10 days incubation, embryos were examined for viability. The yolk sacs were then harvested, pooled for each group and a 10% suspension (v/v) subpassaged into ten additional seven day old embryonated eggs per group. Each egg was candled for viability at 24 and 48 hours post-injection. After 9 days, the embryos were examined for viability.

In either the yolk sac or the allantoic assays, fluid from each embryonated egg which contained a non-viable embryo was plated onto two blood agar plates. One plate was incubated aerobically at 36°C ± 1°C. The second plate was incubated anaerobically at 36°C ± 1°C. The plates were examined for growth after a minimum of 24 hours.

Animal Husbandry

All animals were fed the following diet ad libitum:

Guinea pigs - Teklad Certified Guinea Pig Chow.

Mice – 2018S 18% Protein Rodent diet (sterilizable) – Harlan Teklad

Water was supplied ad libitum via water bottles. Water for guinea pigs was disinfected with 7 ppm chlorine. Water for mice was autoclaved.

Bedding - Corncob, Harlan Tekald. Cages were changed as necessary, usually twice per week.

Animal facilities are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International.

RESULTS

All adult mice injected with the test article or the negative control article appeared normal and healthy for the twenty-eight day observation period.

All suckling mice injected with the test article or the negative control article appeared normal and healthy after 14 days. The surviving mice of each group were homogenized and the homogenate of each group was passaged into a new group of suckling mice. The remainder of the homogenates was frozen at -70°C ± 10°C.

In the blind passage, all suckling mice injected with the test article homogenate or the negative control article homogenate appeared normal and healthy after 14 days.

All of the test article and all of the negative control article injected guinea pigs remained normal and healthy during the twenty-eight day test period.

See Tables 2 and 3 for a summary of the data discussed above.

Ten nine day old embryonated hens' eggs were injected by the allantoic route with the test article or the negative control article. These eggs were examined for viability at 24 hours and three days post-injection and allantoic fluids from day 3 were tested for hemagglutination using chicken, human O, and guinea pig erythrocytes. All eggs were viable and fluids were negative for hemagglutination.

The day 3 fluids from each group were pooled and injected into ten new nine day old eggs using the same route of injection. These eggs were examined for viability at 24 hours and three days post-injection. All eggs were viable. Allantoic fluids from all subpassage eggs were tested for hemagglutination using chicken, guinea pig, and human O erythrocytes. All fluids were negative for hemagglutination. See Tables 4 and 5 for a summary of the data.

Ten seven day old embryonated hens' eggs were injected by the yolk sac route with the test article or the negative control article. These eggs were examined for viability at 24 and 48 hours and 10 days post-injection. Nine of the test article injected eggs and all of the negative control article injected eggs appeared viable at 24 hours post injection. At 48 hours post injection, eight of the test article injected eggs and all of the negative control article injected eggs appeared viable. At examination on day 10 post injection, eight of the ten test article injected eggs and all of the negative control article injected eggs contained viable embryos. Two of the test article injected eggs contained non-viable embryos. No growth was observed on blood agar plates streaked with fluid from the non-viable eggs. The cause of death of these embryos most likely resulted from injection related trauma as the eggs appeared non-viable at 24 and/or 48 hours post injection. (Evaluation of Test Results.)

The yolk sac material from all viable eggs in each group was pooled. A 10% suspension of pooled yolk sac material was injected into ten new seven day old embryonated eggs using the same route of injection. These eggs were examined for viability at 24 and 48 hours and 9 days post-injection. All eggs appeared viable at 24 and 48 hours post injection. At examination on day 9 post injection, all of the test article subpassage eggs and nine of the ten negative control article subpassage eggs contained viable embryos. One of the negative control article subpassage eggs contained a non-viable embryo. No growth was observed on blood agar plates streaked with fluid from the

non-viable egg. The cause of death of this embryo could not be determined. See Table 4 for a summary of the data.

CONCLUSION

No evidence of viral contamination was observed due to the test article, H1-MCB.1.

CRITERIA FOR A VALID TEST

The test will be considered valid if ninety percent of the control adult mice, eighty percent of the control suckling mice, eighty percent of the control embryonated hen's eggs, and seventy-five percent of the control guinea pigs survive the observation period, show no lesions at the site of injection or signs of viral infection.

There may be instances when the test article animals meet the evaluation criteria, but the negative control animals do not meet the criteria detailed above, yet the assay will be considered valid. This determination will be made by the study director and based on the evaluation of the assay data.

EVALUATION OF TEST RESULTS

The test cells, or other test material, will be considered not contaminated if 80% of the animals remain healthy and survive the entire observation period, and if all the animals used in the test fail to show lesions of any kind at the site of injection and fail to show evidence of any viral infection. Statistical evaluation is not required.

REFERENCE

Jacobs, J.P., D.I. Magrath, A.J. Garrett, and G.C. Schild. Guidelines for the acceptability, management and testing of serially propagated human diploid cells for the production of live virus vaccines for use in man. J. Biol. Stand. 9:331-342, 1981.

APPROVAL

I accept responsibility for the conduct of this study which was performed in compliance with the U.S. Food and Drug Administration Good Laboratory Practice Regulations (21 CFR 58), the UK GLP Compliance Programme, the Japanese GLP Standard and the OECD Principles of Good Laboratory Practice.

9 Jun 07
Date

Study Director

TABLE 1

Summary of Experimental Procedures for H1-MCB.1

Adult Mice						
Cage No.	**Number of Animals**	**Sex**	**Test Material**	**Volume(s) of Test Material**	**Route(s) of Injection**	**Treatments Post-Injection**
AM 1	5	Male	Test Article	0.5 ml	i.p	Animals were observed for illness every working day for 28 days.
AM 2	5	Female		0.05 ml	p.o	
AM 3	5	Male	Negative Control	0.05 ml	i.n	
AM 4	5	Female		0.03 ml	i.c	
Guinea Pigs						
Cage No.	**Number of Animals**	**Sex**	**Test Material**	**Volume(s) of Test Material**	**Route(s) of Injection**	**Treatments Post-Injection**
GP 1	1	Male	Test Article	5.0 ml 0.1 ml	i.p. i.c.	Animals were observed for illness every working day for 28 days.
GP 2	1					
GP 3	1					
GP 4	1	Female				
GP 5	1					
GP 6	1					
GP 7	1	Male	Negative Control			
GP 8	1					
GP 9	1	Female				
GP 10	1					

i.p. = Intraperitoneal injection
p.o. = Per os injection (by mouth)
i.n. = Intranasal injection
i.c. = Intracranial injection

TABLE 1 (Continued)

Summary of Experimental Procedures for H1-MCB.1

Suckling Mice						
Cage No.	**Number of Animals per cage**	**Sex**	**Test Material**	**Volume(s) of Test Material**	**Route(s) of Injection**	**Treatments Post-Injection**
SM1	1	Female (adult lactating)	None	None	None	None
SM2	+10	Various (suckling)	Test Article	0.1 ml 0.01 ml 0.01 ml	i.p. p.o. i.c.	Suckling mice were observed for illness. After 14 days, a single pool of emulsified tissue (minus skin and gastrointestine) of all surviving suckling mice was passaged into ten additional suckling mice. Same routes and volumes as in the original procedure were used.
SM3	1	Female (adult lactating)	None	None	None	None
SM4	+10	Various (suckling)	Negative Control	0.1 ml 0.01 ml 0.01 ml	i.p. p.o. i.c.	Suckling mice were observed for illness. After 14 days, a single pool of emulsified tissue (minus skin and gastrointestine) of all surviving suckling mice was passaged into ten additional suckling mice. Same routes and volumes as in the original procedure were used.

i.p. = Intraperitoneal injection
p.o. = Per os injection (by mouth)
i.c. = Intracranial injection

TABLE 2

Survival Summary for H1-MCB.1

	ANIMAL SPECIES			
	Guinea Pigs[a]	Adult Mice[a]	Suckling Mice[b]	
			Primary Injection	Blind Passage
Test Article	6/6	10/10	20/20	20/20
Negative Control Article	4/4	10/10	20/20	20/20

[a] Number of surviving animals after 28 days/Number of animals injected.

[b] In the suckling mice portion of the assay, animals are injected and observed for 14 days. On day 14 post-injection a homogenate was prepared from the surviving sucklings from each group. This homogenate was used to inject another group of suckling mice which was observed for an additional 14 days.

TABLE 3

Summary of Daily Observations for H1-MCB.1

Guinea Pigs

Test Material	Animal Number	Clinical Signs	Day of Onset (Post-Injection)	Day of Death/Sacrifice (Post-Injection)
Test Article	7651	Normal		
	7652	Normal		
	7653	Normal		
	7654	Normal		
	7655	Normal		
	7656	Normal		
Negative Control	7657	Normal		
	7658	Normal		
	7659	Normal		
	7660	Normal		

TABLE 3 (Continued)

Summary of Daily Observations for H1-MCB.1

Adult Mice

Test Material	Animal Number	Clinical Signs	Day of Onset (Post-Injection)	Day of Death/Sacrifice (Post-Injection)
Test Article	7601	Normal		
	7602	Normal		
	7603	Normal		
	7604	Normal		
	7605	Normal		
	7606	Normal		
	7607	Normal		
	7608	Normal		
	7609	Normal		
	7610	Normal		
Negative Control	7611	Normal		
	7612	Normal		
	7613	Normal		
	7614	Normal		
	7615	Normal		
	7616	Normal		
	7617	Normal		
	7618	Normal		
	7619	Normal		
	7620	Normal		

TABLE 3 (Continued)

Summary of Daily Observations for H1-MCB.1

Suckling Mice

	Test Material	Cage No. (No. suckling mice/group)[a]	Clinical Signs	Day of Onset (Post-injection)	Day of Death/Sacrifice (Post-injection)
Primary Injection	Test Article	SM1 (10)	Normal		
		SM2 (10)	Normal		
	Negative Control	SM3 (10)	Normal		
		SM4 (10)	Normal		
Blind Passage[b]	Test Article	SM1 (10)	Normal		
		SM2 (10)	Normal		
	Negative Control	SM3 (10)	Normal		
		SM4 (10)	Normal		

[a] Ten suckling mice injected per cage.

[b] Surviving suckling mice from the primary injection were sacrificed on day 14 for preparation of blind passage tissue homogenate.

TABLE 4

Survival Summary for H1-MCB.1

Embryonated Hens' Eggs

	Allantoic Route		Yolk Sac Route	
	Primary Injection	Blind Passage	Primary Injection	Blind Passage
Test Article	10/10[a]	10/10	8/10	10/10
Negative Control	10/10	10/10	10/10	9/10

[a] Number of viable embryos/number of eggs injected.

TABLE 5

Hemagglutination Observed by the Allantoic Route of Injection-Day 3 for H1-MCB.1

	PRIMARY INJECTION						BLIND PASSAGE					
	4°C			25°C			4°C			25°C		
	C[a]	G[b]	H[c]	C	G	H	C	G	H	C	G	H
Test Article	0/10[d]	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Negative Control Article	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10

[a] Chick erythrocytes
[b] Guinea pig erythrocytes
[c] Human type O erythrocytes
[d] No. positive/number tested

Quality Assurance Statement

Study Title: TEST FOR THE PRESENCE OF INAPPARENT VIRUSES

Study Number: AC01UH.005002.BSV

Study Director:

Quality Assurance performed the inspections listed below for this study. Verification of the study protocol was also performed and documented by QA. Procedures, documentation, equipment records, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the UK GLP Regulations, the Japanese GLP Standard, and the OECD Principles of Good Laboratory Practice and to assure that the study is conducted according to the protocol and relevant Standard Operating Procedures.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

** Inspect On	04-Jun-07 - 05-Jun-07 To Study Dir 05-Jun-07 To Mgmt 08-Jun-07
Phase	Final Report and data audit
* Inspect On	29-Mar-07 - 29-Mar-07 To Study Dir 29-Mar-07 To Mgmt 29-Mar-07
Phase	Systems Inspection - Administration of Test Substance to Test System
* Inspect On	01-May-07 - 01-May-07 To Study Dir 01-May-07 To Mgmt 01-May-07
Phase	Systems Inspection - Observation of Test System/Data Collection and/or Analysis
* Inspect On	22-May-07 - 22-May-07 To Study Dir 22-May-07 To Mgmt 22-May-07
Phase	Systems Inspection - Manipulation of Test System
* Inspect On	06-Jun-07 - 06-Jun-07 To Study Dir 06-Jun-07 To Mgmt 06-Jun-07
Phase	Systems Inspection - Test System Preparation

** Inspection specific for this study
* Systems Inspection

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

8 June 07
DATE

QUALITY ASSURANCE

E-FR-1-07-002040.30610

FINAL STUDY REPORT

STUDY TITLE: **Ultrastructural Evaluation of Cell Culture Morphology, with Characterization and Tabulation of Retrovirus-like Particles**

TEST PROTOCOL NUMBER: 30610.05

TEST ARTICLE IDENTIFICATION	APPTEC ACCESSION NUMBER
H1 MCB.1 1 passage in TeSR1	07-002040

SPONSOR:

WiCell Research Institute

PERFORMING LABORATORY: AppTec, Inc.

APPTEC ACCESSION NUMBER	RESULTS
07-002040	Transmission electron microscopic examination revealed no identifiable virus-like particles, nor did it reveal any other identifiable microbial agents.

QUALITY ASSURANCE UNIT SUMMARY

STUDY: Ultrastructural Evaluation of Cell Culture Morphology, with Characterization and Tabulation of Retrovirus-like Particles

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. This study has been performed under Good Laboratory Practice regulations (21 CFR Part 58) and in accordance with standard operating procedures and a standard test protocol. The Quality Assurance Unit maintains a copy of the test protocol and standard operating procedures. The Quality Assurance Unit for the subcontractor used in this study was responsible for a study inspection performed on the dates listed below. Each inspection was performed to assure the quality and integrity of the study.

Phase Inspected	Date	Study Director	Management
Processing and embedding Test Article 07-002040	October 10-11, 2007	November 29, 2007	November 29, 2007

The findings of these inspections have been reported to Management and the Study Director.

Quality Assurance

30NOV07
Date

GOOD LABORATORY PRACTICES STATEMENT

This study referenced in this report was conducted in accordance with Good Laboratory Practices for Nonclinical Laboratory Studies as found in Title 21 Code of Federal Regulations Part 58. The study was inspected during at least one phase and AppTec Quality Assurance will audit the final report.

Study Director

30NOV07
Date

Professional Personnel involved in study:

1.0 PURPOSE

The purpose of this study was to use thin-section electron microscopy to describe the ultrastructural morphological characteristics of the Sponsor's test article and to determine if viral or viral-like particles or other contaminants are present in the Sponsor's test article.

2.0 SPONSOR: WiCell Research Institute

3.0 TEST FACILITY: **AppTec, Inc.**

4.0 SCHEDULING

DATE SAMPLES RECEIVED: September 25, 2007
STUDY INITIATION DATE: September 26, 2007
STUDY COMPLETION DATE: See page 2 for Study Director's signature and date.

5.0 TEST ARTICLE CHARACTERIZATION

Determinations of strength, homogeneity, purity and stability of the test article are solely the responsibility of the Sponsor. The Sponsor is responsible for supplying to the testing laboratory results of these determinations and any others that may directly impact the testing performed by the testing laboratory, prior to initiation of testing.

6.0 TEST ARTICLE IDENTIFICATION: H1 MCB.1 1 passage in TeSR1

7.0 TEST SYSTEM DESCRIPTION

As described in the Points to Consider (May 1993), the morphological and growth characteristics of cell lines used for the production of biologics need to be monitored. Cells in culture possess inherent qualities, some of which are amenable to study by transmission electron microscopy. The use of electron microscopy allows for the visualization of cellular components, which help in the identification of cell type and may aid in describing any cellular changes that could occur during biopharmaceutical production.

Preparation of thin sections of virus-infected cells and tissues is an indispensable technique for the study of those aspects of virus-cell interaction that are accessible to direct examination by electron microscopy.[1,2,3] Thin sectioning is also of value in elucidating the structure of viruses; the information obtained often complements that provided by a negative staining procedure. This protocol can be utilized to visualize a variety of viral types including retroviruses, herpesviruses, adenoviruses, picornaviruses, parvoviruses, orthomyxo- and paramyxoviruses, reoviruses, and many other common viral agents. Contamination by other microbial agents such as yeast, fungi and bacteria may also be detected.

If retroviruses are detected they will be evaluated on the basis of A-, B-, C-, D-, and R-type retrovirus-like morphologies. *A-type* viral particles are characterized as either (1) intracytoplasmic particles, 60-90 nanometers (nm) in diameter, with an electron-dense core; (2) intracisternal particles, 60-90 nm in diameter, found within the endoplasmic reticulum, with 2 dense concentric shells surrounding an electron-lucent core. *B-type* particles are spherical, enveloped particles that arise by budding at the plasma membrane. They display an eccentric, electron-dense core surrounded by an intermediate layer, and an envelope with prominent projections. *C-type* viral particles are 90-130 nm in diameter, enveloped, and contain an internal nucleoid of variable electron density and shape. They occur either within cytoplasmic vacuoles, on the cell surface, or extracellularly. *D-type* particles are spherical, enveloped particles that bud from the plasma membrane and frequently exhibit an electron-dense bar- or tube-shaped core. *R-type* particles are enveloped, spherical particles, 70-100 nm in diameter, with a central core of variable density from which characteristic spokes extend into the envelope, and are found in the cisternae of the endoplasmic reticulum.

8.0 EXPERIMENTAL DESIGN

For most purposes, optimum preservation of fine structure in animal cells, viruses, and other microbial agents is the prime consideration, and procedures for ensuring this are now fairly well standardized. The cells were thawed and grown at AppTec Laboratories. When an optimal level of $1 - 2 \times 10^7$ cells was available, the cells were harvested and a cell count determined.

8.1 The cells were pelleted, and McDowell-Trump's fixative was added to the pellet.

8.2 The pellet was shipped to the subcontractor Charles River Laboratories Pathology Associates (CRLPA), where typically (if enough cells are available) one-half of the cell pellet(s) were processed and embedded for transmission electron microscopy (TEM).

8.3 Thin sections were cut and mounted on 200-mesh copper grids.

8.4 The samples were stained with 5% methanolic uranyl acetate and Reynold's lead citrate.

8.5 The cells were examined by TEM to characterize morphologically the cell type comprising the culture. Cell characteristics were documented by labeled electron micrographs.

8.6 200 cells were evaluated for the presence of any type of particle with virus-like morphology, and appropriate documentation was provided for any particles found using labeled electron micrographs.

8.7 Retrovirus-like particles for each of the 200 cells were tabulated as follows: (1) no particles, (2) 1 to 5 particles, (3) 6 to 20 particles, (4) more than 20 particles.

8.8 200 cells were evaluated for particles with A-, B-, C-, D-, and R-type retrovirus-like morphology as described in Section 3. Electron micrographs were made to document representative examples of any virus-like particles observed. Except where noted otherwise, a bar denoting 100 nanometers was placed on each micrograph for size reference.

9.0 TEST ARTICLE PREPARATION

On September 25, 2007, AppTec, Inc. received 1 vial containing 1 mL of "Fixed human embryonic stem cells grown in feeder free conditions," cold on cold packs and designated for use in this assay. The test article was stored at 2-8°C until shipment to the subcontractor.

On September 26, 2007, 1 vial containing a fixed and pelleted cell culture was shipped, on ice packs, in storage conditions of 2-8°C, via overnight courier to the subcontractor.

10.0 NEGATIVE CONTROLS

A blank water sample was run in parallel with the test article.

11.0 ASSAY VALIDITY

The following validity criteria are evaluated:

12.1 The test is valid if the test article cells are well preserved and at least 200 cells are examined.

12.0 TEST EVALUATION

Detailed description of unique or distinguishing characteristics of cell ultrastructure will be included and documented by labeled electron micrographs. The general appearance or preservation of the cells will be noted.

Analysis of the photomicrograph from the thin sections will provide the opportunity to observe contaminating viruses or other microbial agents and the morphological responses of the host cell. 200 cells will be examined. The type of viral particles and percentage of cells containing the particles will be enumerated.

13.0 RESULTS

The test was valid. The test article cells were well preserved, and at least 200 cells were examined.

13.1 Cellular Ultrastructure

Cells in the section were of moderate size and polygonal to irregular in shape (L22944). Cells had microvilli (MV: L22944)[1] unevenly distributed on the surface. Nuclei (N: L22944) tended to be polygonal to irregular, with chromatin relatively evenly dispersed or clumped along the periphery. Nuclei often had one or more nucleoli (NS: L22944) that were variably located. Some cells were observed to be under mitosis with chromosomes (CH: L22948) visible.

The cytoplasm of most cells contained varying numbers of dense mitochondria (MI: L22950). Profiles of rough endoplasmic reticulum (RER: L22946), sometimes distended with fine granular material, were seen among the mitochondria. Ribosomes (RB: L22946) were abundant in the cytoplasm of most cells. Cells were observed to contain filaments (F: L22943), desmosomes (D: L22950) and glycogen (G: L22947). Centrioles (CN: L22945), microtubles (T: L22940) and autophagic vacuoles (AV: L22949) were also seen.

13.2 General Viral Particle Evaluation

Transmission electron microscopic examination revealed no identifiable virus-like particles, nor did it reveal any other identifiable microbial agents.

13.3 Retrovirus-like Particle Evaluation and Tabulation

Results of retrovirus-like particle counts in the specimen were as follows:

Test Article: 07-002040
PAI EM Number: 07.501-1

Number of cells with:

Particle Type	No Particles	1-5 Particles	6-20 Particles	20 or more Particles
A-Type	200	0	0	0
B-Type	200	0	0	0
C-Type	200	0	0	0
Extracellular C-type	200	0	0	0
D-Type	200	0	0	0
R-Type	200	0	0	0

Percentage of cells with each type of retrovirus-like particle:

A-Type	0%
B-Type	0%
C-Type	0%
D-Type	0%
R-Type	0%

Four percent of the cells were necrotic.

[1] Numbers in parentheses are specific micrographs in which the designated structures were found.

14.0 CONCLUSION

Evidence of viral or viral-like particles or other contaminants was not detected in the test article.

15.0 STATISTICAL DATA ANALYSIS

Statistical analysis of the data is not required.

16.0 DEVIATIONS / AMENDMENTS

No deviations from the protocol were encountered during the conduct of this study.

No amendments to the protocol were generated.

17.0 RECORD RETENTION

The testing facility will retain all records involving the study for seven (7) years including, but not limited to: the signed test protocol with all amendments, any written communication concerning the conduct of the study, test article accountability record, raw data, worksheets, and an official copy of the final study report.

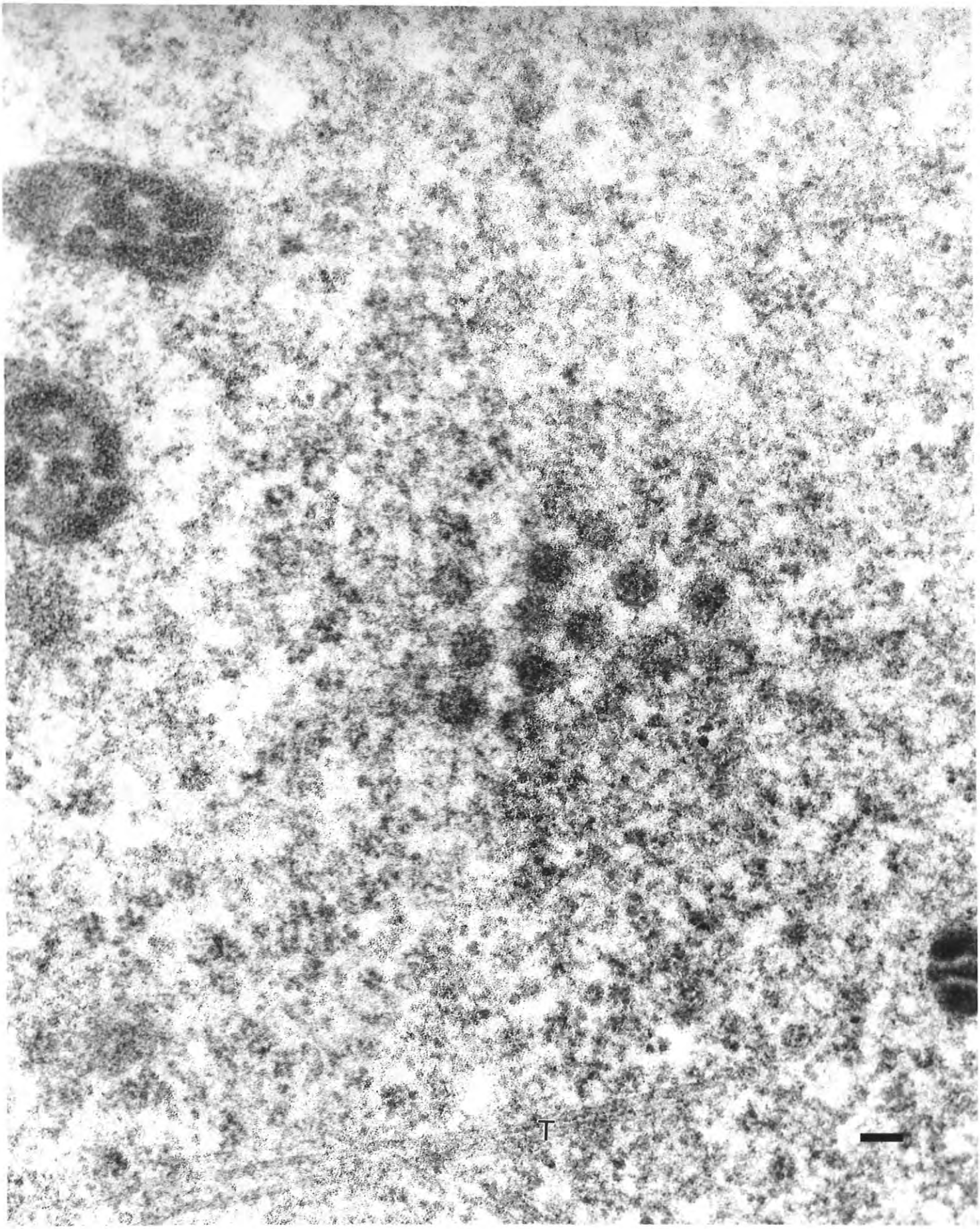
18.0 REFERENCES

1. Morgan C and Rose HM (1967). "The Application of Thin Sectioning," *Methods in Virology* Vol. **3** (Maramorosch K and Koprowski H, eds.), Academic Press, New York, NY, pp. 576-616.

2. Palmer E and Martin M (1988). Retroviridae in "Electron Microscopy in Viral Diagnosis", CRC Press, Boca Raton, FL, pp. 91-103.

3. Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals (1993). Office of Biologics Research and Review, Food and Drug Administration.

4. Jawetz E, Melnick JL, and Adelberg, EA, eds. (1984). Tumor Viruses in: "Review of Medical Microbiology," 16th Edition, Lange Medical Publications, Drawer L, Los Altos, CA, pp. 495-498.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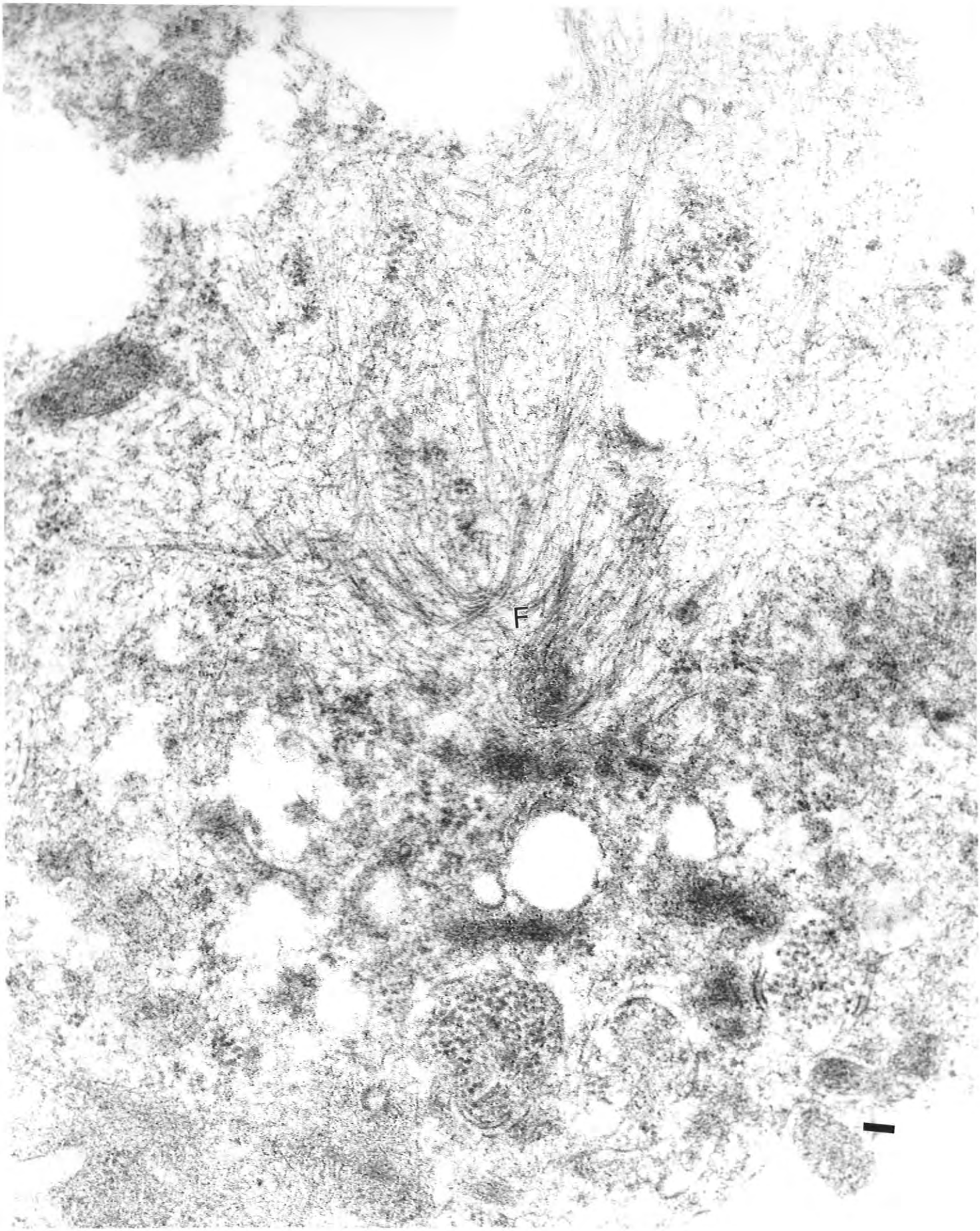
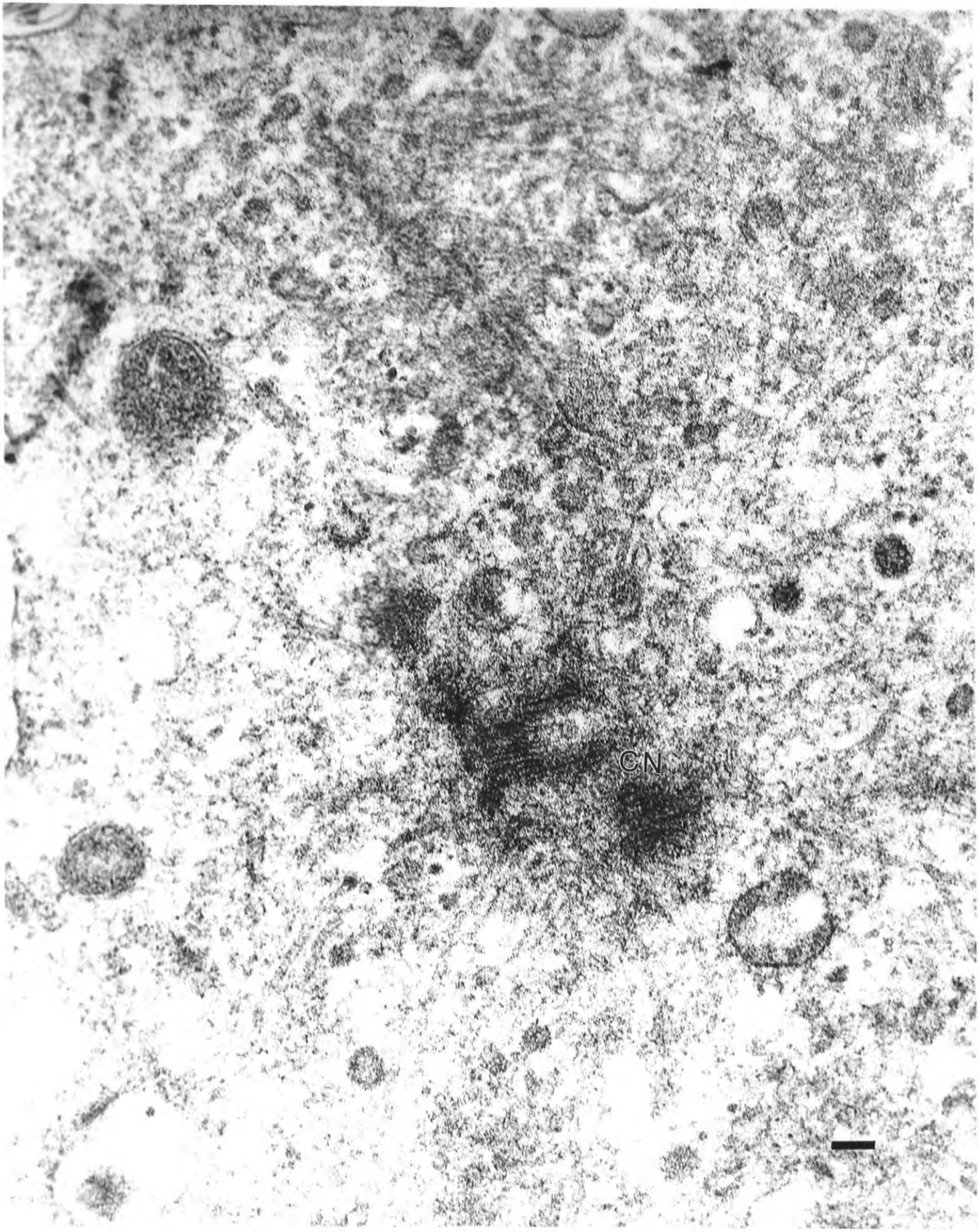

F


N
MV
NS
1.0μm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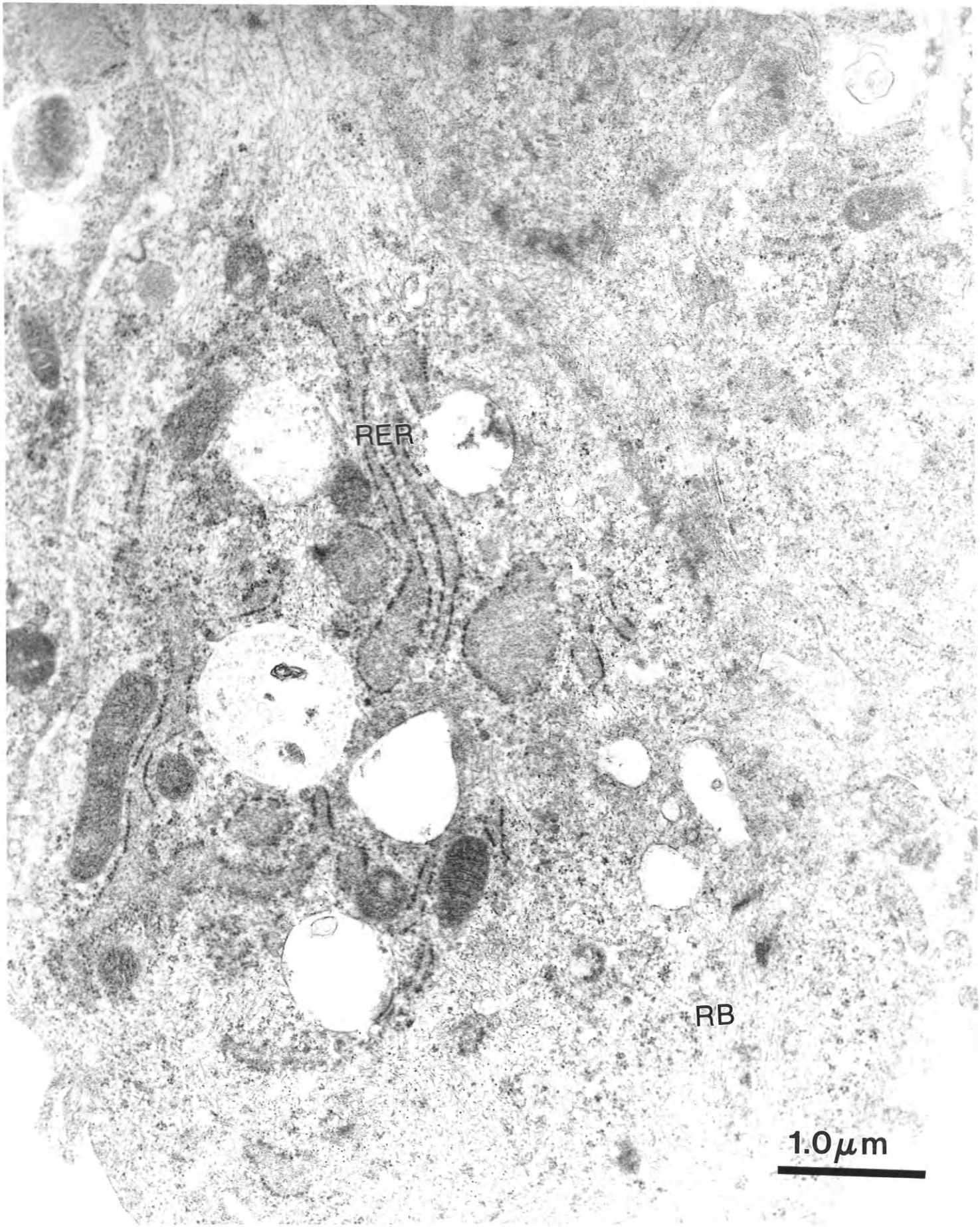
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RB
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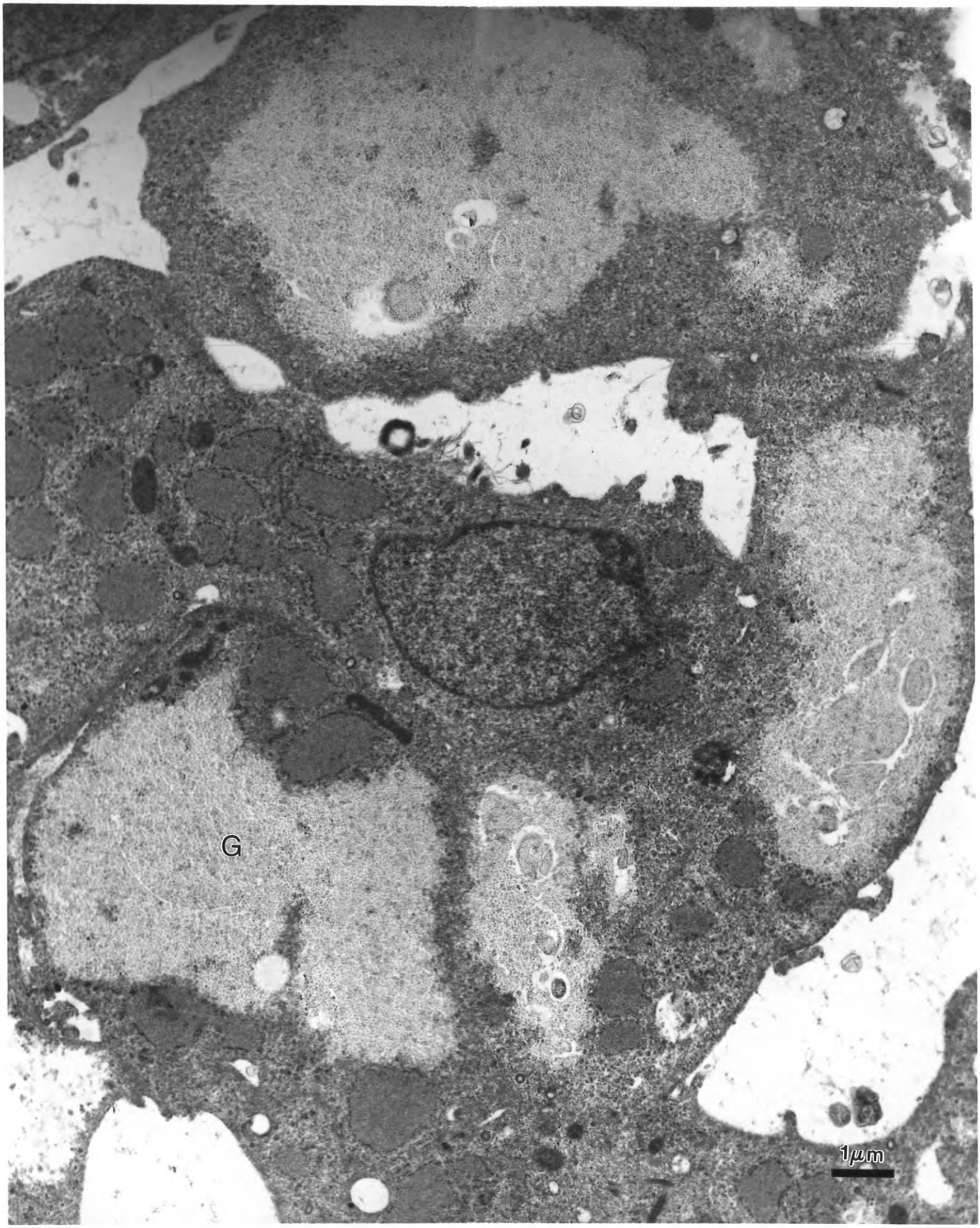
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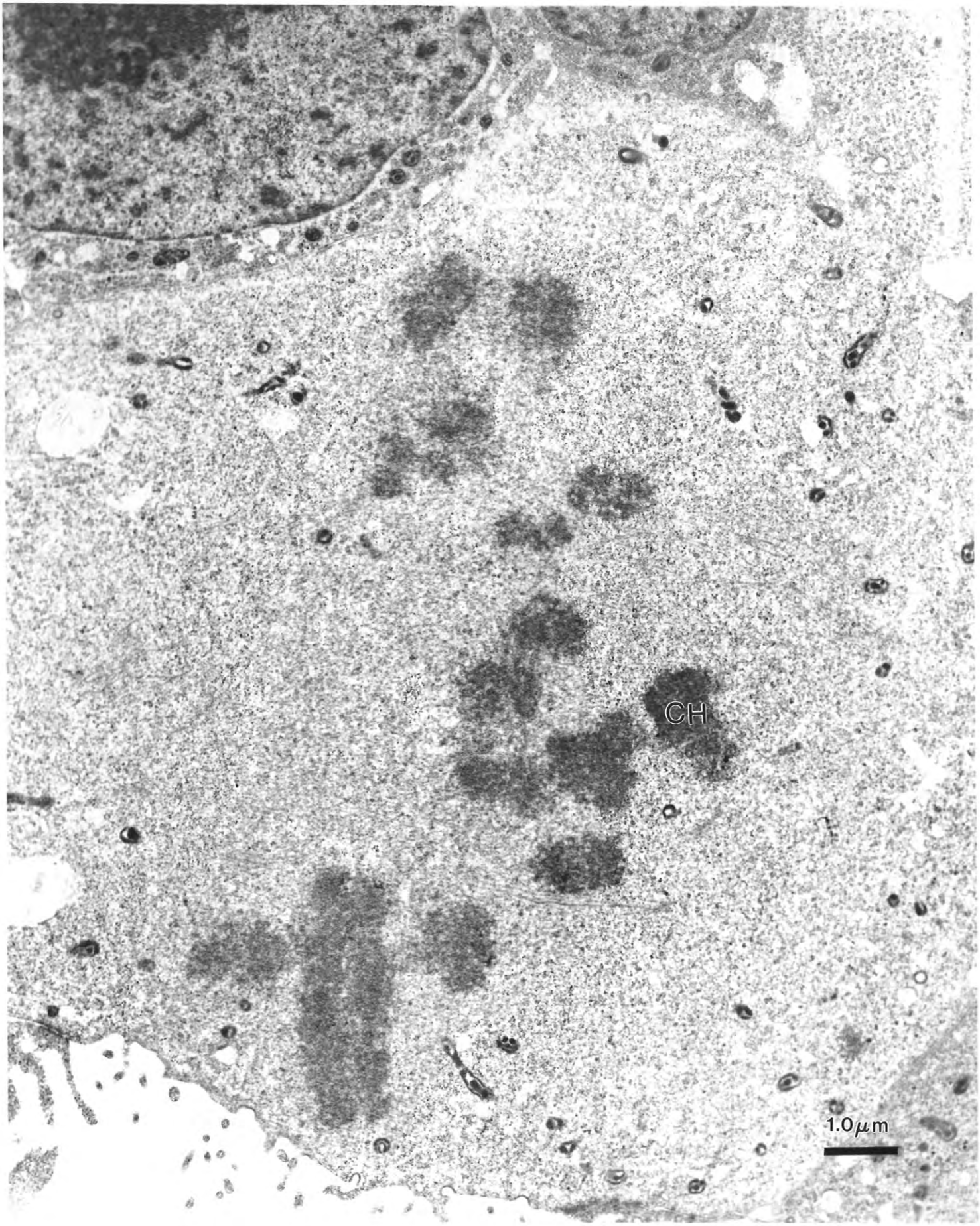
1μm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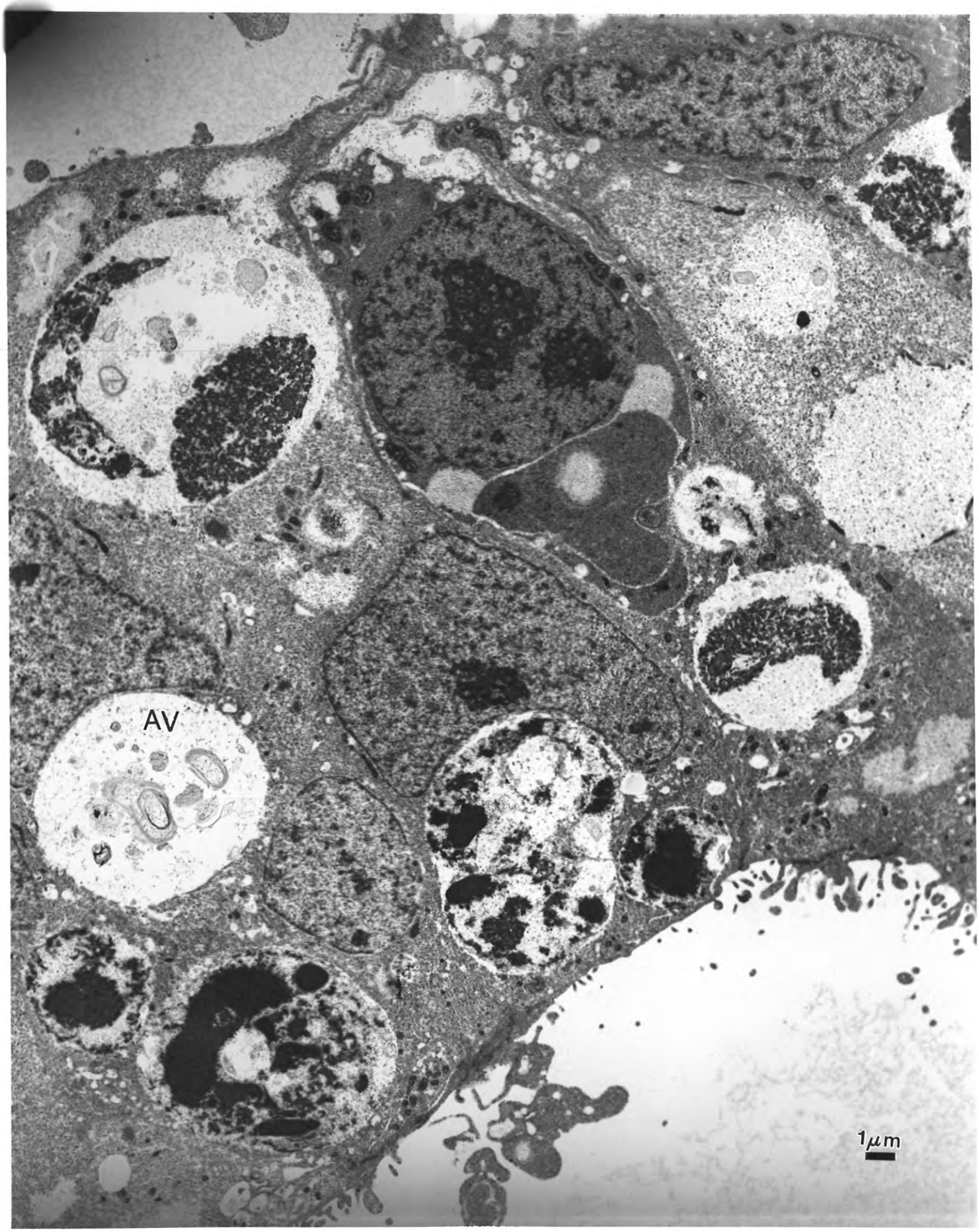
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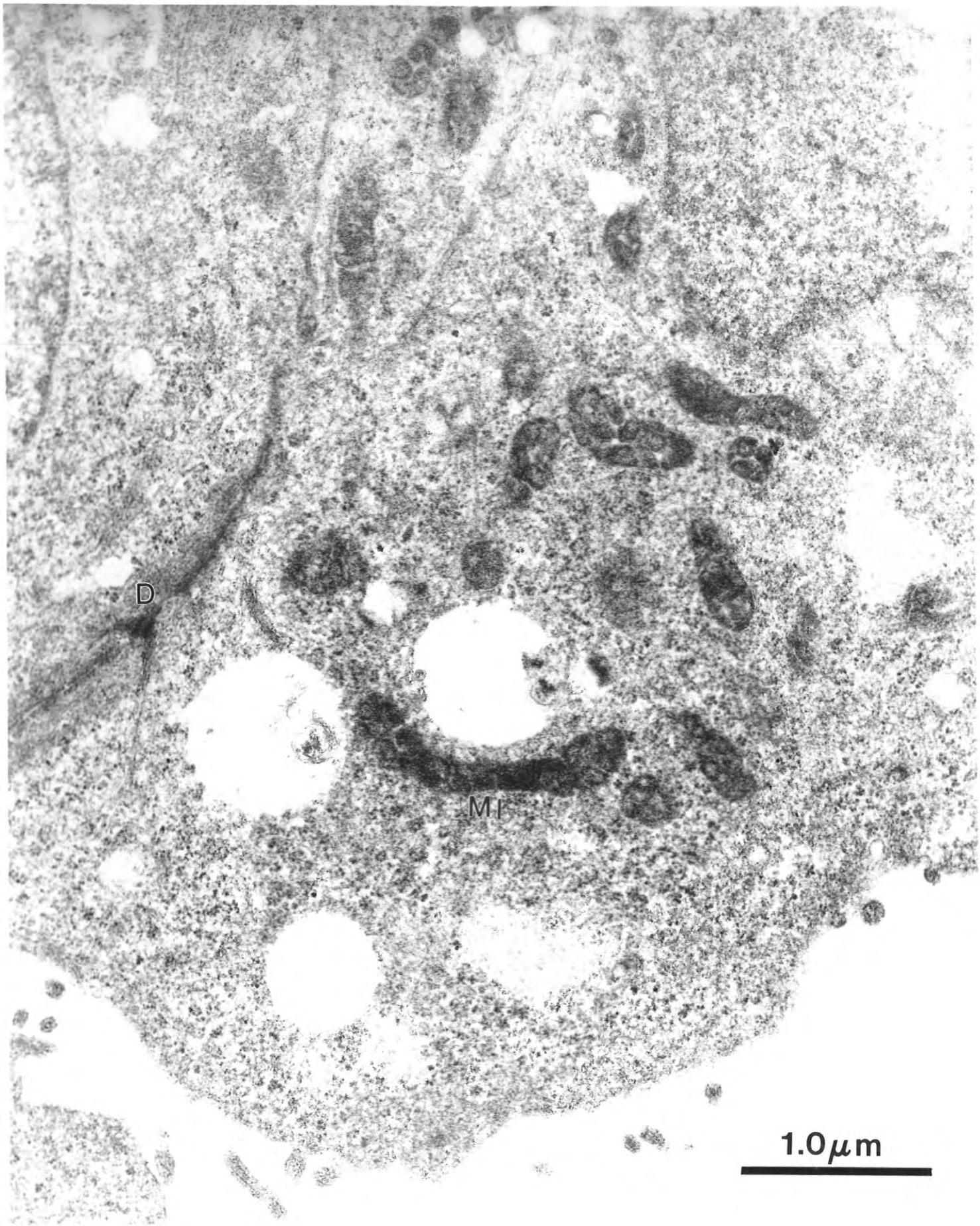
1μm


D
MI
1.0μm

E-FR-1-07-002234.30610

FINAL STUDY REPORT

STUDY TITLE: **Ultrastructural Evaluation of Cell Culture Morphology, with Characterization and Tabulation of Retrovirus-like Particles**

TEST PROTOCOL NUMBER: 30610.05

TEST ARTICLE IDENTIFICATION	APPTEC ACCESSION NUMBER
H1 MCB.1 5 passage in TeSR1	07-002234

SPONSOR: WiCell Research Institute

PERFORMING LABORATORY: AppTec, Inc.

APPTEC ACCESSION NUMBER	RESULTS
07-002234	Transmission electron microscopic examination revealed no identifiable virus-like particles, nor did it reveal any other identifiable microbial agents.

QUALITY ASSURANCE UNIT SUMMARY

STUDY: Ultrastructural Evaluation of Cell Culture Morphology, with Characterization and Tabulation of Retrovirus-like Particles

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. This study has been performed under Good Laboratory Practice regulations (21 CFR Part 58) and in accordance with standard operating procedures and a standard test protocol. The Quality Assurance Unit maintains a copy of the test protocol and standard operating procedures and has inspected this study on the dates listed below. Each inspection was performed to assure the quality and integrity of the study.

Phase Inspected	Date
Processing and embedding Test Article 07-002234	October 23, 2007

The findings of these inspections have been reported to Management and the Study Director.

Quality Assurance — 28 Dec 07
Date

GOOD LABORATORY PRACTICES STATEMENT

This study referenced in this report was conducted in accordance with Good Laboratory Practices for Nonclinical Laboratory Studies as found in Title 21 Code of Federal Regulations Part 58. The study was inspected during at least one phase and AppTec Quality Assurance will audit the final report.

Study Director — 31DEC07
Date

Professional Personnel involved in study:

1.0 PURPOSE

The purpose of this study was to use thin-section electron microscopy to describe the ultrastructural morphological characteristics of the Sponsor's test article and to determine if viral or viral-like particles or other contaminants are present in the Sponsor's test article.

2.0 SPONSOR: WiCell Research Institute

3.0 TEST FACILITY: **AppTec, Inc.**

4.0 SCHEDULING

DATE SAMPLES RECEIVED: October 23, 2007
STUDY INITIATION DATE: October 23, 2007
STUDY COMPLETION DATE: See page 2 for Study Director's signature and date.

5.0 TEST ARTICLE CHARACTERIZATION

Determinations of strength, homogeneity, purity and stability of the test article are solely the responsibility of the Sponsor. The Sponsor is responsible for supplying to the testing laboratory results of these determinations and any others that may directly impact the testing performed by the testing laboratory, prior to initiation of testing.

6.0 TEST ARTICLE IDENTIFICATION: H1 MCB.1 5 passage in TeSR1

7.0 TEST SYSTEM DESCRIPTION

As described in the Points to Consider (May 1993), the morphological and growth characteristics of cell lines used for the production of biologics need to be monitored. Cells in culture possess inherent qualities, some of which are amenable to study by transmission electron microscopy. The use of electron microscopy allows for the visualization of cellular components, which help in the identification of cell type and may aid in describing any cellular changes that could occur during biopharmaceutical production.

Preparation of thin sections of virus-infected cells and tissues is an indispensable technique for the study of those aspects of virus-cell interaction that are accessible to direct examination by electron microscopy.[1,2,3] Thin sectioning is also of value in elucidating the structure of viruses; the information obtained often complements that provided by a negative staining procedure. This protocol can be utilized to visualize a variety of viral types including retroviruses, herpesviruses, adenoviruses, picornaviruses, parvoviruses, orthomyxo- and paramyxoviruses, reoviruses, and many other common viral agents. Contamination by other microbial agents such as yeast, fungi, and bacteria may also be detected.

If retroviruses are detected they will be evaluated on the basis of A-, B-, C-, D-, and R-type retrovirus-like morphologies. *A-type* viral particles are characterized as either (1) intracytoplasmic particles, 60-90 nanometers (nm) in diameter, with an electron-dense core; (2) intracisternal particles, 60-90 nm in diameter, found within the endoplasmic reticulum, with 2 dense concentric shells surrounding an electron-lucent core. *B-type* particles are spherical, enveloped particles that arise by budding at the plasma membrane. They display an eccentric, electron-dense core surrounded by an intermediate layer, and an envelope with prominent projections. *C-type* viral particles are 90-130 nm in diameter, enveloped, and contain an internal nucleoid of variable electron density and shape. They occur either within cytoplasmic vacuoles, on the cell surface, or extracellularly. *D-type* particles are spherical, enveloped particles that bud from the plasma membrane and frequently exhibit an electron-dense bar- or tube-shaped core. *R-type* particles are enveloped, spherical particles, 70-100 nm in diameter, with a central core of variable density from which characteristic spokes extend into the envelope, and are found in the cisternae of the endoplasmic reticulum.

8.0 EXPERIMENTAL DESIGN

For most purposes, optimum preservation of fine structure in animal cells, viruses, and other microbial agents is the prime consideration, and procedures for ensuring this are now fairly well standardized. The cells were submitted as live cells in a flask. When an optimal level of $1 - 2 \times 10^7$ cells was available, the cells were harvested and a cell count determined.

8.1 The cells were pelleted, and McDowell-Trump's fixative was added to the pellet.

8.2 The pellet was shipped to the subcontractor Charles River Laboratories Pathology Associates (CRLPA), where typically (if enough cells are available) one-half of the cell pellet(s) were processed and embedded for transmission electron microscopy (TEM).

8.3 Thin sections were cut and mounted on 200-mesh copper grids.

8.4 The samples were stained with 5% methanolic uranyl acetate and Reynold's lead citrate.

8.5 The cells were examined by TEM to characterize morphologically the cell type comprising the culture. Cell characteristics were documented by labeled electron micrographs.

8.6 200 cells were evaluated for the presence of any type of particle with virus-like morphology, and appropriate documentation was provided for any particles found using labeled electron micrographs.

8.7 Retrovirus-like particles for each of the 200 cells were tabulated as follows: (1) no particles, (2) 1 to 5 particles, (3) 6 to 20 particles, (4) more than 20 particles.

8.8 200 cells were evaluated for particles with A-, B-, C-, D-, and R-type retrovirus-like morphology as described in Section 3. Electron micrographs were made to document representative examples of any virus-like particles observed. Except where noted otherwise, a bar denoting 100 nanometers was placed on each micrograph for size reference.

9.0 TEST ARTICLE PREPARATION

On October 23, 2007, AppTec, Inc. received 1 vial containing 1 mL of "Fixed human embryonic stem cells grown in feeder free conditions," cold on cold packs and designated for use in this assay. The test article was stored at 2-8ºC until shipment to the subcontractor.

On October 23, 2007, 1 vial containing a fixed and pelleted cell culture was shipped, on ice packs, in storage conditions of 2-8°C, via overnight courier to the subcontractor.

10.0 NEGATIVE CONTROLS

A blank water sample was run in parallel with the test article.

11.0 ASSAY VALIDITY

The following validity criteria are evaluated:

12.1 The test is valid if the test article cells are well preserved and at least 200 cells are examined.

12.0 TEST EVALUATION

Detailed description of unique or distinguishing characteristics of cell ultrastructure will be included and documented by labeled electron micrographs. The general appearance or preservation of the cells will be noted.

Analysis of the photomicrograph from the thin sections will provide the opportunity to observe contaminating viruses or other microbial agents and the morphological responses of the host cell. 200 cells will be examined. The type of viral particles and percentage of cells containing the particles will be enumerated.

13.0 RESULTS

The test was valid. The test article cells were well preserved, and at least 200 cells were examined.

13.1 Cellular Ultrastructure

Cells in the section were of moderate size and polygonal to irregular in shape (Z30363). Cells had microvilli (MV: Z30364)[1] unevenly distributed on the surface. Nuclei (N: Z30363) tended to be polygonal to irregular, with chromatin relatively evenly dispersed or clumped along the periphery. Nuclei often had one or more nucleoli (NS: Z30363) that were variably located, and nuclear pores (NP: Z30367) were seen.

The cytoplasm of most cells contained varying numbers of dense mitochondria (MI: Z30365). Ribosomes (RB: Z30366) were abundant in the cytoplasm of most cells. Cells were observed to contain centrioles (CN: Z30366), Golgi complexes (GO: Z30368), microtubules (T: Z30365), coated vesicles (CV: Z30368), desmosomes (D: Z30367) and autophagic vacuoles (AV: Z30364).

13.2 General Viral Particle Evaluation

Transmission electron microscopic examination revealed no identifiable virus-like particles, nor did it reveal any other identifiable microbial agents.

13.3 Retrovirus-like Particle Evaluation and Tabulation

Results of retrovirus-like particle counts in the specimen were as follows:

Test Article: 07-002234
PAI EM Number: 07.544-1

Number of cells with:

Particle Type	No Particles	1-5 Particles	6-20 Particles	20 or more Particles
A-Type	200	0	0	0
B-Type	200	0	0	0
C-Type	200	0	0	0
Extracellular C-type	200	0	0	0
D-Type	200	0	0	0
R-Type	200	0	0	0

Percentage of cells with each type of retrovirus-like particle:

A-Type	0%
B-Type	0%
C-Type	0%
D-Type	0%
R-Type	0%

Four and one-half percent of the cells were necrotic.

[1] Numbers in parentheses are specific micrographs in which the designated structures were found.

14.0 CONCLUSION

Evidence of viral or viral-like particles or other contaminants was not detected in the test article.

15.0 STATISTICAL DATA ANALYSIS

Statistical analysis of the data is not required.

16.0 DEVIATIONS / AMENDMENTS

No deviations from the protocol were encountered during the conduct of this study.

No amendments to the protocol were generated.

17.0 RECORD RETENTION

The testing facility will retain all records involving the study for seven (7) years including, but not limited to: the signed test protocol with all amendments, any written communication concerning the conduct of the study, test article accountability record, raw data, worksheets, and an official copy of the final study report.

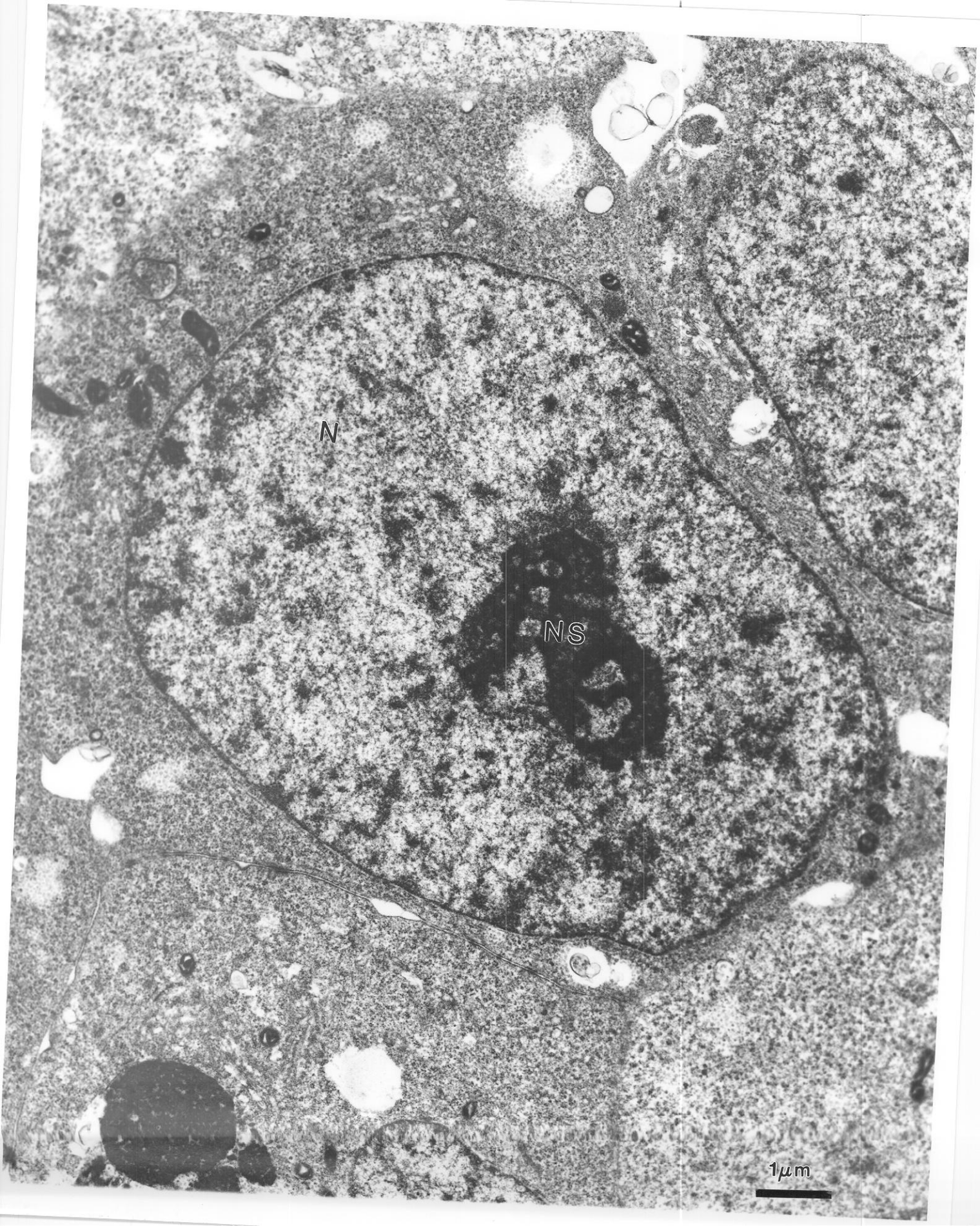
18.0 REFERENCES

1. Morgan C and Rose HM (1967). "The Application of Thin Sectioning," *Methods in Virology* Vol. **3** (Maramorosch K and Koprowski H, eds.), Academic Press, New York, NY, pp. 576-616.

2. Palmer E and Martin M (1988). Retroviridae in "Electron Microscopy in Viral Diagnosis", CRC Press, Boca Raton, FL, pp. 91-103.

3. Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals (1993). Office of Biologics Research and Review, Food and Drug Administration.

4. Jawetz E, Melnick JL, and Adelberg, EA, eds. (1984). Tumor Viruses in: "Review of Medical Microbiology," 16th Edition, Lange Medical Publications, Drawer L, Los Altos, CA, pp. 495-498.


N
NS
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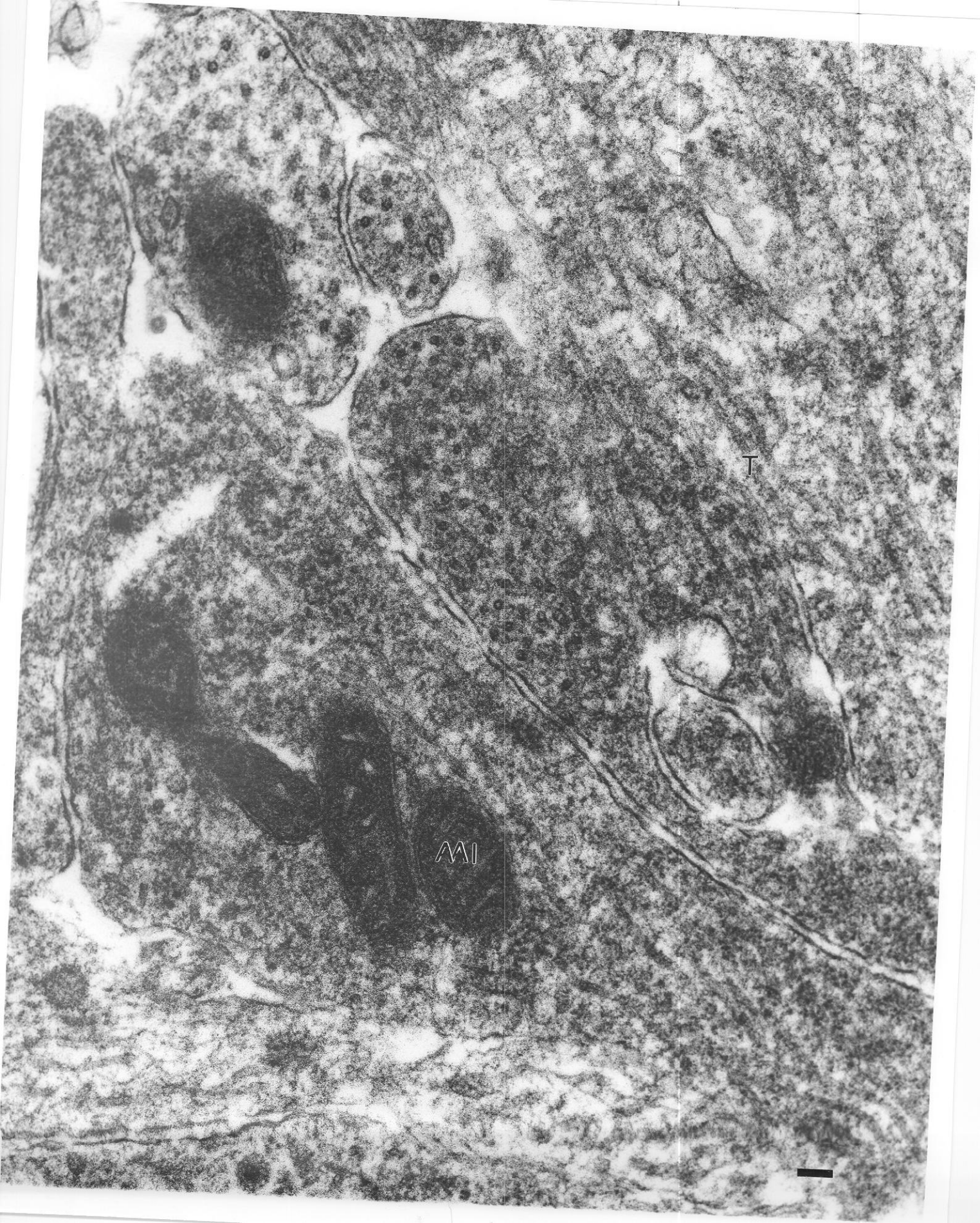
MV
AV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CV
GO


RB
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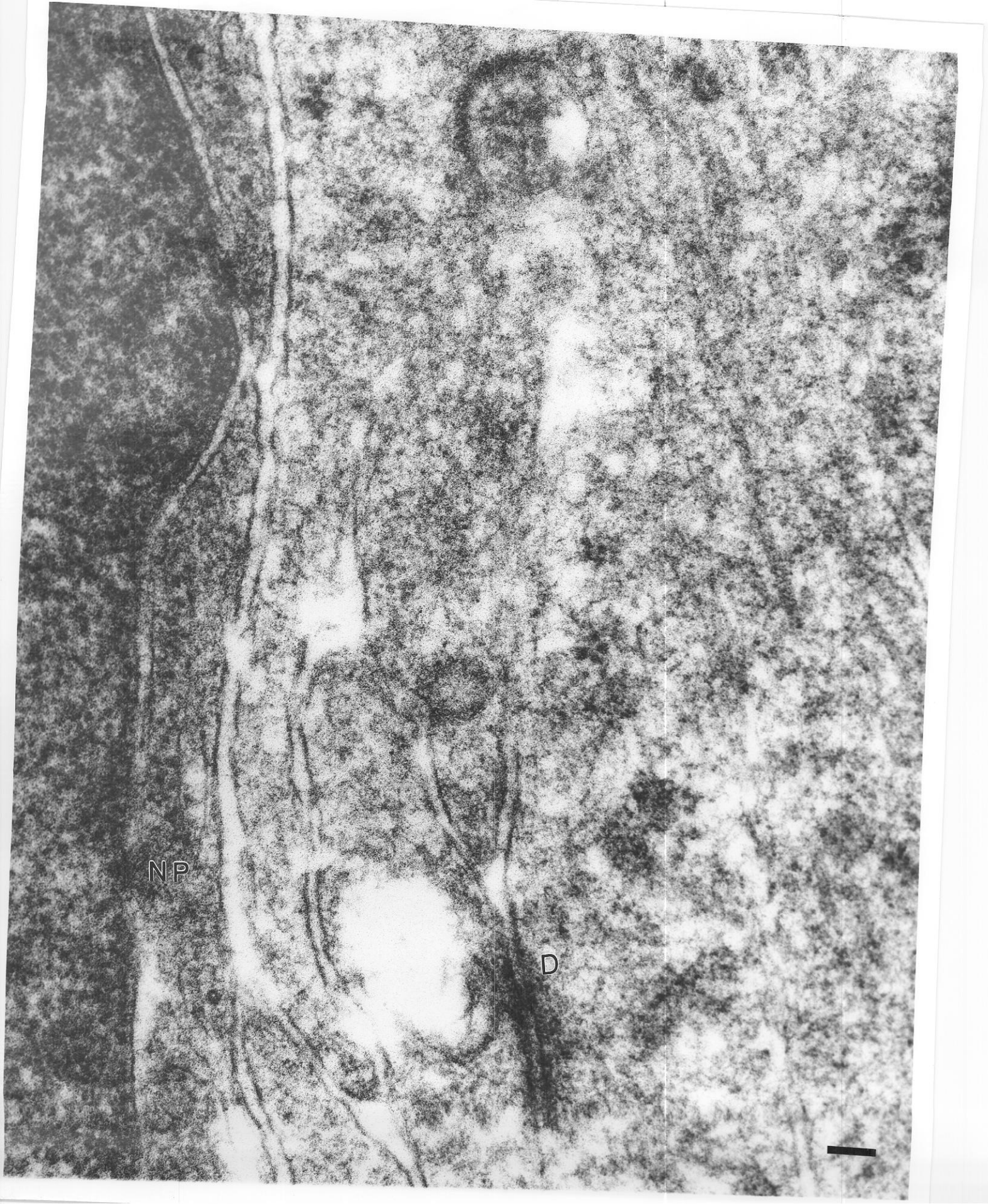
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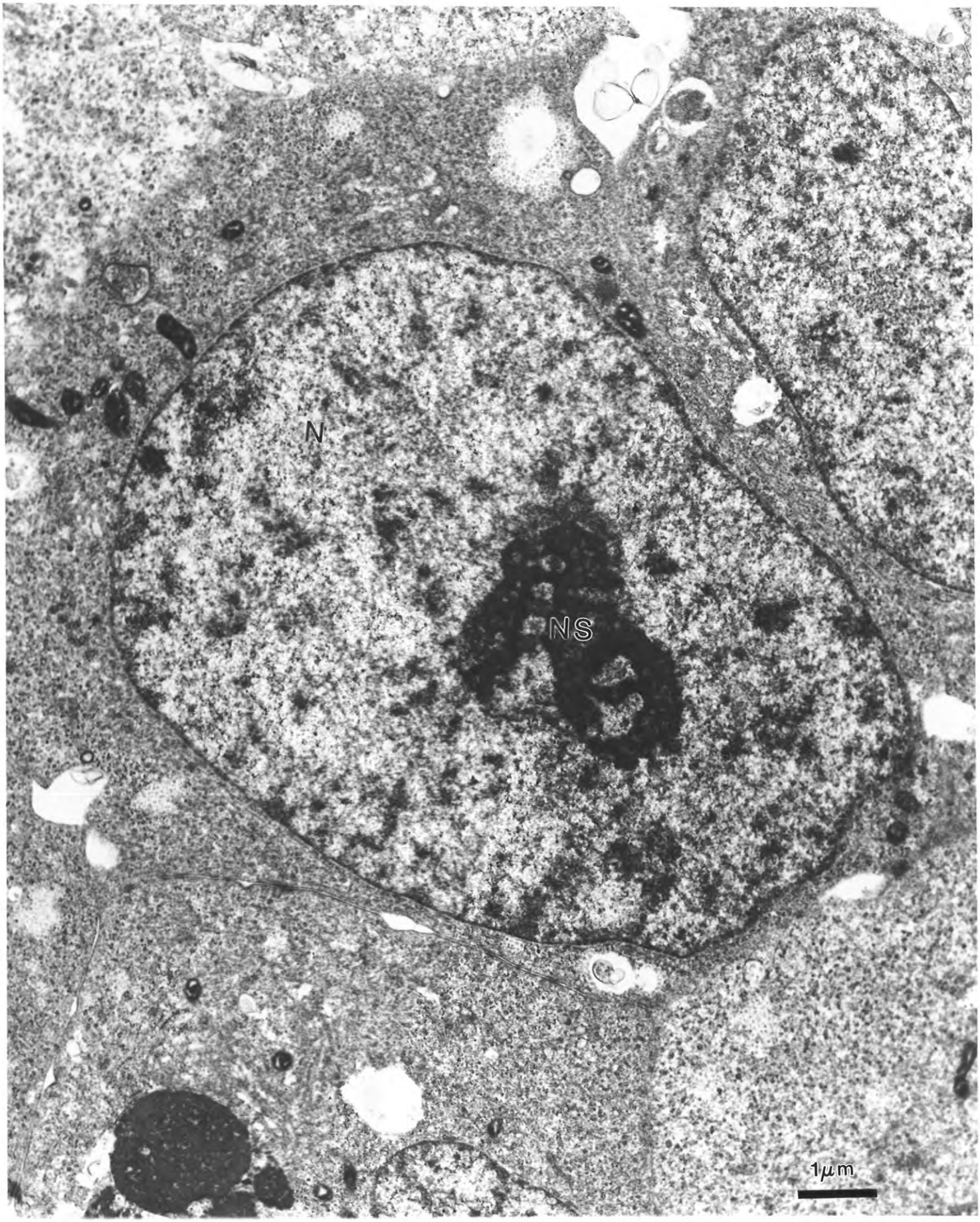
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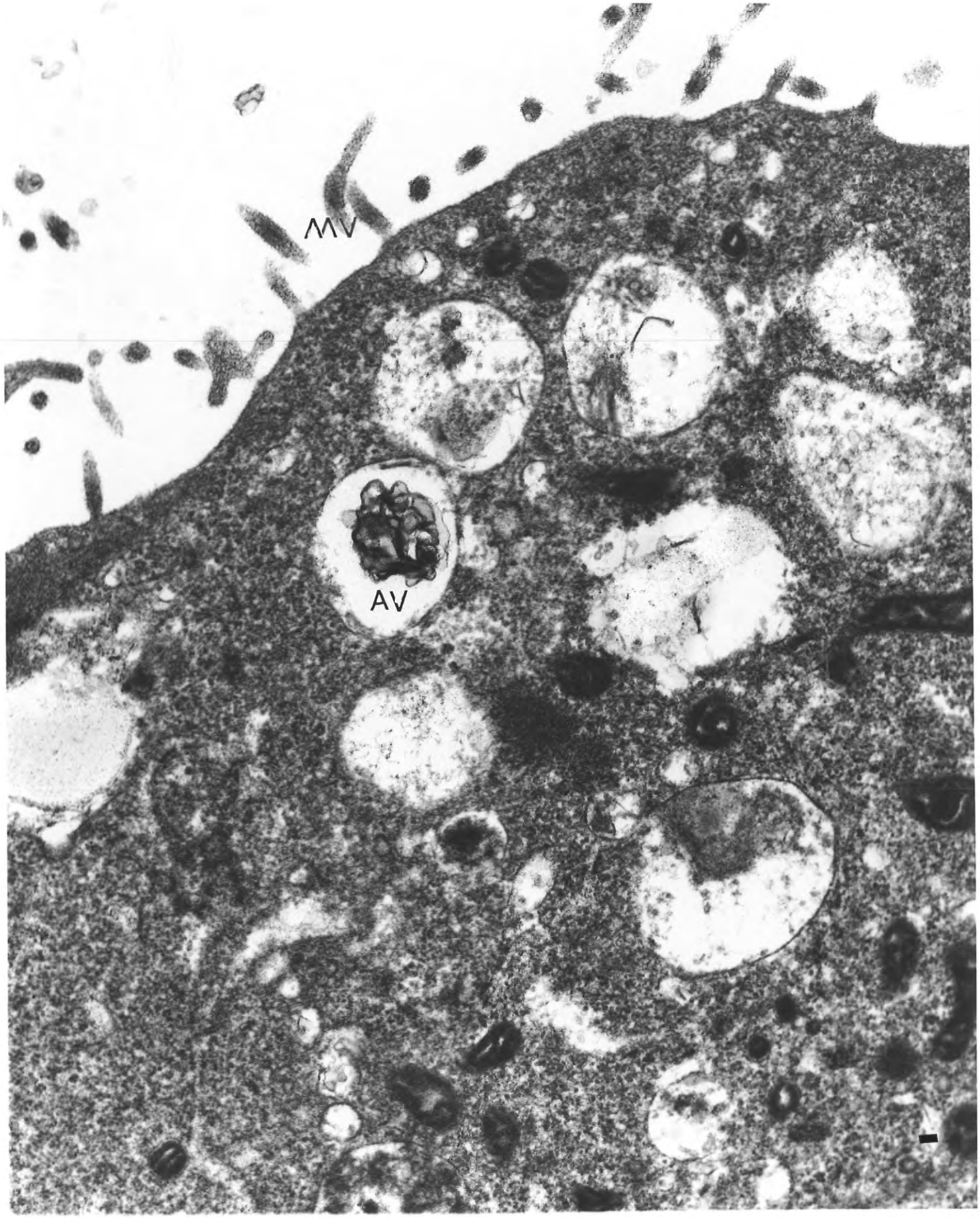
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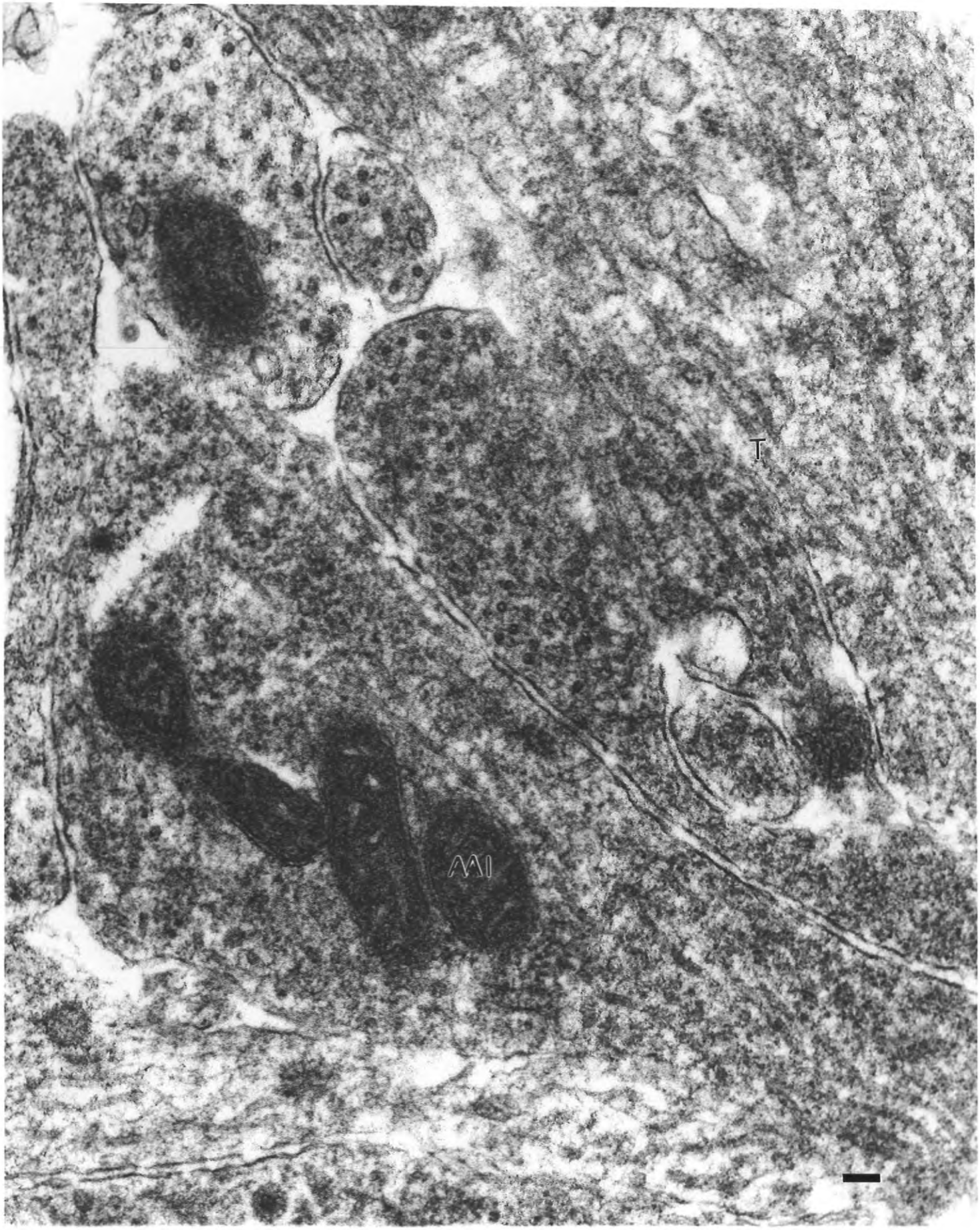
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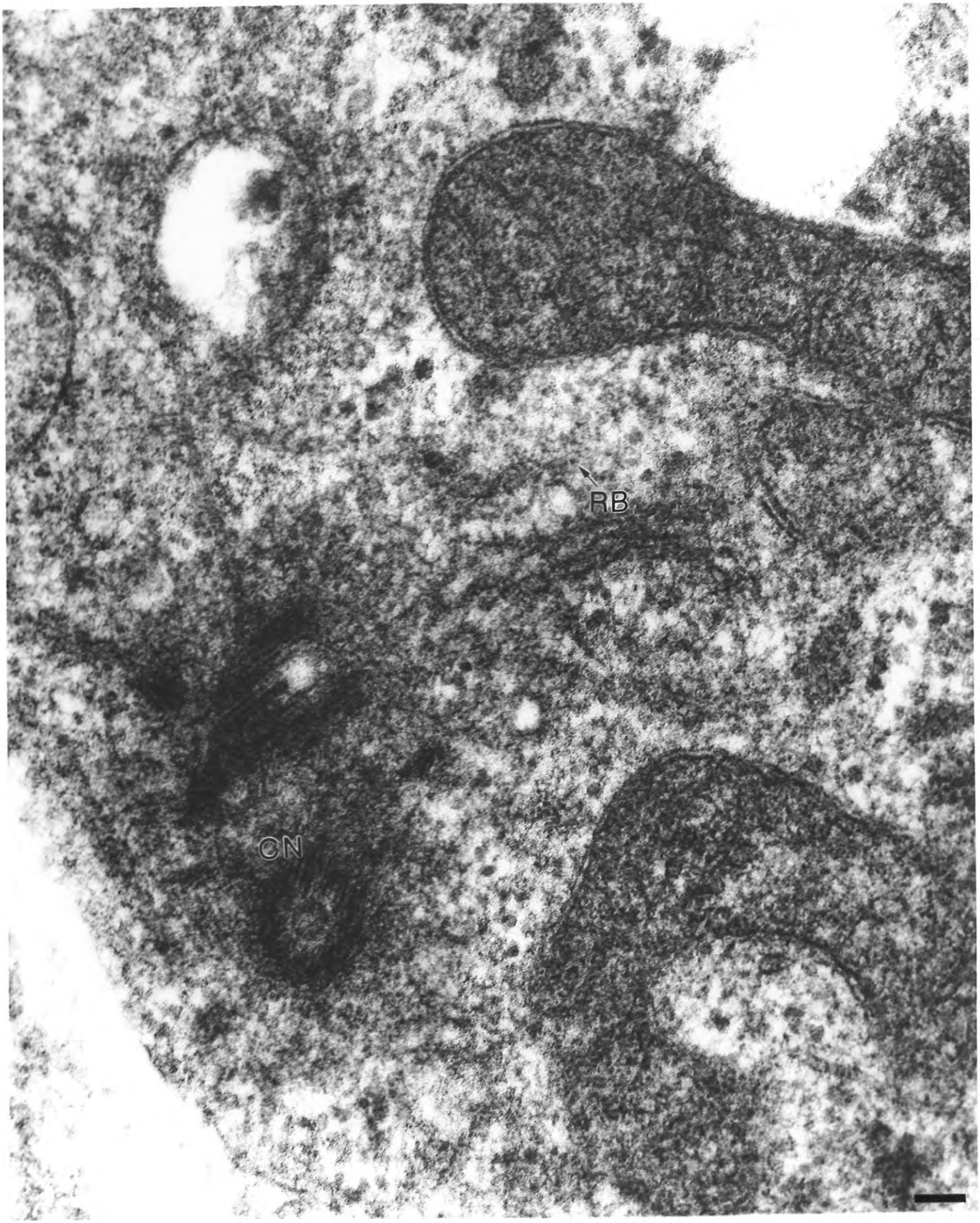
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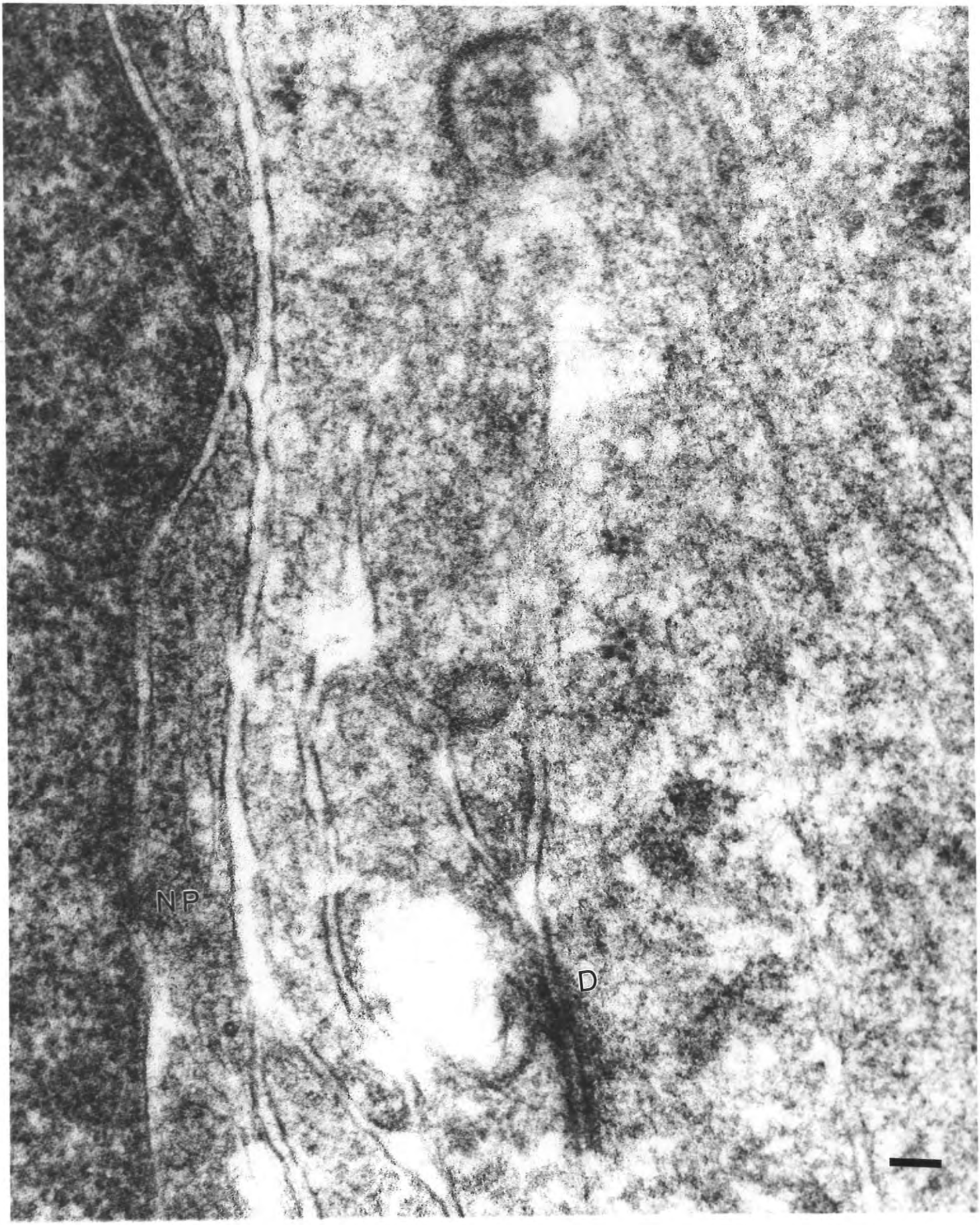
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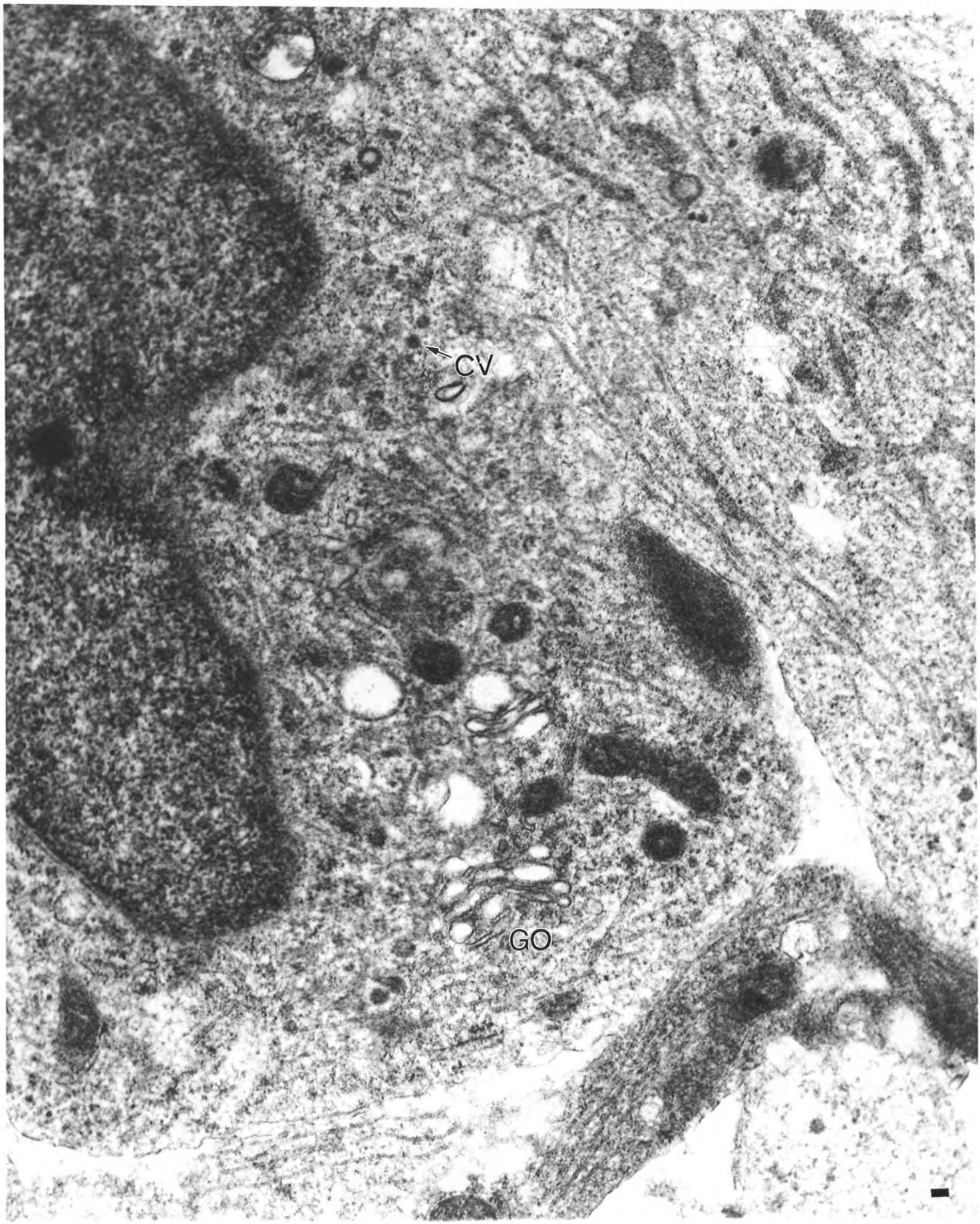

CV
GO

E-FR-1-07-001213.30610

FINAL STUDY REPORT

STUDY TITLE: **Ultrastructural Evaluation of Cell Culture Morphology, with Characterization and Tabulation of Retrovirus-like Particles**

TEST PROTOCOL NUMBER: 30610.04

TEST ARTICLE IDENTIFICATION	APPTEC ACCESSION NUMBER
H1-MCB.1	07-001213

SPONSOR: [redacted]

PERFORMING LABORATORY: AppTec, Inc.

APPTEC ACCESSION NUMBER	RESULTS
07-001213	Cells demonstrated C-type and extracellular C-type retrovirus-like particles. The combined percentage of cells demonstrating this results was 5.5%. Given the presence of mouse cells in the culture, this result is considered to be typical and expected.

QUALITY ASSURANCE UNIT SUMMARY

STUDY: Ultrastructural Evaluation of Cell Culture Morphology, with Characterization and Tabulation of Retrovirus-like Particles

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. This study has been performed under Good Laboratory Practice regulations (21 CFR Part 58) and in accordance with standard operating procedures and a standard test protocol. The Quality Assurance Unit maintains a copy of the test protocol and standard operating procedures. The Quality Assurance Unit for the subcontractor used in this study was responsible for a study inspection performed on the dates listed below. Each inspection was performed to assure the quality and integrity of the study.

Phase Inspected	Date	Study Director	Management
Specimen Processing and Embedding	June 7, 2007	June 22, 2007	June 22, 2007

The findings of these inspections have been reported to Management and the Study Director.

Quality Assurance — 05JUL07 Date

GOOD LABORATORY PRACTICES STATEMENT

This study referenced in this report was conducted in accordance with Good Laboratory Practices for Nonclinical Laboratory Studies as found in Title 21 Code of Federal Regulations Part 58. The subcontractor inspected the study at least once and AppTec Quality Assurance will audit the final report.

Study Director _____ 05JUL07 Date

Professional Personnel involved in study:

1.0 PURPOSE

The purpose of this study was to use thin-section electron microscopy to describe the ultrastructural morphological characteristics of the Sponsor's test article and to determine if viral or viral-like particles or other contaminants are present in the Sponsor's test article.

2.0 SPONSOR: WiCell

3.0 TEST FACILITY: **AppTec, Inc.**

4.0 SCHEDULING

DATE SAMPLES RECEIVED: May 22, 2007
STUDY INITIATION DATE: May 31, 2007
STUDY COMPLETION DATE: See page 2 for Study Director's signature and date.

5.0 TEST ARTICLE CHARACTERIZATION

Determinations of strength, homogeneity, purity and stability of the test article are solely the responsibility of the Sponsor. The Sponsor is responsible for supplying to the testing laboratory results of these determinations and any others that may directly impact the testing performed by the testing laboratory, prior to initiation of testing.

6.0 TEST ARTICLE IDENTIFICATION: H1-MCB.1

7.0 TEST SYSTEM DESCRIPTION

As described in the Points to Consider (May 1993), the morphological and growth characteristics of cell lines used for the production of biologics need to be monitored. Cells in culture possess inherent qualities, some of which are amenable to study by transmission electron microscopy. The use of electron microscopy allows for the visualization of cellular components, which help in the identification of cell type and may aid in describing any cellular changes that could occur during biopharmaceutical production.

Preparation of thin sections of virus-infected cells and tissues is an indispensable technique for the study of those aspects of virus-cell interaction that are accessible to direct examination by electron microscopy.[1,2,3] Thin sectioning is also of value in elucidating the structure of viruses; the information obtained often complements that provided by a negative staining procedure. This protocol can be utilized to visualize a variety of viral types including retroviruses, herpesviruses, adenoviruses, picornaviruses, parvoviruses, orthomyxo- and paramyxoviruses, reoviruses, and many other common viral agents. Contamination by other microbial agents such as yeast, fungi, and bacteria may also be detected.

If retroviruses are detected they will be evaluated on the basis of A-, B-, C-, D-, and R-type retrovirus-like morphologies. *A-type* viral particles are characterized as either (1) intracytoplasmic particles, 60-90 nanometers (nm) in diameter, with an electron-dense core; (2) intracisternal particles, 60-90 nm in diameter, found within the endoplasmic reticulum, with 2 dense concentric shells surrounding an electron-lucent core. *B-type* particles are spherical, enveloped particles that arise by budding at the plasma membrane. They display an eccentric, electron-dense core surrounded by an intermediate layer, and an envelope with prominent projections. *C-type* viral particles are 90-130 nm in diameter, enveloped, and contain an internal nucleoid of variable electron density and shape. They occur either within cytoplasmic vacuoles, on the cell surface, or extracellularly. *D-type* particles are spherical, enveloped particles that bud from the plasma membrane and frequently exhibit an electron-dense bar- or tube-shaped core. *R-type* particles are enveloped, spherical particles, 70-100 nm in diameter, with a central core of variable density from which characteristic spokes extend into the envelope, and are found in the cisternae of the endoplasmic reticulum.

8.0 EXPERIMENTAL DESIGN

For most purposes, optimum preservation of fine structure in animal cells, viruses, and other microbial agents is the prime consideration, and procedures for ensuring this are now fairly well standardized. The cells were submitted as live cells in a flask. When an optimal level of $1 - 2 \times 10^7$ cells was available, the cells were harvested and a cell count determined.

8.1 The cells were pelleted, and McDowell-Trump's fixative was added to the pellet.

8.2 The pellet was shipped to the subcontractor Charles River Laboratories Pathology Associates (CRLPA), where typically (if enough cells are available) one-half of the cell pellet(s) were processed and embedded for transmission electron microscopy (TEM).

8.3 Thin sections were cut and mounted on 200-mesh copper grids.

8.4 The samples were stained with 5% methanolic uranyl acetate and Reynold's lead citrate.

8.5 The cells were examined by TEM to characterize morphologically the cell type comprising the culture. Cell characteristics were documented by labeled electron micrographs.

8.6 200 cells were evaluated for the presence of any type of particle with virus-like morphology, and appropriate documentation was provided for any particles found using labeled electron micrographs.

8.7 Retrovirus-like particles for each of the 200 cells were tabulated as follows: (1) no particles, (2) 1 to 5 particles, (3) 6 to 20 particles, (4) more than 20 particles.

8.8 200 cells were evaluated for particles with A-, B-, C-, D-, and R-type retrovirus-like morphology as described in Section 7. Electron micrographs were made to document representative examples of any virus-like particles observed.

9.0 TEST ARTICLE PREPARATION

On May 22, 2007, AppTec, Inc. received 1 vial containing 5 mL of "human embryonic stem cell line H9 grown on mouse embryonic Feeders Fixed in McDowell-Trump's," cold on cold packs and designated for use in this assay. The cells were already fixed by the client. The test article was stored at 2-8°C until May 31, 2007 when 1 vial containing a fixed and pelleted cell culture was shipped, on ice packs, in storage conditions of 2-8°C, via overnight carrier to the subcontractor.

10.0 NEGATIVE CONTROLS

A blank water sample was run in parallel with the test article.

11.0 ASSAY VALIDITY

The following validity criteria are evaluated:

12.1 The test is valid if the test article cells are well preserved and at least 200 cells are examined.

12.0 TEST EVALUATION

Detailed description of unique or distinguishing characteristics of cell ultrastructure will be included and documented by labeled electron micrographs. The general appearance or preservation of the cells will be noted.

Analysis of the photomicrograph from the thin sections will provide the opportunity to observe contaminating viruses or other microbial agents and the morphological responses of the host cell. 200 cells will be examined. The type of viral particles and percentage of cells containing the particles will be enumerated.

13.0 RESULTS

The test was valid. The test article cells were well preserved, and at least 200 cells were examined.

13.1 Cellular Ultrastructure

Cells in the section were of moderate size and polygonal to irregular in shape (L22204). Cells had microvilli (MV: L22204) unevenly distributed on the surface. Nuclei (N: L22204) tended to be polygonal to irregular, with chromatin relatively evenly dispersed or clumped along the periphery. Nuclei often had one or more nucleoli (NS: L22204) that were variably located. Some cells were observed to be under mitosis with chromosomes (CH: L22202) visible.

The cytoplasm of most cells contained varying numbers of condensed mitochondria (MI: L22203). Profiles of rough endoplasmic reticulum (RER: L22198) sometimes distended with fine granular material were seen among the mitochondria. Ribosomes (RB: L22198) were abundant in the cytoplasm of most cells. Cells were observed to contain autophagic vacuoles (AV: L22201), Golgi complexes (GO: L22194) and lipid droplets (L: L22204).

13.2 General Viral Particle Evaluation

Retrovirus-like particles observed in the sample were C-type particles (C in micrographs) seen both budding from cell membranes and extracellularly.

13.3 Retrovirus-like Particle Evaluation and Tabulation

Results of retrovirus-like particle counts in the specimen were as follows:
Test Article: 07-001213
PAI EM Number: 07.274-1

Number of cells with:

Particle Type	No Particles	1-5 Particles	6-20 Particles	20 or more Particles
A-Type	200	0	0	0
B-Type	200	0	0	0
C-Type	193	3	4	0
Extracellular C-type	193	5	1	1
D-Type	200	0	0	0
R-Type	200	0	0	0

Percentage of cells with each type of retrovirus-like particle:

A-Type	0%
B-Type	0%
C-Type	5.5%
D-Type	0%
R-Type	0%

The percentage of cells containing C-type particles is obtained by adding the number of cells with budding and those with extracellular C-type particles, subtracting the number of cells that contained both budding and extracellular C-type particles, then dividing by the total number of 200 cells examined.

Three cells had both budding and extracellular C-type particles.

26% of the cells were necrotic.

14.0 CONCLUSION

Cells demonstrated C-type and extracellular C-type retrovirus-like particles. The combined percentage of cells demonstrating this results was 5.5%.

Given the presence of mouse cells in the culture, this result is considered to be typical and expected.

15.0 STATISTICAL DATA ANALYSIS

Statistical analysis of the data is not required.

16.0 DEVIATIONS / AMENDMENTS

No deviations from the protocol were encountered during the conduct of this study.

No amendments to the protocol were generated.

17.0 RECORD RETENTION

The testing facility will retain all records involving the study for seven (7) years including, but not limited to: the signed test protocol with all amendments, any written communication concerning the conduct of the study, test article accountability record, raw data, worksheets, and an official copy of the final study report.

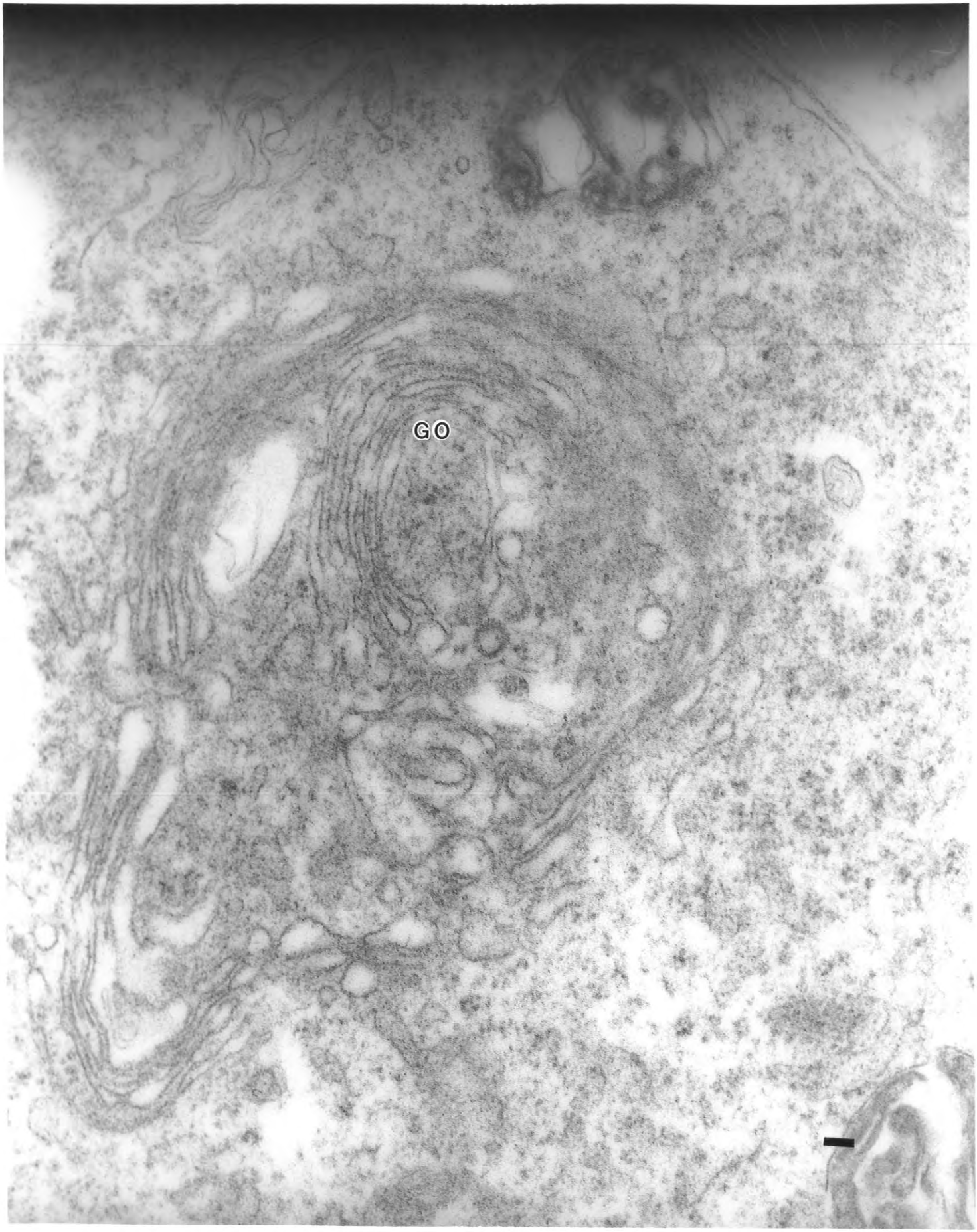
18.0 REFERENCES

1. Morgan C and Rose HM (1967). "The Application of Thin Sectioning," *Methods in Virology* Vol. **3** (Maramorosch K and Koprowski H, eds.), Academic Press, New York, NY, pp. 576-616.

2. Palmer E and Martin M (1988). Retroviridae in "Electron Microscopy in Viral Diagnosis", CRC Press, Boca Raton, FL, pp. 91-103.

3. Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals (1993). Office of Biologics Research and Review, Food and Drug Administration.

4. Jawetz E, Melnick JL, and Adelberg, EA, eds. (1984). Tumor Viruses in: "Review of Medical Microbiology," 16th Edition, Lange Medical Publications, Drawer L, Los Altos, CA, pp. 495-498.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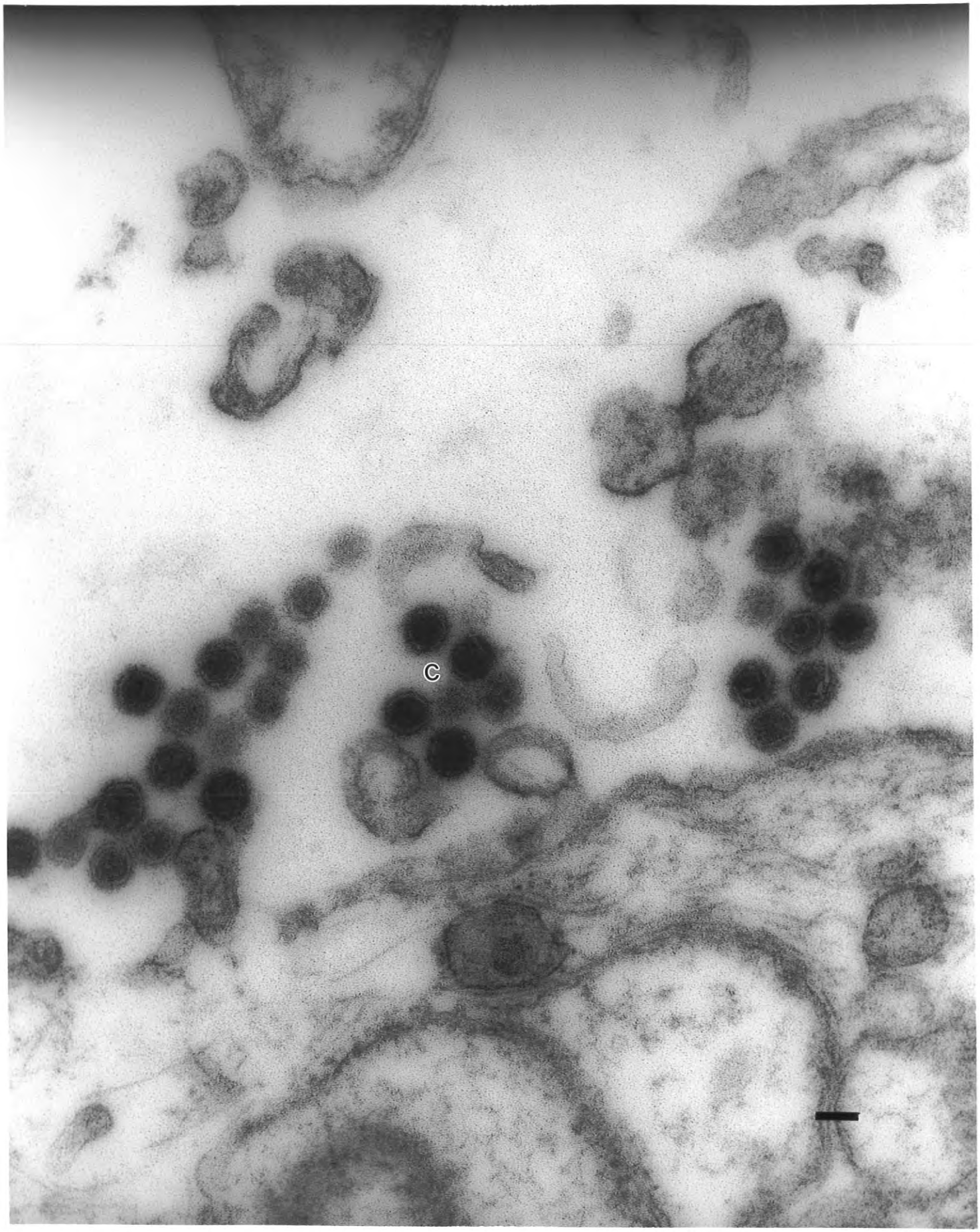
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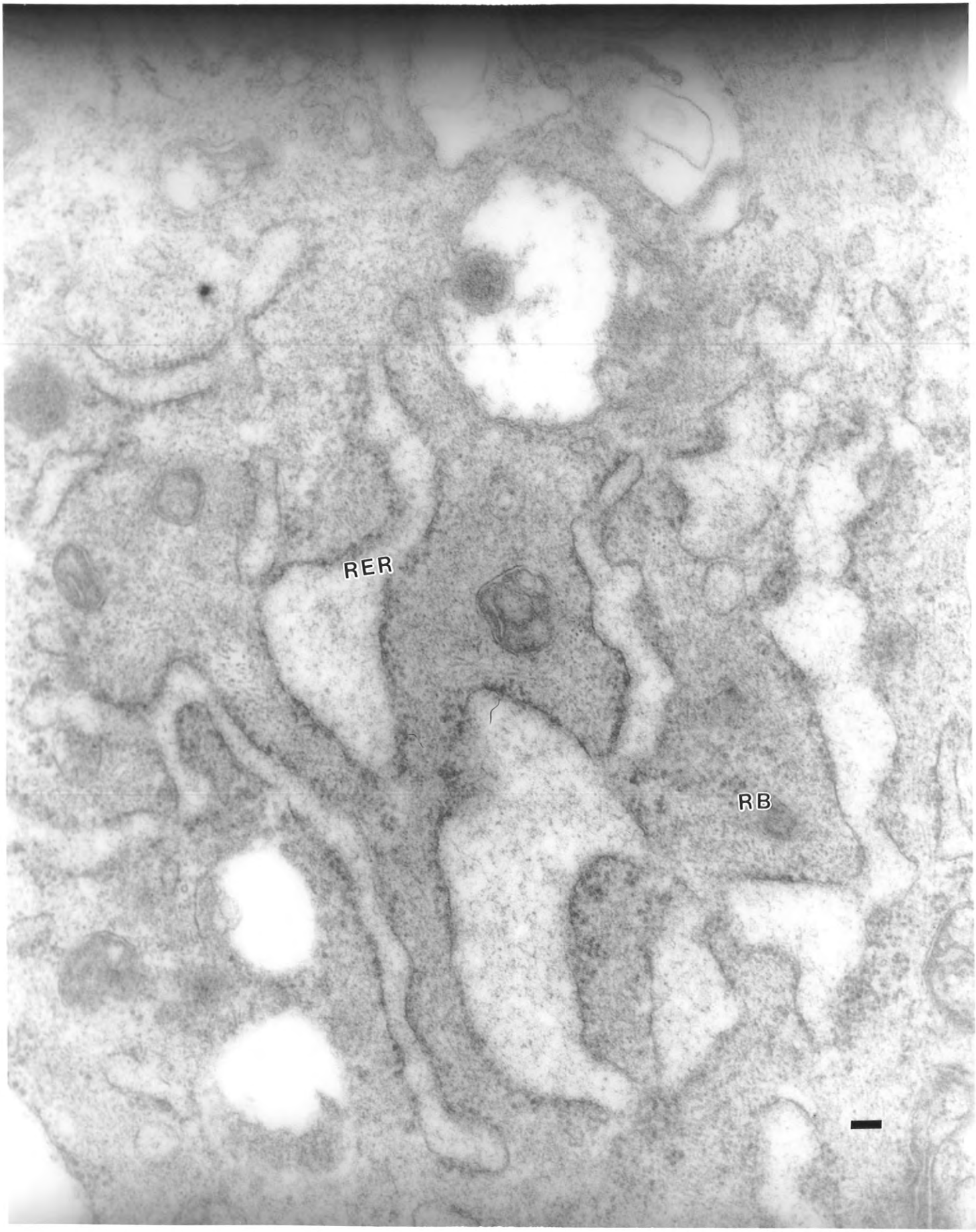
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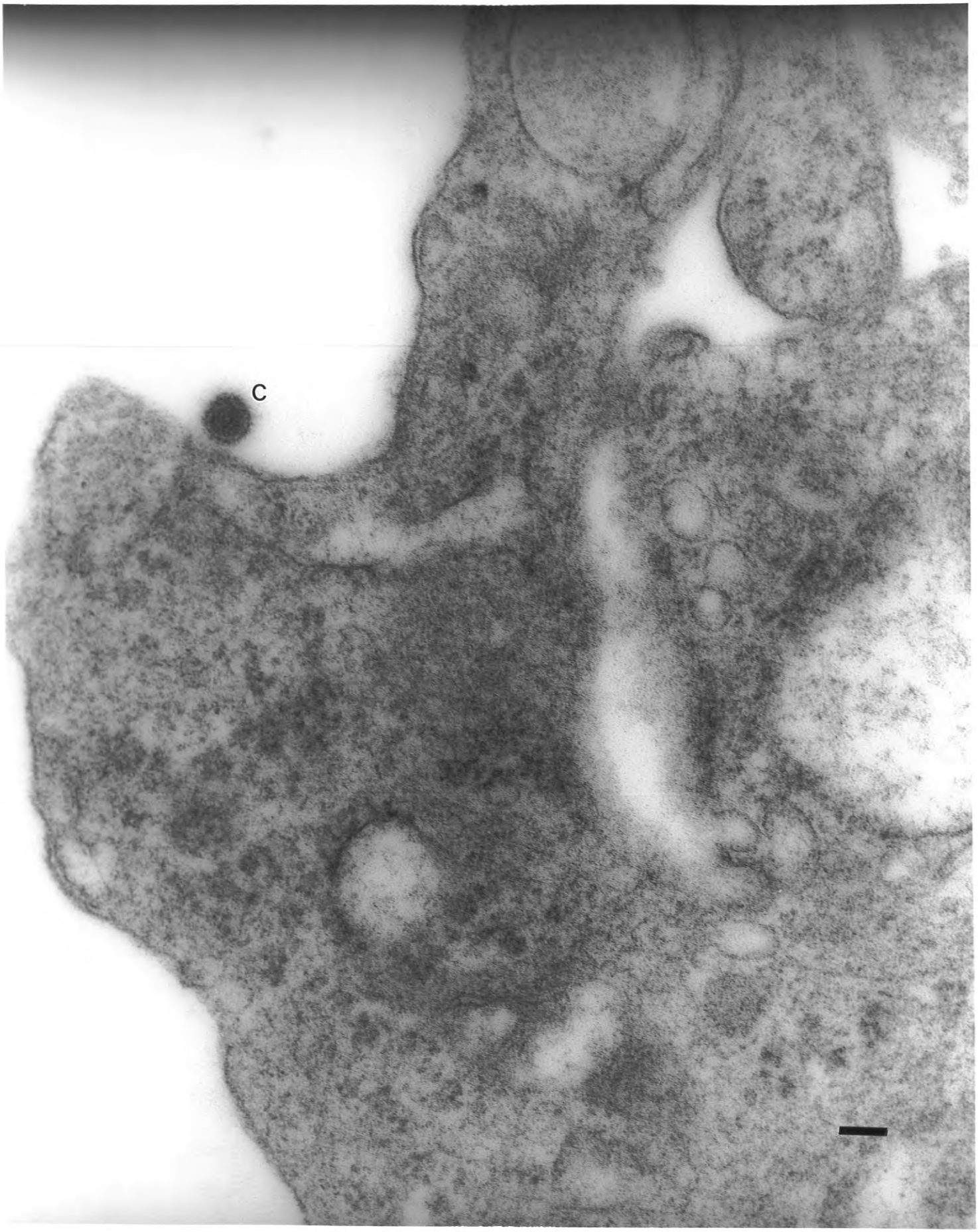
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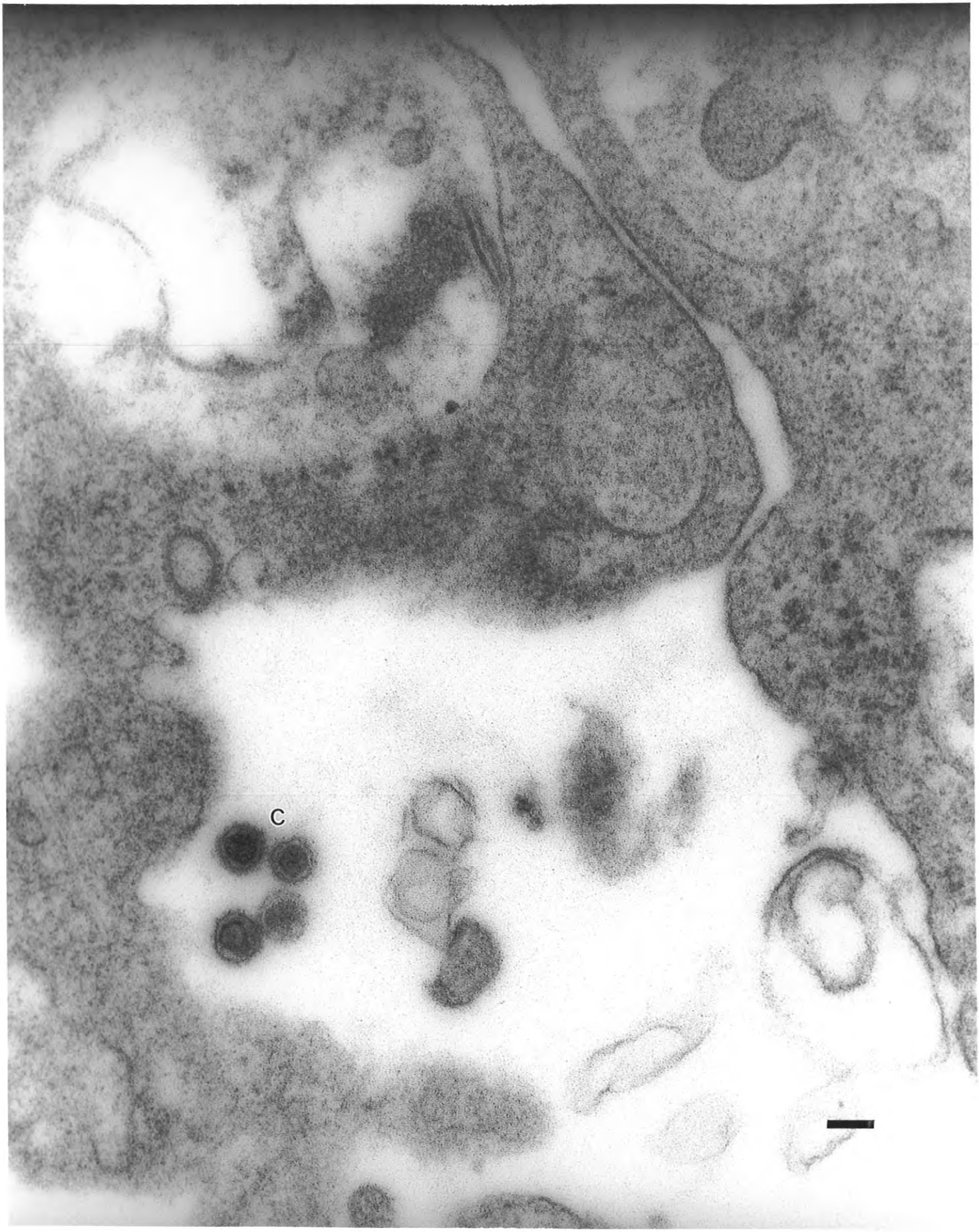
C


AV
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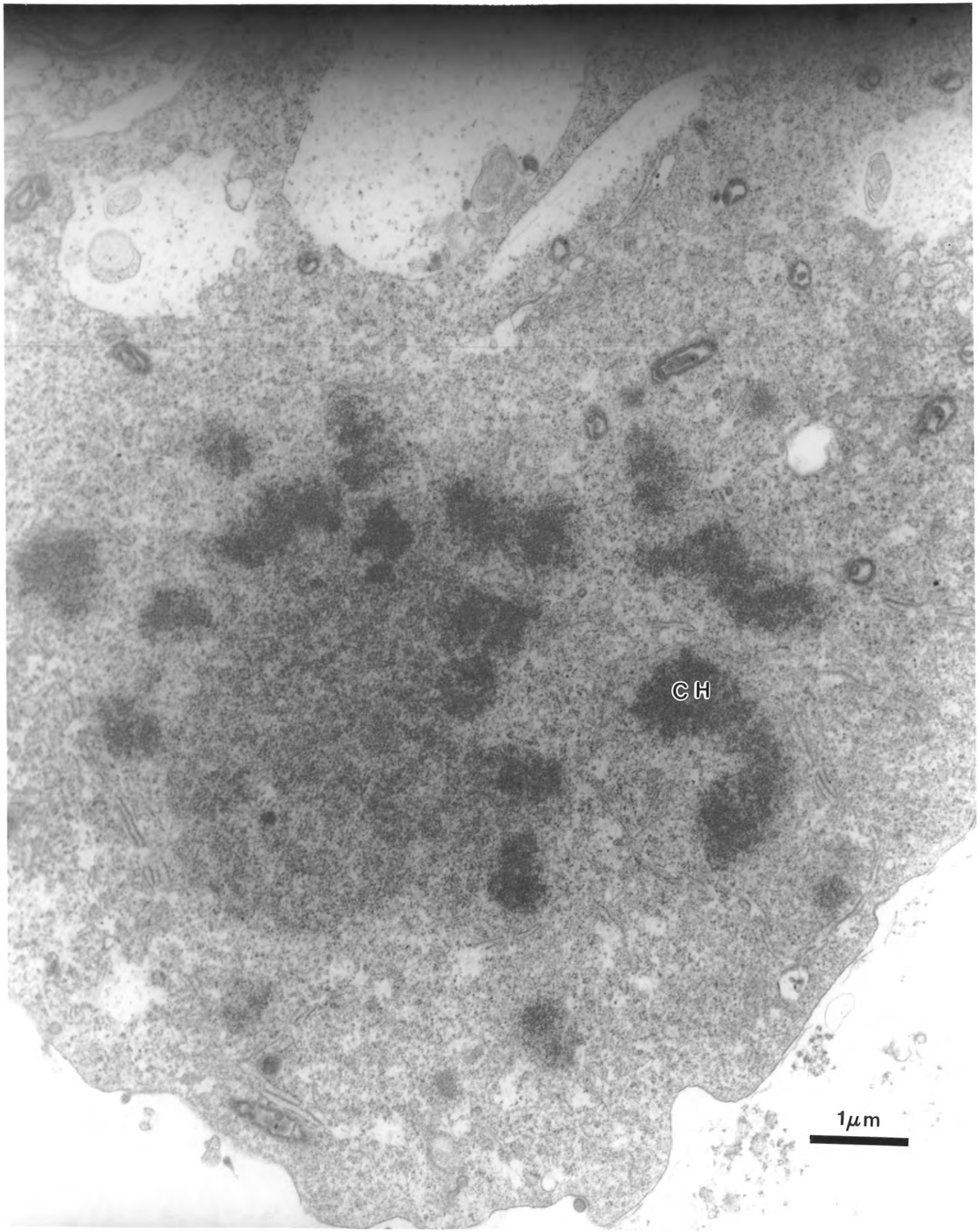
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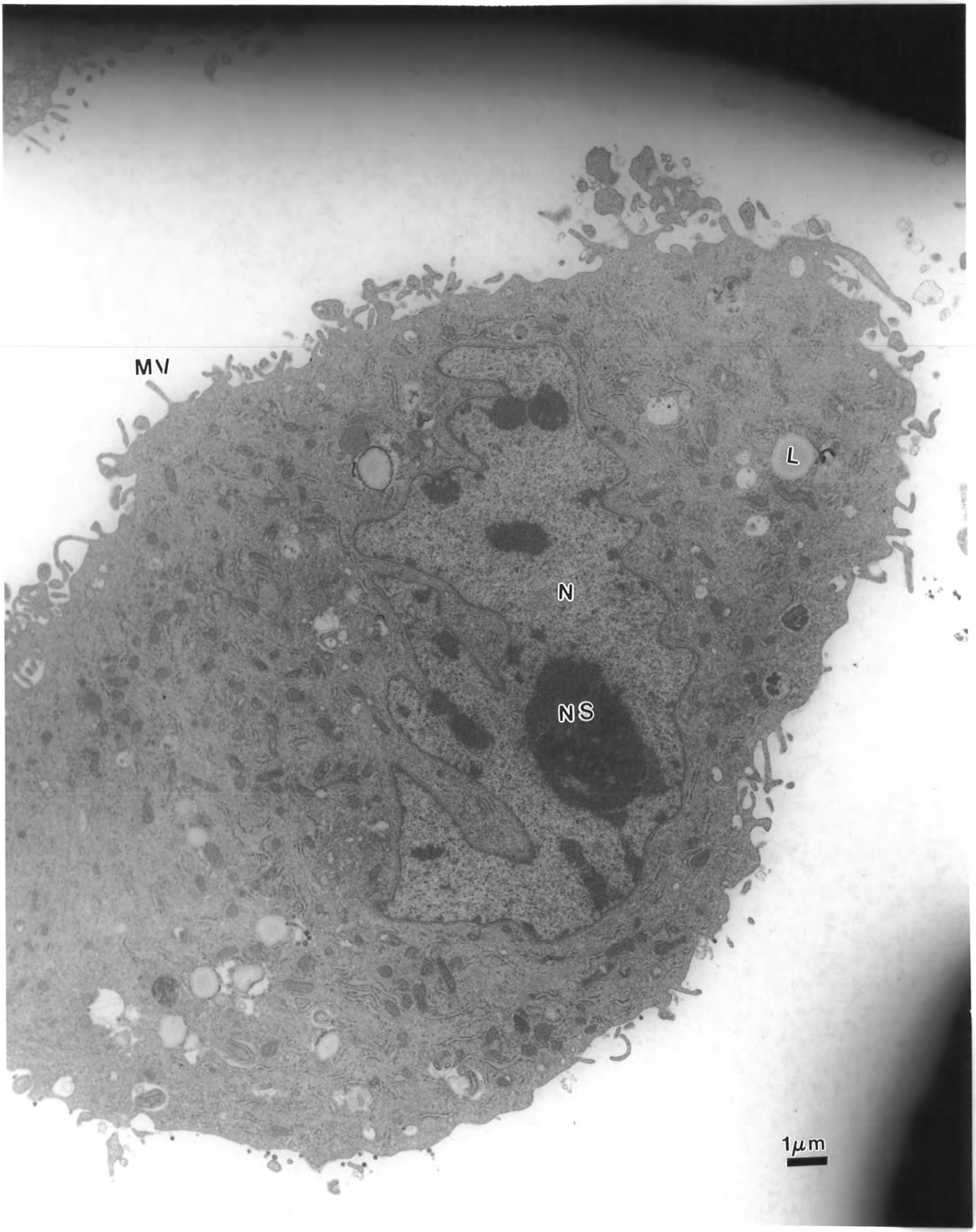
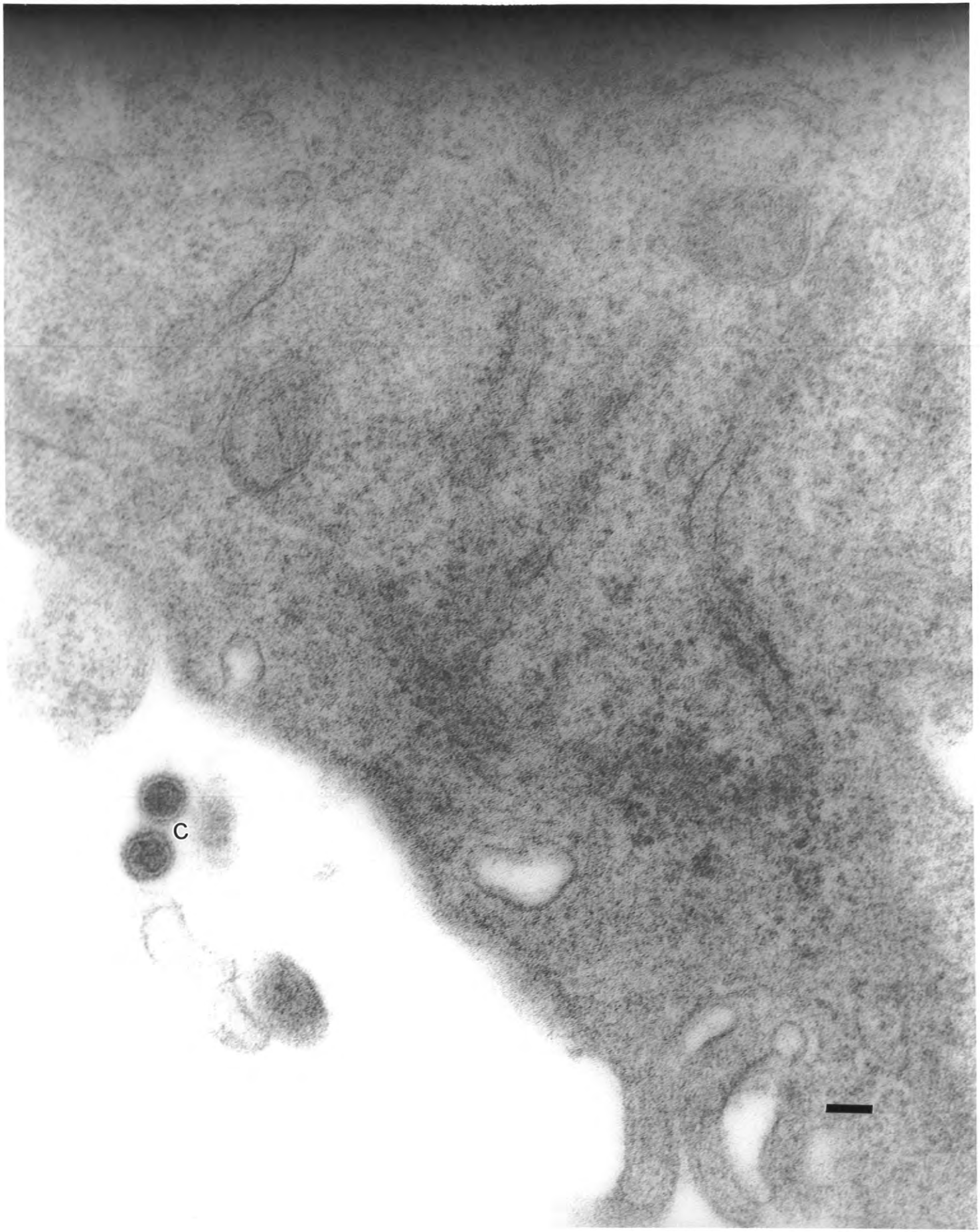

MV
L
N
NS
1μm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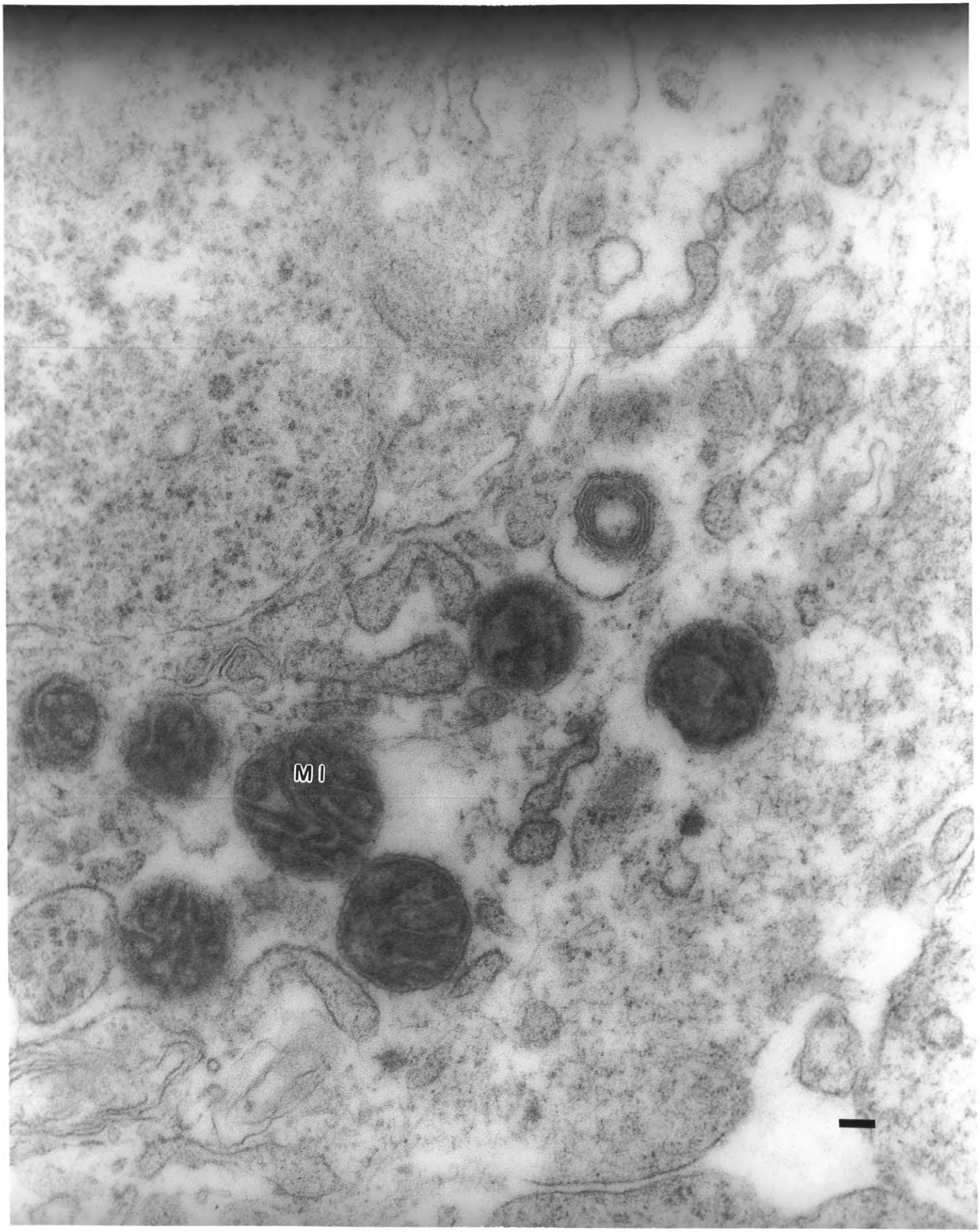
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E-FR-1-07-001215.30201

FINAL STUDY REPORT

STUDY TITLE: **Co-Cultivation of Test Article Cells with *Mus dunni* Cells: 2 Passes**

PROTOCOL: 30201.04

TEST ARTICLE IDENTIFICATION	APPTEC ACCESSION NUMBER
H1-MCB.1	07-001215

SPONSOR: WiCell

PERFORMING LABORATORY: AppTec, Inc.

APPTEC ACCESSION NUMBER	RESULTS
07-001215	No evidence for xenotropic, amphotropic, or MCF MuLV retroviral contamination was found in the test article. Following co-cultivation the test article demonstrated a negative response in the PG4 $S^{+}L^{-}$ assay.

QUALITY ASSURANCE UNIT SUMMARY

STUDY: Co-Cultivation of Test Article Cells with *Mus dunni* Cells: 2 Passes

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. This study has been performed under Good Laboratory Practice regulations (21 CFR Part 58) and in accordance with standard operating procedures and a standard test protocol. The Quality Assurance Unit maintains a copy of the test protocol and standard operating procedures and has inspected this study on the dates listed below. Each inspection was performed to assure the quality and integrity of the study.

Phase Inspected	Date	Study Director	Management
BR# 30201 04 Step 4.8.4 Remove the growth medium from all test article flasks	May 29, 2007	June 12, 2007	June 12, 2007

The findings of these inspections have been reported to Management and the Study Director

Quality Assurance

24Jul07
Date

GOOD LABORATORY PRACTICES STATEMENT

The study referenced in this report was conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice (GLP) regulations as set forth in 21 CFR Part 58. Test article characterization is the responsibility of the Sponsor

Study Director

7-24-07
Date

Personnel involved in study:

1.0 PURPOSE

The purpose of this study was to detect replication-competent retroviruses from the Sponsor's test article cells by co-cultivation with *Mus dunni* cells for at least 14 days with 2 passages of the cultures At the conclusion of the co-cultivation, the supernatants were tested in PG4 $S^{+}L^{-}$ assay (30165) for detection of xenotropic, amphotropic and mink cell focus-forming or polytropic viruses

2.0 SPONSOR: WiCell

3.0 TEST FACILITY: **AppTec, Inc.**

4.0 SCHEDULING

DATE SAMPLES RECEIVED: May 22, 2007
STUDY INITIATION DATE: May 23, 2007
STUDY COMPLETION DATE: See page 2 for Study Director's signature and date

5.0 TEST ARTICLE CHARACTERIZATION

Determinations of strength, homogeneity, purity, and stability of the test article are solely the responsibility of the Sponsor The Sponsor is responsible for supplying to the testing laboratory results of these determinations and any others that may directly impact the testing performed by the testing laboratory, prior to initiation of testing

6.0 TEST ARTICLE IDENTIFICATION: H1-MCB 1

7.0 TEST SYSTEM DESCRIPTION

In the generation of retroviral vectors for gene therapy it has become necessary to assay for replication competent retroviruses (RCR's) that may have been produced through recombination during the viral stock preparation process This testing should include examining the master cell bank (MCB), the manufacturer's working cell bank (MWCB), the production lots, and the transduced target cells if *ex vivo* technology is utilized Co-cultivation with cell lines that are sensitive to various classes of the murine retroviruses is the method of choice to detect any potential RCR's that may have arisen The *Mus dunni* cells are a well characterized cell line that will support the replication of most classes of murine leukemia viruses (MuLV) including Ecotropic, Amphotropic, Xenotropic and Mink Cell Focus-Forming (MCF or Polytropic) viruses[1] (The ecotropic Moloney MuLV will not however replicate in the *Mus dunni* cells: if an RCR is suspected that may have generated a Moloney MuLV envelope, co-cultivation should be performed on

NIH/3T3 or SC-1 cells [30024]). In contrast to other mouse, rat or mink cell lines typically used in co-cultivation, the *Mus dunni* line has demonstrated little cross-reactivity between MuLV and endogenous *Mus dunni* DNA sequences. This property reduces the possibility of aberrant results arising as a consequence of recombination between input virus and the endogenous viral sequences

This protocol should be performed only for Sponsors who have a CHO cell line or CHO derived vector or other non-gene therapy based product. Sponsors who require MuLV testing and do not need to conform to the FDA guidelines for gene therapy vector testing can also use this protocol

The test article cells are co-cultivated with detector cells for up to two weeks in culture with two passages of the cells to increase the ability of any potential retroviruses to replicate. The original test article (if available) and the cell culture supernatants collected after day 14 are tested for the presence of RCR's by the PG4 S^+L^- assay (outlined below, and as described further in protocol 30165). The PG4 S^+L^- assay is a very sensitive S^+L^- assay that can detect amphotropic, xenotropic and MCF viruses. If necessary, the presence of ecotropic viruses can be detected by the XC Plaque Assay (30015). This optional determination for ecotropic viruses would be performed on the original test article (if available) and the supernatant from day 14

8.0 EXPERIMENTAL DESIGN

The test article was maintained according to the Sponsor's instructions. Indicator cell lines were maintained by the Cell Biology Laboratory.

8.1 Co-Cultivation with *Mus dunni* Cells

8.1.1 *Mus dunni* cells alone served as the negative control and were run in parallel with the test article for 14 days. A sample of the conditioned medium was reserved as a time zero (T_0) time point for testing in the PG4 S^+L^- assay.

8.1.2 An aliquot of the test article supernatant was saved for testing in the PG4 S^+L^- assay as a T_0 time point.

8.1.3 To initiate the co-cultivation, 1 mL of test article cells were added to a flask of *Mus dunni* cells ($10x10^5$ cells)

8.1.4 Positive controls were established last, using viral amphotropic murine retrovirus stocks inoculated between 10 and 100 FFU.

8.1.5 All cultures were plated in a suitable growth medium supplemented with fetal bovine serum and antibiotics and maintained at 37±2°C with 5±2% CO_2 humidified atmosphere

8.1.6 Cultures were passaged on days 6 and 12 post-inoculation. The negative cultures were handled first, followed by the test article cultures, and finally the positive controls.

8.1.7 Cell culture supernatants were collected from the negative control, test article, and positive control cultures on day 14. The supernatants were frozen at -60°C or below until tested.

8.2 PG4 S^+L^- Assay (30165)

8.2.1 The PG4 cells were set up 1 day prior to inoculation. The cells were set up in 6-well plates using media containing polybrene to increase viral uptake

8.2.2 On the day of inoculation, the cells were inoculated (0.5 mL per well) starting first with the assay negative controls plates, which were inoculated with Eagle's Minimum Essential Medium (EMEM). The co-cult test samples were then added, 0.5 mL per well at a 1:2 dilution, in triplicate, starting first with the negative control and followed by the test article. The co-cult positive samples were inoculated last onto the PG4 cells, at three dilutions (1:10, 1:100 and 1:1000)

8.2.3 The assay positive control was inoculated onto PG4 S^+L^- cells, utilizing a few dilutions (1:1000 and 1:10000) of the virus. Positive virus was an amphotropic virus

8.2.4 After incubation, the inoculum was removed, and the cells were fed with fresh media and incubated at 37±2°C in a 5±2% CO_2 atmosphere

8.2.5 On days 1 and 4 after the inoculation, the cultures were fed with fresh media. The negative cultures were fed first, followed by the test article samples, and finally the positive cultures.

8.2.6 The plates were read on day 5. All samples were read on the same day. The data was presented as focus forming units (FFU) per well and reported as the average FFU/mL for 3 wells

9.0 TEST ARTICLE PREPARATION

On May 22, 2007, AppTec, Inc. received 1 flask of "Human embryonic stem cell line H1 on a mouse embryonic feeder layer" at room temperature and designated for use in this assay. The test article was stored at 37±2°C / 5±2% CO_2 atmosphere until the assay was initiated.

10.0 POSITIVE CONTROLS

10.1 Co-Cultivation Controls

As a positive infectious retrovirus control, *Mus dunni* cells inoculated with an amphotropic retrovirus (A-MuLV) were run in parallel with the test article cells in the co-cultivation assay for 14 days. These were assayed in the PG4 S^+L^- assay on day 14 to confirm the replication of these viruses.

10.2 Controls for PG4 S^+L^- Assay

Known positive amphotropic murine leukemia virus was run along with the test samples in each assay as positive controls

11.0 NEGATIVE CONTROLS

11 1 Co-Cultivation Controls

Mus dunni cells alone served as the negative control These negative control cultures were run in parallel with the test article cells in the co-cultivation assay for 14 days

11 2 Controls for PG4 S^+L^- Assay

Negative (EMEM) samples were run along with the test samples in each assay as negative controls.

12.0 ASSAY VALIDITY

12 1 Validity Criteria for Co-Cultivation

The test was considered valid if supernatant samples derived from negative control co-cultivation cultures were negative for retroviral growth in the PG4 S^+L^-, and if the positive cultures inoculated with A-MuLV demonstrated a positive reaction in the PG4 S^+L^- assay

12 2 Validity Criteria for PG4 S^+L^- Assay

The test was considered valid if no foci were observed in the negative control and the positive control displayed viral-specific focus formation.

13.0 TEST EVALUATION

Co-cultivation of the test article cells with detector cells was considered positive if cell culture supernatants harvested after day 14 demonstrated a positive reaction in the PG4 S^+L^- assay

14.0 RESULTS

The test was valid The supernatant samples derived from negative control co-cultivation cultures were negative for retroviral growth in the PG4 S^+L^- assay, and the positive control co-cultivation cultures inoculated with A-MuLV demonstrated a positive reaction in the PG4 S^+L^- assay. No foci were observed in the negative assay control for the PG4 S^+L^- assay, and the positive assay control displayed viral-specific focus formation

The test article supernatant from T_0 produced a negative PG4 S^+L^- result Following co-cultivation with *Mus dunni* cells, the test article supernatants from post-passage 2 produced a negative PG4 S^+L^- result

TABLE 1: Observation of PG4 S^+L^- - Assay

	Culture Inoculum	Time	FFU/mL
Co-Cultivation Samples	**Accession # 07-001215[1] (diluted 1:2)**	**T_0**	**ND**
	Accession # 07-001215 (diluted 1:2)	**PP2**	**ND**
	Negative control[2] (diluted 1:2)	T_0	ND
	Negative control (diluted 1:2)	PP2	ND
	Positive control (A-MuLV)[3] (diluted 1:10)	PP2	TNTC
	Positive control (A-MuLV)[3] (diluted 1:100)	PP2	TNTC
	Positive control (A-MuLV)[3] (diluted 1:1000)	PP2	TNTC
PG4 S^+L^- - Assay Controls	Negative control (EMEM)	NA	ND
	High positive control (A-MuLV) (diluted 1:1000)	NA	TNTC
	Low positive control (A-MuLV) (diluted 1:10000)	NA	TNTC

Legend:

T_0	-	Time 0
PP2	-	Post passage 2
NA	-	Not applicable
ND	-	None detected
TNTC	-	Too numerous to count

[1] Supernatant collected from initial test article cultures used to prepare cultures for this assay.

[2] Controls prepared from supernatant taken from fresh *M. dunni* cultures used to prepare cultures for assay

[3] Stock virus used to initiate positive control in co-cultivation assay

NOTE: While not all significant figures were documented in the table, during calculation the numbers were not rounded until the final operation to determine the FFU/mL.

15.0 CONCLUSION

No evidence of xenotropic, amphotropic, or MCF MuLV retroviral contamination was detected in the test article

16.0 STATISTICAL DATA ANALYSIS

Statistical analysis of the data was not required.

17.0 DEVIATIONS / AMENDMENTS

No deviations from the protocol were encountered during the conduct of this study.

No amendments to the protocol were generated.

18.0 RECORD RETENTION

The testing facility will retain all records involving the study for seven (7) years including, but not limited to: the signed testing protocol with all amendments, any written communication concerning the conduct of the study, test substance accountability record, raw data worksheets, and an official copy of the final study report.

19.0 REFERENCES

1 Lander, MR, and Chattopadhyay, SK, (1984) "A *Mus Dunni* Cell Line That Lacks Sequences Closely Related to Endogenous Murine Leukemia Viruses and Can Be Infected by Ecotropic, Amphotropic, Xenotropic, and Mink Cell Focus-Forming Viruses." *J. Virol.* **52**: 695-698.

2 Morse III, HC, and Hartley, JW, (1986) "Murine Leukemia Viruses," in Viral and Mycoplasmal Infections of Laboratory Rodents. Academic Press, Orlando, FL. pp 349-388.

3 Kuta, A. "Presentation to the Vaccine Committee by the FDA" (October, 1993).

4 "Supplemental Guidance on Testing for Replication Competent Retrovirus in Retroviral Vector Based Gene Therapy Products and During Follow-up of Patients in Clinical Trials Using Retroviral Vectors," FDA/CBER (October 2000).

COVANCE
THE DEVELOPMENT SERVICES COMPANY

RETURN OF DATA TO SPONSOR

STUDY NUMBER(S): 2823/001 + 002

DELIVERY ADDRESS:

DATE OF DESPATCH: 04th August 2008

TYPE OF DATA TO BE TRANSFERRED:	NUMBER OF BOXES
Documents	2

1287a (May 2007)

COVANCE
THE DEVELOPMENT SERVICES COMPANY

RECORD OF DATA ACCEPTANCE BY SPONSOR

STUDY NUMBER(S): 2823/001+002

The data detailed in Page 1 have been transferred following instructions received from the Study Sponsor/Representative.

The Study Sponsor/Representative accepts total responsibility for the security and completeness of the records transferred, except as notified in writing at the time of transfer.

Transfer approved on behalf of Covance Management:

Name:

Position in Company: Archivist/Manager

Date: 31st July 2008

Verified by:

Position in Company: Records Officer

Date: 31st July 2008

Data accepted on behalf of Sponsor by:*

Name: _____

Signature: _____

Position in Company: _____

Date: _____

*Please sign and return one copy to Central Scientific Records, Covance Laboratories Ltd., [redacted] HG3 1PY. United Kingdom.

1287b (May 2007)

COVANCE
THE DEVELOPMENT SERVICES COMPANY

RECORD OF DATA ACCEPTANCE BY SPONSOR

STUDY NUMBER(S): 2823/001+002

The data detailed in Page 1 have been transferred following instructions received from the Study Sponsor/Representative.

The Study Sponsor/Representative accepts total responsibility for the security and completeness of the records transferred, except as notified in writing at the time of transfer.

Transfer approved on behalf of Covance Management:

Name:

Position in Company: Archivist/Manager

Date: 31st July 2008

Verified by:

Position in Company: Records Officer

Date: 31st July 2008

Data accepted on behalf of Sponsor by:*

Name: _____

Signature: _____

Position in Company: _____

Date: _____

*Please sign and return one copy to Central Scientific Records, Covance Laboratories Ltd., [redacted] HG3 1PY. United Kingdom.

1287b (May 2007)

Sponsor Information Listings

Covance Study Number 2823/001	Test Article: H1-MCB.1	
Covance Study Director:	Sponsor's Representative:	Study Type: In Vitro Adventitious

Study Title: In vitro evaluation of adventitious viruses in cell cultures - 28 day assay

Report Date: 02/10/2007

Archival Expiry Date: 01/10/2008

Please tick only one ( ) for each box

Box Number: 90118611 **( ) Retain (✓) Return ( ) Destroy**

Type of Data: Archive Confirmation Form
Final Report
Protocol File
Study File
eNotes

Total Number of boxes for this study: 1

Please complete and return this form to Covance Laboratories Ltd. Central Scientific Records, Otley Road, Harrogate, North Yorkshire. United Kingdom. HG3 1PY

Signed..... ..... Date.....7/28/08.....

Print Name.... ..... Position.....Test Directo

Covance Study Number 2823/002	Test Article: H9-MCB.1	
Covance Study Director: [redacted]	Sponsor's Representative: [redacted]	Study Type: In vitro Adventitious

Study Title: In vitro evaluation of adventitious viruses in cell cultures - 28 day assay

Report Date: 02/10/2007

Archival Expiry Date: 01/10/2008

Please tick only one ( ) for each box

Box Number: 90118612 **( ) Retain (✓) Return ( ) Destroy**

Type of Data: Archive Confirmation Form
Final Report
Protocol File
Study File
eNotes

Total Number of boxes for this study: 1

Please complete and return this form to Covance Laboratories Ltd. Central Scientific Records, [redacted] United Kingdom. HG3 1PY

Signed... ..... Date.....7/28/08.....

Print Name..... Position..... Tech Director

18/12/2007

Data Index Listings

Study Number: 2823/001

Study Title: In vitro evaluation of adventitious viruses in cell cultures - 28 day assay

Contact Name: **Study Director**

Study Type: In Vitro Adventitious **Test Article:** H1-MCB.1

Report Date: 02/10/2007

Box Number:	**90118611**	Type of Data:	Archive Confirmation Form Final Report Protocol File Study File eNotes

Total Number of boxes for this study: 1

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Archival Confirmation

Study Number:	2823-001	Study Director/Manager:
Department:	Biotechnology	
Study Title:	*In Vitro* Evaluation of Adventitious Viruses in Cell Cultures – 28 day assay	

Please complete one of the following, and enter any additional relevant information:-

☑ I hereby confirm that this study has been finalised. The protocol and all data including E notes for the above study are now lodged in Central Scientific Records (CSR)

☐** I hereby confirm that the protocol and all data for this study, for which a formal claim of FDA/EPA GLP compliance is to be made, has been submitted to CSR.

☐ I hereby confirm that the above study has been cancelled/aborted, and no final report is to be issued. I confirm that the necessary amendments have been made to the protocol. The protocol including E notes and any data generated during the study are now lodged in CSR, and the archive period should commence from the following date

☐* I hereby confirm that the above study was a non-regulatory study, therefore no final report was issued. All study data, protocol ,E notes and letter report (delete if not applicable) is now lodged in CSR and the archiving period should commence from the following date

☐* I hereby confirm that the above study was a non-GLP study and that all reports, protocol and all study data including E notes are now lodged in CSR. The archiving period should commence from the following date

☐* I hereby confirm that the study did not commence and an unsigned protocol and/or study correspondence including E notes are lodged in CSR, and this may now be destroyed.

☐* If any of these options are marked then the Study Director/Manager must send a copy of this form to QA

☐** Confirmed by CSR dated/Signature .....

Additional Information:

Study Director/Manager / Head of Department — 12/Dec/07 Date

N.B. - This form must be signed by the Study Director/Manager, and lodged with CSR as soon as one of the above criteria has been met, but not later than 3 months after issue of the final report. Where the Study Director/Manager has left the employment of Covance, the Head of Department should sign.

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Archive Summary

Study Number 2823-001		Responsible Person	Department Biotechnology
Item	**Type**	**Comment**	**Packet Bar Code**
1.	Protocol File	Protocol Reading List Client Protocol	00140653
2.	Study File	TAD Working Documents	00140654

Lodged By	**Accepted By**
Signature	**Signature**
Printed Name	**Printed Name**
Date	**Date**

Box 90118611.

Final Report

Study Title	*In Vitro* Evaluation of Adventitious Viruses in Cell Cultures – 28 day assay
Test Article	H1-MCB.1
Author	
Test Facility	Covance Laboratories Ltd
Sponsor Representative	
Sponsor	
Covance Study Number	2823/001
Covance Report Number	2823/001-D5141
Report Issued	October 2007
Page Number	1 of 28

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STUDY DIRECTOR AUTHENTICATION AND GLP COMPLIANCE STATEMENT

In Vitro Evaluation of Adventitious Viruses in Cell Cultures – 28 day assay

I, the undersigned, hereby declare that the work was performed by me or under my supervision and that the findings provide a true and accurate record of the results obtained.

The study was performed in accordance with the agreed Protocol and with Covance Laboratories Limited Standard Operating Procedures, unless otherwise stated, and the study objectives were achieved.

The study was conducted in compliance with:

The United Kingdom Statutory Instrument 1999 No.3106, the Good Laboratory Practice Regulations 1999, as amended by the Good Laboratory Practice (Codification Amendments Etc.) Regulations 2004.

The Organisation for Economic Co-operation and Development (OECD) Principles on Good Laboratory Practice (revised 1997, issued January 1998). ENV/MC/CHEM(98)17.

02/Oct/07
Date

Study Director

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QUALITY ASSURANCE STATEMENT

In Vitro Evaluation of Adventitious Viruses in Cell Cultures – 28 day assay

This study has been reviewed by the Quality Assurance Unit of Covance Laboratories Ltd. and the report accurately reflects the raw data. The following inspections were conducted and findings reported to the study director (SD) and associated management.

Critical procedures, which are performed routinely in an operational area, may be audited as part of a "process" inspection programme. This can be in addition to phases scheduled on an individual study basis. Selected process inspections conducted and considered applicable to this study are included below.

In addition to the inspection programmes detailed below, a facility inspection programme is also operated. Details of this programme, which covers all areas of the facility annually (at a minimum), are set out in standard operating procedures.

Inspection Dates		Phase	Date Reported to SD and SD Management
From	To		
11 Jun 2007	11 Jun 2007	Protocol Review	11 Jun 2007
10 Aug 2007	10 Aug 2007	Draft Report and Data Review	10 Aug 2007
02 Oct 2007	02 Oct 2007	Final Report Review	02 Oct 2007

Process

Inspection Dates		Phase	Date Reported to SD and SD Management
From	To		
04 Jul 2007	04 Jul 2007	Test Article Receipt	04 Jul 2007

2 Oct 07
Date

Quality Assurance Unit

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RESPONSIBLE PERSONNEL

In Vitro Evaluation of Adventitious Viruses in Cell Cultures – 28 day assay

The following personnel were responsible for key elements of the study:

Study Director:

Study Supervisor:

STUDY SCHEDULE

The study schedule was as follows:

Study initiation date:	6th June 2007 (Date Study Director signed Client Protocol).
Assay initiation date:	6th June 2007 (Date of the first study specific data capture).
Assay completion date:	10th July 2007 (Date of final data capture).
Study completion date:	Date Study Director signed Final Report.

ARCHIVE STATEMENT

In Vitro Evaluation of Adventitious Viruses in Cell Cultures – 28 day assay

All primary data, or authenticated copies thereof, specimens and the Final Report will be retained in the Covance Laboratories Limited archives for one year after issue of the Final Report. At this time, the Sponsor will be contacted to determine whether the data should be returned, retained or destroyed on their behalf. Sponsors will be notified of the financial implications of each of these options at that time.

Specimens or samples requiring frozen storage at Covance are specifically excluded from the above. These will be retained for as long as the material permits further evaluation, up to three months after issue of the Draft Report. At this time, the Sponsor will be contacted to determine whether samples should be returned, retained or destroyed on their behalf. Any financial implications of these options will also be notified at this time. Samples will not be destroyed without prior approval of the Study Director.

CONTENTS

SUMMARY

Four indicator cell lines (Vero, MRC-5, HeLa and NIH 3T3) were inoculated with either negative control (diluent), positive control virus or test article and incubated for 28 days. All positive controls turned positive for cytopathic effect (CPE). A fresh positive control for the MRC-5 cell line was set up on day 23 and this was positive for haemadsorption, as was the original MRC-5 positive control. All test article and negative control inoculated cells were negative for CPE and haemadsorption.

INTRODUCTION AND OBJECTIVE

The objective of this study was to determine the viral status of the test article. The assay was to detect the presence of viruses that induce CPE in culture cells; furthermore, the assay was to detect viruses capable of inducing haemadsorption.

MATERIALS

Protocol Adherence

The study described in this report was carried out according to the agreed Client Protocol, see Annex for details. Minor deviations, which are deemed not to have affected the study, are presented in the Appendix.

Test Article

The test article was received at Covance Laboratories Ltd on 29th March 2007 in two 15 ml centrifuge tubes each containing approximately 11 mls of a red/pink frozen material. The sample was received on dry ice and stored according to Sponsor instructions until required for the assay.

Identification:	H1-MCB.1
Source:	Sponsor.
Details on Test Article Vessel:	Covance 10 ml @ $1x10^6$ c/ml MCB.A.H1p30. 24 JAN07. DF

Appearance:	Red/pink frozen material.
Description:	Cell suspension.
Storage conditions:	< – 70°C.
Sterility check performed:	No.

This study to determine the presence of extraneous agents was conducted to define the purity of the test substance therefore, information regarding the purity of the test article was not available at study initiation. Stability of the test article was not considered relevant, as the objective of the study was to test for extraneous agents (adventitious viruses) that may be present in the test material.

Test Article Preparation

Prior to the assay starting, a cell lysate was prepared by freeze-thawing the test article three times in liquid nitrogen and a waterbath set at 37°C. The test article was then clarified by centrifugation.

TEST SYSTEM

Positive control virus:	*Parainfluenza 3* (PI3) strain SF-4 used at approximately $1x10^4$ $TCID_{50}$/ml (control for Vero, HeLa and MRC-5 cells). *Minute virus of mice* (MVM) used at approximately $1x10^4$ $TCID_{50}$/ml (control for CPE on NIH 3T3)
Source:	Maintained as laboratory stocks, original stocks supplied by ATCC.
Negative control (virus diluent):	Minimal essential medium + 5% tryptose phosphate broth.
Source:	Minimum essential medium. Tryptose phosphate broth. Gentamycin. Fungizone. Supplied by Invitrogen.

Indicator cell lines:	MRC-5. Vero. HeLa. NIH 3T3.
Source:	Maintained as laboratory stocks, original stocks supplied by ATCC or ECACC.
Growth medium:	Minimal essential medium containing Earles salts, non-essential amino acids plus 10% foetal calf serum. Minimum essential medium containing Earles Salts, non-essential amino acids plus 5% foetal calf serum for the re-feed.
Source:	Minimum essential medium. Non-essential amino acids. Gentamycin. Fungizone. Supplied by Invitrogen. Foetal calf serum. Supplied by PAA.

EXPERIMENTAL PROCEDURES AND DATA ANALYSIS

The experimental procedures were performed as outlined in the Client Protocol, see Annex for details. Minor deviations deemed not to have affected the study are presented in the Appendix.

The assay acceptance and evaluation criteria as detailed in the Client Protocol were achieved, see Annex for details.

RESULTS

Four indicator cell lines (Vero, MRC-5, HeLa and NIH 3T3) were inoculated with either negative control (diluent), positive control virus, test article or test article spiked with positive control and incubated for 28 days. MRC-5 cells that were negative for CPE were refed, and Vero, HeLa and NIH 3T3 cells that were negative for CPE were subcultured on day 7. On day 13 and on day 20 the NIH 3T3 cells were looking unhealthy so were refed to revive them. On day 14 and day 21 all cells that were negative for CPE were subcultured. They were observed for CPE and haemadsorption. Positive controls and spiked test article-inoculated cells for Vero and HeLa cells were positive for CPE by day 7. The positive control and spiked test article-inoculated cells for MRC-5 and NIH 3T3 cells were positive by day 28. A fresh positive control for the MRC-5 cell line was set up on day 23 and this was positive for haemadsorption with Guinea Pig, Human O and Adult Chicken blood at 7.6°C and 24.0°C. All test article and negative control-inoculated cells were negative for CPE and haemadsorption.

TABLES

Table 1: Day 14 and 28 Observation for Cytopathic Effect using Indicator Cells Inoculated with Test Article, Spike Test Article, Positive and Negative Controls

Sample	Indicator cell lines			
	Vero	MRC-5	HeLa	NIH 3T3
Indicator Assay First 14-Day Period Observations				
Negative Control	–	–	–	–*[1]
Test Article	–	–	–	–*[1]
Spiked Test Article	+	–*	+	–*[1]
Positive Control	+	–*	+	–*[1]
Indicator Assay Second 14-Day Period Observations				
Negative Control	–	–	–*[4]	–*[1]*[4]
Test Article	–	–*[2]	–*[4]	–*[1]*[4]
Spiked Test Article	N/A	+*[3]	N/A	+*[1]
Positive Control	N/A	+*[5]	N/A	+*[1]

\+ = Some or all flasks exhibited CPE.
– = Flasks did not exhibit CPE (normal morphology observed).
N/A = Not applicable as cells were discarded in first 14 days
* = Some vacuolation observed but not positive for CPE
*[1] = Cells were very overgrown and starting to die so were refed on day 13 and day 20.
*[2] = Some rounded cells observed on day 17 due to overgrowth and not CPE
*[3] = Early signs of CPE observed, which was confirmed as viral in haemadsorption assay (Table 2)
*[4] = Floating cells observed due to overgrowth
*[5] = Both original and fresh positive control (for haemadsorption assay) turned positive for CPE

Table 2: Day 28 Observation for Haemadsorption using Indicator Cells Inoculated with Test Article, Spiked Test Article, Positive and Negative Controls

Sample	Indicator cell lines			
	Vero	MRC-5	HeLa	NIH 3T3
	1-10°C Incubation			
Negative Control	–*[1]	–	–	–
Test Article	–*[1]	–	–	–
Spiked Test Article	N/A	+	N/A	N/A
Positive Control	N/A	+	N/A	N/A
Fresh Positive Control	N/A	+*	N/A	N/A
	37 ± 1°C Incubation			
Negative Control	–*[1]	–	–	–
Test Article	–*[1]	–	–	–
Spiked Test Article	N/A	+	N/A	N/A
Positive Control	N/A	+	N/A	N/A
Fresh Positive Control	N/A	+*	N/A	N/A

All Indicator cell lines were tested with a mixture of Adult Chicken blood, Guinea Pig blood and Human O blood.
+ = Haemadsorption observed.
– = No haemadsorption observed.
N/A = Not applicable.
* = Two fresh positive controls were set up, one inoculated with $1x10^4$ $TCID_{50}$/ml and one with $1x10^5$ $TCID_{50}$/ml, both were positive for haemadsorption.
*[1] = Some non-specific binding observed.

CONCLUSION

The test article (H1-MCB.1) was assessed for presence of adventitious viruses that are capable of causing cytopathic effects or haemadsorption within this system. The results show that no evidence of viral contamination was observed in the test article.

APPENDIX

Minor Deviations from the Protocol

1. DMEM was used to culture the 3T3 cell line during this study. The Protocol states MEM should be used, but DMEM is the preferred medium for this cell line. This deviation should improve the growth of the cells and would therefore not impact on the outcome of the study.

2. The NIH 3T3 cells were refed on day 13 and day 20 and then subcultured on day 14 and day 21. The refeed is in deviation to the Protocol but was necessary has cells had overgrown and the media had changed colour. This is a minor deviation to the Protocol that would not affect the outcome of the study.

3. The fresh positive control for the MRC-5 cell line was set up on day 23 instead of day 14 as stated in the Protocol. This is a minor deviation to the Protocol that did not affect the outcome of the study as the cells were positive for haemadsorption on day 28.

4. Medium containing 10% FCS was used for the subculture of cells on day 21. This is a deviation to the Protocol that states 5% FCS should be used following inoculation. This is considered a minor deviation to the Protocol that did not affect the outcome of the study as the cells were at a suitable concentration of the haemadsorption on day 28.

ANNEX

The Annex consists of 13 pages, including this one, and includes:

- Client Protocol (12 pages)

CLIENT PROTOCOL

Procedure Number	49001
Version Number	00
Supersedes	N/A
Study Title	In Vitro Evaluation of Adventitious Viruses in Cell Cultures – 28 day assay
Test Facility	Covance Laboratories Ltd.,

Protocol Produced on: 29 May 2007

Prepared at Covance Laboratories by

29/May/07
Date

INTRODUCTION

Rationale

The cell lines utilised in this assay are sensitive to a wide variety of viruses. The end-points utilised, cytopathic effect (CPE) and haemadsorption with three types of erythrocytes, provide a series of methods for evaluating the viral status of the test article cells [1, 2 & 3].

Objective

The objective of this assay is to evaluate the adventitious viral status of the test article.

TEST ARTICLE

The test article will be supplied by the Sponsor and all relevant information completed on the Test Article Safety & Pre-Study Questionnaire.

This study for the presence of extraneous agents will be conducted to define the purity of the test substance; therefore, information regarding the purity of the test article is not available at study initiation. Stability of the test article is not considered relevant, as the objective of this study is to test for extraneous agents (adventitious viruses) that may be present in the test material.

TEST SYSTEM

Positive control virus:	*Parainfluenza type 3* (PI3) strain SF-4 used at approximately $1x10^4$ $TCID_{50}$/ml (control for Vero, HeLa and MRC-5 cells). *MVM virus* used at approximately $1x10^4$ $TCID_{50}$/ml (control for CPE on NIH 3T3).
Source:	Maintained as laboratory stocks, original stocks supplied by ATCC.
Negative control:	Minimal essential medium + 5% tryptose

(virus diluent)	phosphate broth.
Source:	Minimum essential medium. Tryptose phosphate broth. Gentamycin. Fungizone. Supplied by Invitrogen.
Indicator cell lines:	MRC-5. Vero. HeLa NIH 3T3.
Source:	Maintained as laboratory stocks, original MRC-5, Vero, HeLa and NIH 3T3 stocks supplied by ATCC or ECACC.
Growth medium:	Minimal essential medium containing Earles salts, non-essential amino acids plus 10% foetal calf serum for culture establishment. Minimum essential medium containing Earles Salts, non-essential amino acids plus 5% foetal calf serum will be used for growth of cultures post inoculation.
Source:	Minimum essential medium. Non-essential amino acids. Gentamycin. Fungizone. Supplied by Invitrogen. Foetal calf serum. Supplied by PAA.

EXPERIMENTAL PROCEDURES

Preparation of Test Article

The test article will be prepared according to standard methods. Where needed the inoculation volume and surface of indicator cells, as described below, may be adapted. Any alterations will be noted in study records

Adventitious Virus Assay

For each indicator cell line, the following will be carried out:

Indicator cells will be seeded into T 75 flasks (4 x T 75 flasks). A flask will be labelled as the test article, a flask as the positive control, a flask as the test article interference control and a flask as the negative control. All flasks will be labelled with the cell type, the study number, and any other relevant information. The flasks will be incubated at 37 ± 1°C in a humidified 5% CO_2 in air incubator until required for assay.

For each indicator cell type, the medium will be aspirated from sub-confluent cultures and the cell sheets will be washed with a balanced salt solution. The final wash will be aspirated from the wells and 5ml of test article will be inoculated into a flask of each indicator cell line. To determine if the test article interferes with the assays ability to detect the relevant viruses, 5ml of test article spiked with the relevant positive control at an appropriate concentration will be inoculated into a flask of each indicator cell line. Appropriate positive and negative controls will be similarly inoculated into a flask of each indicator cell line. Flasks will be incubated at 37 ± 1°C for 60-90 minutes. The inoculum will be aspirated from the flasks and cells washed in a balanced salt solution, then refed with the appropriate growth medium. Flasks will be incubated and observed for cytopathic effect over 14 days. Each culture will be refed or subcultured on approximately day 7 with the appropriate growth medium.

If the test article inoculated indicator cells give a negative result on day 14, the test article and any other cultures negative for CPE will be split and seeded appropriately into fresh T75 flask. If positive controls turn positive they will be discarded. A fresh positive control will also be set up for the MRC-5 cell line and inoculated with the appropriate positive control virus. These flasks will then be incubated and observed for cytopathic effect over a further 14 days. Each culture will be refed or subcultured on approximately day 21 with the appropriate growth medium If the positive control shows CPE too early a fresh positive control may be set up for the MRC-5 cell line inoculated with the appropriate positive control virus. On day 28, a haemadsorption assay will be

carried out on the flasks inoculated with test article, the flasks inoculated with negative control and the MRC-5 flask inoculated with positive control.

For each indicator cell type, the haemadsorption assay will be carried out as follows:

The cell sheets will be washed with Dulbecco's Phosphate Buffered Saline and after aspirating the final wash, a mix of adult chicken, guinea pig and human type O blood will be added to each flask. Firstly, the flasks will be placed in the refrigerator for 30 ± 5 minutes and then incubated at room temperature for 30 ± 5 minutes. At the end of each incubation period the cell sheets will be observed for haemadsorption and the results recorded.

All culture vessels and plates will be discarded by the end of the experiment.

DATA ANALYSIS

Assay Acceptance Criteria

The assay will be considered acceptable if:

1. The positive control and test article spiked with positive control viruses cause CPE in all the indicator cells used.

2. The positive control inoculated cells cause haemadsorption in the indicator cells used.

3. The negative control inoculated indicator cells show normal morphology.

4. The negative inoculated indicator cells show no evidence of haemadsorption.

Evaluation Criteria

1. The assay will be considered as positive if any test article inoculated indicator cells or test article cells show a cytopathic effect or haemadsorption with any of the blood types assayed.

2. The test article will be considered to have interfered with the assay for the detection of adventitious viruses if CPE is not detected in all of the indicator cell lines inoculated with test article spiked with the appropriate positive control.

GLP COMPLIANCE

Following completion of the study a draft report will be issued. Client comments should be supplied for inclusion into a final document within six weeks of receipt of the draft document. If no client comments are received within six weeks of issue, the report will be finalised. Any further changes after this time will be addressed as an amended final report which may result in additional costs.

The study will be performed in compliance with:

United Kingdom Statutory Instrument 1999 No. 3106, The Good Laboratory Practice Regulations 1999, as amended by the Good Laboratory Practice (Codification Amendments Etc.) Regulations 2004.

OECD Principles on Good Laboratory Practice (revised 1997, Issued Jan 1998) ENV/MC/CHEM(98)17

All procedures will be performed in accordance with Covance Laboratories standard operating procedures (SOPs). The study will be subject to Quality Assurance evaluation by the Covance Laboratories Quality Assurance Department (QAD) in accordance with SOPs.

REFERENCES

[1] Jacobs J P, Magrath D I, Garrett A J and Schild G C (1981) Guidelines for the acceptability, management, and testing of serially propagated human diploid cells for the production of live virus vaccines for use in man. J Biol Standard *9*, 331-342.

[2] Schmidt N J (1979) Cell culture techniques for diagnostic virology. In: Diagnostic procedures for viral, rickettsial and chlamydial infections 5th edition. E H Lennette and N J Schmidt, editors. American Public Health Association, Washington, DC.

[3] Poiley JA (1990) Methods for the detection of adventitious viruses in cell culture used in the production of biotechnology products. In: Large-scale mammalian cell culture technology. (AS Lubiniecki, ed) Marcel Dekker Inc.

ARCHIVE STATEMENT

All primary data, or authenticated copies thereof, specimens and the Final Report will be retained in the Covance Laboratories Limited archives for one year after issue of the Final Report. At this time, the Sponsor will be contacted to determine whether the data should be returned, retained or destroyed on their behalf. Sponsors will be notified of the financial implications of each of these options at that time.

Specimens or samples requiring frozen storage at Covance are specifically excluded from the above. These will be retained for as long as the material permits further evaluation, up to three months after issue of the Draft Report. At this time, the Sponsor will be contacted to determine whether samples should be returned, retained or destroyed on their behalf. Any financial implications of these options will also be notified at this time. Samples will not be destroyed without prior approval of the Study Director.

APPENDIX

Study Records

The study records will be prepared to contain the following information:

Client Protocol, file note(s)*, study related correspondence*, Test Article Safety and Pre-Study Questionnaire, Test article receipt and utilisation, metrology#, records for reagents and stock solutions#, Test article cell culture records#, work sheets, indicator cell culture records#, positive control culture records#.

* Where appropriate.
# Some records held centrally.

Draft Report

The Draft Report will be prepared to contain but not be limited to the following information:

- The identity of the test substance as specified by the Sponsor.
- Any unforeseen circumstances which may have affected the quality or integrity of the study.

The following items will be presented in the Draft Report:

- Summary.
- Results.
- Conclusion.
- The name and address of the testing facility and the dates, on which the study was initiated, assay initiation and assay completion.

The Protocol will be attached as an Annex to the Report detailing procedures, assay acceptance criteria, evaluation criteria and references. Minor deviation(s) from the Protocol (if applicable) will be presented in the Report.

Final Report

The Final Report will be issued following Quality Assurance Department (QAD) evaluation of the Draft Report. The report will include all details described above and will include but not be limited to the following additions:

- Signed Quality Assurance statement.

- The signature of the Study Director for date of study completion and authentication of the Report.

PROTOCOL REVISION SUMMARY

Protocol Number: 49001

Protocol Title: In Vitro Evaluation of Adventitious Viruses in Cell Cultures – 28 day assay

Version Number	Revision Description	Authorisation Date
00	First issue	29 May 2007

TEST ARTICLE DETAILS AND STUDY AUTHORISATION

Test Article(s):
(As it should appear
on all documentation) HI-MCB.1

Experimental Phase

Start Date: 6th June 2007

End Date: 10th August 2007

6th June 2007
Date

Study Director

6th Jun 2007
Date

Covance Biotechnology Management

SPONSOR ACCEPTANCE SHEET

Sponsor Name

Title

Sponsors Company

Sponsor Address

Sponsor Contact Details

Telephone

e-mail

5/29/07
Date

Sponsor Approval

6/4/07
Date

Sponsor QA

This form must be completed, signed and returned to Biotechnology Services Administration, Covance Laboratories by post.

A faxed copy sent to, can be used for assay initiation.

THE DEVELOPMENT SERVICES COMPANY

Report Copying Request

Department:	**Biotechnology**	Cost Centre:	**D5141**
Report Number:	**2823-001**	Report Type:	**Final**
Study Director:		Extension:	**8335**
Study Co-ordinator:		Extension:	**8930**

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Letter Included ☒	Letter to Follow ☐	Letter N/A ☐	

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For Japanese clients a hard copy survey should be supplied to Admin Services and CLJ are copied into emails.

Please type an email address:

Please give a telephone number of the sponsor(s) receiving the report

Any other comments: **Please PDF and forward to**

PLEASE STATE REQUIRED DATE OF DESPATCH, ON/BY
(PLEASE GIVE SPECIFIC DATE IF NECESSARY):

COVANCE
THE DEVELOPMENT SERVICES COMPANY

Report Copying Request

Admin Use Only

Report Study Number	28231001	Report Type	Final

Report Checks	**1st Check**	**2nd Check**
Contents page checked/headers, date and pages counted	✓	
SC/SD informed of errors	—	
Errors logged on spreadsheet	—	
Checked CD for correct study number	—	
Report Scanned as PDF file onto CD	—	
CD Labels, Trays and covers made for PDF report	—	
Printing of report on Doc90/Fiery 2101	☑	
Checked for marks	CC	
Total pages counted/Checked pages are in numerical order	CC	
Bound/Unbound (If bound see reverse for type of binding)	L-W	
Dates and details added to CMS	☑	
Shipping labels and Shipping request form	☑	
Customer Satisfaction Survey/Email sent	☑	
PDF version of report sent electronically to sponsor with Disclaimer	—	
PDF version of report sent electronically to SD/SC	☑	

Any other comments:	
TOTAL AMOUNT OF COPIES 4	***TOTAL AMOUNT OF PAGES*** 28

PLEASE STAMP DATE OF DESPATCH HERE: 04 OCT 2007

OVERALL SIGN OFF CHECK ..... MJ

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Archive Packet Contents

Study Number	2823-002	**Responsible Person**		**Department**	Biotechnology
Item Number	1	**Type**	Protocol File		
Description				**Comments**	
Protocol Reading List Client Protocol					

Prepared by		**Date**	11-12-07	**Checked by**	**Date** 12/12/2007

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PROTOCOL READING LIST

Study Title: In vitro Evaluation of Adventitious Viruses in cell cultures -28day Assay.

Covance Study Number: 2823-002

Name	Definitive protocol read	Amendment number read	Signature/date
	✓	N/A	AD 12/6/07
	✓	NA	RL 18/6/07
	✓	N/A	DF 19.6.07
	✓	N/A	[illegible] 19/06/07
	✓	N/A	EAG 4/7/07
	✓	N/A	[illegible] 9/10/07
	✓	NA	[illegible] 11.07.07
	✓	NA	[illegible] 11.07.07

Page completion check by .....CH..... date .....27/7/07.....

CLIENT PROTOCOL

Procedure Number	49001
Version Number	00
Supersedes	N/A
Study Title	In Vitro Evaluation of Adventitious Viruses in Cell Cultures – 28 day assay
Test Facility	Covance Laboratories Ltd.,

Protocol Produced on: 29 May 2007

Prepared at Covance Laboratories by _____ Date 29/May/07

INTRODUCTION

Rationale

The cell lines utilised in this assay are sensitive to a wide variety of viruses. The end-points utilised, cytopathic effect (CPE) and haemadsorption with three types of erythrocytes, provide a series of methods for evaluating the viral status of the test article cells [1, 2 & 3].

Objective

The objective of this assay is to evaluate the adventitious viral status of the test article.

TEST ARTICLE

The test article will be supplied by the Sponsor and all relevant information completed on the Test Article Safety & Pre-Study Questionnaire.

This study for the presence of extraneous agents will be conducted to define the purity of the test substance; therefore, information regarding the purity of the test article is not available at study initiation. Stability of the test article is not considered relevant, as the objective of this study is to test for extraneous agents (adventitious viruses) that may be present in the test material.

TEST SYSTEM

Positive control virus:	*Parainfluenza type 3* (PI3) strain SF-4 used at approximately $1x10^4$ $TCID_{50}$/ml (control for Vero, HeLa and MRC-5 cells). *MVM virus* used at approximately $1x10^4$ $TCID_{50}$/ml (control for CPE on NIH 3T3).
Source:	Maintained as laboratory stocks, original stocks supplied by ATCC.
Negative control:	Minimal essential medium + 5% tryptose

(virus diluent)	phosphate broth.
Source:	Minimum essential medium. Tryptose phosphate broth. Gentamycin. Fungizone. Supplied by Invitrogen.
Indicator cell lines:	MRC-5. Vero. HeLa NIH 3T3.
Source:	Maintained as laboratory stocks, original MRC-5, Vero, HeLa and NIH 3T3 stocks supplied by ATCC or ECACC.
Growth medium:	Minimal essential medium containing Earles salts, non-essential amino acids plus 10% foetal calf serum for culture establishment. Minimum essential medium containing Earles Salts, non-essential amino acids plus 5% foetal calf serum will be used for growth of cultures post inoculation.
Source:	Minimum essential medium. Non-essential amino acids. Gentamycin. Fungizone. Supplied by Invitrogen. Foetal calf serum. Supplied by PAA.

EXPERIMENTAL PROCEDURES

Preparation of Test Article

The test article will be prepared according to standard methods. Where needed the inoculation volume and surface of indicator cells, as described below, may be adapted. Any alterations will be noted in study records

Adventitious Virus Assay

For each indicator cell line, the following will be carried out:

Indicator cells will be seeded into T 75 flasks (4 x T 75 flasks). A flask will be labelled as the test article, a flask as the positive control, a flask as the test article interference control and a flask as the negative control. All flasks will be labelled with the cell type, the study number, and any other relevant information. The flasks will be incubated at 37 ± 1°C in a humidified 5% CO_2 in air incubator until required for assay.

For each indicator cell type, the medium will be aspirated from sub-confluent cultures and the cell sheets will be washed with a balanced salt solution. The final wash will be aspirated from the wells and 5ml of test article will be inoculated into a flask of each indicator cell line. To determine if the test article interferes with the assays ability to detect the relevant viruses, 5ml of test article spiked with the relevant positive control at an appropriate concentration will be inoculated into a flask of each indicator cell line. Appropriate positive and negative controls will be similarly inoculated into a flask of each indicator cell line. Flasks will be incubated at 37 ± 1°C for 60-90 minutes. The inoculum will be aspirated from the flasks and cells washed in a balanced salt solution, then refed with the appropriate growth medium. Flasks will be incubated and observed for cytopathic effect over 14 days. Each culture will be refed or subcultured on approximately day 7 with the appropriate growth medium.

If the test article inoculated indicator cells give a negative result on day 14, the test article and any other cultures negative for CPE will be split and seeded appropriately into fresh T75 flask. If positive controls turn positive they will be discarded. A fresh positive control will also be set up for the MRC-5 cell line and inoculated with the appropriate positive control virus. These flasks will then be incubated and observed for cytopathic effect over a further 14 days. Each culture will be refed or subcultured on approximately day 21 with the appropriate growth medium If the positive control shows CPE too early a fresh positive control may be set up for the MRC-5 cell line inoculated with the appropriate positive control virus. On day 28, a haemadsorption assay will be

carried out on the flasks inoculated with test article, the flasks inoculated with negative control and the MRC-5 flask inoculated with positive control.

For each indicator cell type, the haemadsorption assay will be carried out as follows:

The cell sheets will be washed with Dulbecco's Phosphate Buffered Saline and after aspirating the final wash, a mix of adult chicken, guinea pig and human type O blood will be added to each flask. Firstly, the flasks will be placed in the refrigerator for 30 ± 5 minutes and then incubated at room temperature for 30 ± 5 minutes. At the end of each incubation period the cell sheets will be observed for haemadsorption and the results recorded.

All culture vessels and plates will be discarded by the end of the experiment.

DATA ANALYSIS

Assay Acceptance Criteria

The assay will be considered acceptable if:

1. The positive control and test article spiked with positive control viruses cause CPE in all the indicator cells used.

2. The positive control inoculated cells cause haemadsorption in the indicator cells used.

3. The negative control inoculated indicator cells show normal morphology.

4. The negative inoculated indicator cells show no evidence of haemadsorption.

Evaluation Criteria

1. The assay will be considered as positive if any test article inoculated indicator cells or test article cells show a cytopathic effect or haemadsorption with any of the blood types assayed.

2. The test article will be considered to have interfered with the assay for the detection of adventitious viruses if CPE is not detected in all of the indicator cell lines inoculated with test article spiked with the appropriate positive control.

GLP COMPLIANCE

Following completion of the study a draft report will be issued. Client comments should be supplied for inclusion into a final document within six weeks of receipt of the draft document. If no client comments are received within six weeks of issue, the report will be finalised. Any further changes after this time will be addressed as an amended final report which may result in additional costs.

The study will be performed in compliance with:

United Kingdom Statutory Instrument 1999 No. 3106, The Good Laboratory Practice Regulations 1999, as amended by the Good Laboratory Practice (Codification Amendments Etc.) Regulations 2004.

OECD Principles on Good Laboratory Practice (revised 1997, Issued Jan 1998) ENV/MC/CHEM(98)17

All procedures will be performed in accordance with Covance Laboratories standard operating procedures (SOPs). The study will be subject to Quality Assurance evaluation by the Covance Laboratories Quality Assurance Department (QAD) in accordance with SOPs.

REFERENCES

[1] Jacobs J P, Magrath D I, Garrett A J and Schild G C (1981) Guidelines for the acceptability, management, and testing of serially propagated human diploid cells for the production of live virus vaccines for use in man. J Biol Standard *9*, 331-342.

[2] Schmidt N J (1979) Cell culture techniques for diagnostic virology. In: Diagnostic procedures for viral, rickettsial and chlamydial infections 5th edition. E H Lennette and N J Schmidt, editors. American Public Health Association, Washington, DC.

[3] Poiley JA (1990) Methods for the detection of adventitious viruses in cell culture used in the production of biotechnology products. In: Large-scale mammalian cell culture technology. (AS Lubiniecki, ed) Marcel Dekker Inc.

ARCHIVE STATEMENT

All primary data, or authenticated copies thereof, specimens and the Final Report will be retained in the Covance Laboratories Limited archives for one year after issue of the Final Report. At this time, the Sponsor will be contacted to determine whether the data should be returned, retained or destroyed on their behalf. Sponsors will be notified of the financial implications of each of these options at that time.

Specimens or samples requiring frozen storage at Covance are specifically excluded from the above. These will be retained for as long as the material permits further evaluation, up to three months after issue of the Draft Report. At this time, the Sponsor will be contacted to determine whether samples should be returned, retained or destroyed on their behalf. Any financial implications of these options will also be notified at this time. Samples will not be destroyed without prior approval of the Study Director.

APPENDIX

Study Records

The study records will be prepared to contain the following information:

Client Protocol, file note(s)*, study related correspondence*, Test Article Safety and Pre-Study Questionnaire, Test article receipt and utilisation, metrology#, records for reagents and stock solutions#, Test article cell culture records#, work sheets, indicator cell culture records#, positive control culture records#.

* Where appropriate.
# Some records held centrally.

Draft Report

The Draft Report will be prepared to contain but not be limited to the following information:

- The identity of the test substance as specified by the Sponsor.
- Any unforeseen circumstances which may have affected the quality or integrity of the study.

The following items will be presented in the Draft Report:

- Summary.
- Results.
- Conclusion.
- The name and address of the testing facility and the dates, on which the study was initiated, assay initiation and assay completion.

The Protocol will be attached as an Annex to the Report detailing procedures, assay acceptance criteria, evaluation criteria and references. Minor deviation(s) from the Protocol (if applicable) will be presented in the Report.

Final Report

The Final Report will be issued following Quality Assurance Department (QAD) evaluation of the Draft Report. The report will include all details described above and will include but not be limited to the following additions:

- Signed Quality Assurance statement.

- The signature of the Study Director for date of study completion and authentication of the Report.

PROTOCOL REVISION SUMMARY

Protocol Number: 49001

Protocol Title: In Vitro Evaluation of Adventitious Viruses in Cell Cultures – 28 day assay

Version Number	Revision Description	Authorisation Date
00	First issue	29 May 2007

TEST ARTICLE DETAILS AND STUDY AUTHORISATION

Test Article(s):
(As it should appear
on all documentation) H9-MCB.1

Experimental Phase

Start Date: 6th June 2007

End Date: 10th August 2007

6/June/07.
Date

Study Director

6th June 2007
Date

Covance Biotechnology Management

SPONSOR ACCEPTANCE SHEET

Sponsor Name

Title

Sponsors Company

Sponsor Address

Sponsor Contact Details

Telephone

e-mail

5/29/07
Date

Sponsor Approval

6/4/07
Date

Sponsor QA

This form must be completed, signed and returned to Biotechnology Services Administration, Covance Laboratories by post.

A faxed copy sent to, +44 (0)1423 569595, can be used for assay initiation.

Log Out

eNotes User Manual Your password will expire in 13 days.

29 Jul 2008, 04:22 PM (GMT +1) v2.5.6

hide all exit

eNotes Id:	236057
Study Number:	2823-002
Descriptive Title:	MEdia usage
Status:	Archived
	Approved by on 13 Jul 2007, 03:42 PM (GMT +1)

Workflow (hide)

Created by:
Recipient:
Interim Approver:
Approver:

Post Approval:

Summary Information (hide)

Category:	Protocol Deviation
Subcategory:	Assay Criteria
Site:	Harrogate
Department / CC:	Biosafety / D5141

Communication

Previous:

13 Jul 2007, 03:42 PM (GMT +1)

Corrective Action: Documentation of deviation serves as the corrective action.

13 Jul 2007, 03:42 PM (GMT +1)

Impact: No impact to study integrity apparent at this time.

13 Jul 2007, 03:41 PM (GMT +1)

The cell confluence at day 28 was suitable for haemadsorption so this deviation did not affect the outcome of the study.

13 Jul 2007, 03:39 PM (GMT +1)

MEM/10 was used as opposed to MEM/5 for cell splits on day 21.

Attachments (hide)

[No Attachments]

Log Out

eNotes User Manual Your password will expire in 13 days.

29 Jul 2008, 04:22 PM (GMT +1) v2.5.6

hide all exit

eNotes Id:	232899
Study Number:	2823-002
Descriptive Title:	Refeed of 3T3 cells on Day 20
Status:	Archived
	Approved by on 02 Jul 2007, 03:08 PM (GMT +1)

Workflow (hide)

Created by:
Recipient:
Interim Approver:
Approver:

Post Approval:

Summary Information (hide)

Category:	Protocol Deviation
Subcategory:	Test Cells
Site:	Harrogate
Department / CC:	Biosafety / D5141

Communication

Previous:

02 Jul 2007, 03:08 PM (GMT +1)

Corrective Action: Documentation of deviation serves as the corrective action.

02 Jul 2007, 03:08 PM (GMT +1)

Impact: No impact to study integrity apparent at this time.

02 Jul 2007, 03:08 PM (GMT +1)

This colour change in the medium is believed to be due to overgrowth of the cells.

02 Jul 2007, 02:39 PM (GMT +1)

3T3 cells were refed with DMEM/5E as the media was yellow and the cells were looking unhealthy although a monolayer was still present.

Attachments (hide)

[No Attachments]

Log Out

eNotes User Manual Your password will expire in 13 days.

29 Jul 2008, 04:22 PM (GMT +1) v2.5.6

hide all exit

eNotes Id:	231388
Study Number:	2823-002
Descriptive Title:	day 14 positive control not set up
Status:	Archived
	Approved by on 26 Jun 2007, 11:57 AM (GMT +1)

Workflow (hide)

Created by:
Recipient:
Interim Approver:
Approver:

Post Approval:

Summary Information (hide)

Category:	Protocol Deviation
Subcategory:	Methodology and Specifications
Site:	Harrogate
Department / CC:	Biosafety / D5141

Communication

Previous:

26 Jun 2007, 11:57 AM (GMT +1)

Corrective Action: Documentation of deviation serves as the corrective action.

26 Jun 2007, 11:57 AM (GMT +1)

Impact: No impact to study integrity apparent at this time.

26 Jun 2007, 11:24 AM (GMT +1)

Please note that the day 14 fresh positive control was not set up on day 13 or inoculated on day 14 in error. However the original positive control flask is still in use with a good monolayer. A fresh positive control will be set up on approximatly day 21 AS 26 June 07

Attachments (hide)

[No Attachments]

Log Out

eNotes User Manual Your password will expire in 13 days.
29 Jul 2008, 04:22 PM (GMT +1) v2.5.6

hide all | exit

eNotes Id:	231137
Study Number:	2823-002
Descriptive Title:	3T3 cells were refed on day 13
Status:	Archived
	Approved by on 25 Jun 2007, 04:36 PM (GMT +1)

Workflow (hide)

Created by:
Recipient:
Interim Approver:
Approver:

Post Approval:

Summary Information (hide)

Category:	Protocol Deviation
Subcategory:	Methodology and Specifications
Site:	Harrogate
Department / CC:	Biosafety / D5141

Communication

Previous:

25 Jun 2007, 04:36 PM (GMT +1)

Corrective Action: Documentation of deviation serves as the corrective action.

25 Jun 2007, 04:36 PM (GMT +1)

Impact: No impact to study integrity apparent at this time.

25 Jun 2007, 04:35 PM (GMT +1)

On observing the cells there were a lot of floating cells and gaps in the monolayer. This was probably caused by the cells overgrowing over the weekend and starting to die and come off the surface of the flask. This overgrowth can cause the medium to turn yellow due to the metabolism of the cells.

25 Jun 2007, 03:54 PM (GMT +1)

3T3 cell media was yellow on day 13, cells were refed with fresh media.

Attachments (hide)

[No Attachments]

Log Out

eNotes User Manual Your password will expire in 13 days.

29 Jul 2008, 04:22 PM (GMT +1) v2.5.6

hide all exit

eNotes Id:	231132
Study Number:	2823-002
Descriptive Title:	DMEM/10 used as opposed to MEM for 3T3 cell line
Status:	Archived
	Approved by on 25 Jun 2007, 04:38 PM (GMT +1)

Workflow (hide)

Created by:
Recipient:
Interim Approver:
Approver:

Post Approval:

Summary Information (hide)

Category:	Protocol Deviation
Subcategory:	Reagents and Controls
Site:	Harrogate
Department / CC:	Biosafety / D5141

Communication

Previous:

25 Jun 2007, 04:38 PM (GMT +1)

Corrective Action: Documentation of deviation serves as the corrective action.

25 Jun 2007, 04:38 PM (GMT +1)

Impact: No impact to study integrity apparent at this time.

25 Jun 2007, 03:47 PM (GMT +1)

DMEM was used as the growth media for the 3T3 cell line as opposed to MEM as stated in the protocol as this is the preferential growth media for this cell line.

Attachments (hide)

[No Attachments]

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Archive Packet Contents

Study Number	2823-001	**Responsible Person**		**Department**	Biotechnology
Item Number	2	**Type**	Study File		
Description				**Comments**	
TAD Working Documents					

Prepared by		**Date**	11-12-07	**Checked by**	**Date** 12/12/2007

COVANCE
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J7004270

CLE Study No. 2823-001

BIOTECHNOLOGY DEPT.

Test Article Receipt Form

Test article name: H1-MCB.1

Identification on vessel: COVANCE 10 ml @ 1x10⁶ c/ml
MCB.A.H1p30. 24 JAN 07. DF

Date received: 20/3/07 Received by:

How received: By courier on dry ice

Physical description: 2x orange capped 15 ml centrifuge tubes containing approx. 11 ml frozen red/pink material.

Storage location: BS115 -80°C freezer (Box D) X1

Logging in checked by: Date: 26 March

Date used	Material used	Material remaining	By	CLE Study No.
06/06/07	All	used to create cell lysate (20ml) X2	AS	2823-001
12.6.7	ALL	NONE	DF	2823-001

X3

Remaining test article transferred to:
CLE Study number: By: Date:

Remaining test article disposed:
By Covance method: By: Date:

Remaining test article returned to Sponsor:
By: Date: How:
Returned to:

Comments:

X1 Moved to BS152 3rd shelf box 01 LHS AS05Jun07

X2 Entire sample from 2 tubes used to make cell lysate. Subsequent records refer to cell lysate usage AS06/06/07 cell lysate pooled into 1 tube following clarification AS06/06/07

X3 Cell lysate labelling: (2823-001 H1-MCB.1 TEST ARTICLE Cell lysate AS06/06/07) AS06/06/07

Abbreviated name:

Test Article Safety and Pre-Study Questionnaire Biotechnology Based Compounds

Confidential

To be completed by the Study Sponsor and returned to Covance Laboratories

Test Facility Covance Laboratories Ltd

Reception Telephone:
General Fax:
Business Development Fax:

This information is required to comply with UK Health & Safety regulations, IATA regulations (for transport), GLP compliance guidelines, the Animals (Scientific Procedures) Act 1986 and to assist with study design.

Covance Laboratories (Europe) will not accept contracts to conduct *in vivo* experiments on cosmetic products or substances intended to have primary use as an ingredient of a cosmetic product. Under Article 1 of Directive 76/768/EEC a cosmetic product is any substance or preparation intended to be placed in contact with the various external parts of the human body (epidermis, hair system, nails, lips) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance and/or correcting body odours and/or protecting them in good condition.

Please note: Where it is determined that insufficient information is provided to enable Covance Laboratories Ltd fulfil its legal obligations, the commencement of studies/compound development appraisal may be delayed until such time as adequate information is obtained.

Authorised by:
(please sign your name and type or print your company status underneath your signature)

Signature:	
Name and Position:	Quality Assurance Manager
Date:	2/21/01

Where options are given please tick (√) those required or delete those not applicable

1 COMPANY REFERENCE

Name and Address of Company (as it should appear on all documents)	Name and Address of person to whom all financial correspondence should be addressed (if different)
WiCell Research Institute	WiCell Accounts Payable

Name(s) of Study Monitor(s) and contact details			
Name		Name	
Telephone		Telephone	
Fax		Fax	
E-mail		E-mail	

2 TEST ARTICLE INFORMATION

Supplier's Name and Address (if different from above)			
Test article name as it should appear in all documents		H1-MCB.1	
Exact details as presented on the test article vessel		H1-MCB.1	
Batch/Lot Number	H1-MCB.1	Quantity Supplied	2
Appearance (e.g. solid, liquid, lyophilised powder)	liquid	Concentration	$10x10^6$ cells
Despatch Date		Vial size (cryopreserved cells)	15ml conical tube

Where options are given please tick (√) those required or delete those not applicable

3 TEST ARTICLE TYPE

Please indicate type of Test Article in boxes below				
Virus		Vaccine (specify)		
DNA plasmid		Peptide		
Protein		Cells	Cryopreserved	Yes, but no longer viable. Human Embryonic Stem Cells
			Live	
Oligonucleotide		Cell culture Supernatant		

4 STERILITY

Has compound been sterility checked	No-in progress
If known please specify bioburden	

5 STORAGE AND DISPOSAL

Storage temperature:							
Liquid nitrogen (<-150°C)		<-50°C	-70C freezer	<-10°C		1 to 10°C	
Storage conditions (please specify):							
Under Nitrogen		Desiccated		Other	-70C freezer	Other	
Expiry date (if stored under the above conditions) none							
Disposal of unused Test Article	Incinerate: Yes ☒ No ☐		Return to Sponsor: Yes ☐ No ☒		Identify Recipient of Returned Test Article:		

6 FORMULATION

Please indicate optimum formulation conditions:-

Vial composition (e.g. polypropylene, siliconised glass)	n/a
Diluent	n/a
Method of mixing if applicable	n/a
Method of sterilisation (e.g. 0.2µm filter)	n/a

Where options are given please tick (√) those required or delete those not applicable

7 HEALTH AND SAFETY

Please indicate known or suspected hazards. (Do not leave blanks, state "Not Known" if no data available). State LD_{50} and species if known, state "Not known" if this is the case.

Does the test article contain any chemical which may be hazardous, e.g. DMSO	Yes ☐ No ☒
If "yes", please give details Medium contains DMEM-F12, Knock out Serum Replacer, Amino Acids. None of these items are hazardous. Does not contain antibiotics or DMSO.	
Specific handling precautions (please state)	Standard PPE
Known antidote and/or First Aid procedures	Not known
Contact for Safety Information (Name & Telephone Number)	

A Certificate of Analysis confirming the identity and purity of the test article is a requirement of UK GLP, please indicate correct option below:

Certificate of Analysis:	Is attached	Will accompany sample	Will follow later	Is not available
		x		

Cell lines for GMP cell banking and cell banks for GMP storage will be required to meet minimum acceptance testing criteria before being handled/stored in GMP facilities. Requirements will be advised separately:

FOR VIRAL COMPOUNDS AND CELL LINES:

Has the test article been classified as a "Genetically Modified Organism" (EC Directive 90/219/EEC and 94/51/EC)	Yes ☐ No ☒
If "yes", please supply **full** Risk Assessment including whether the test article is classified as group 1 or group 2.	

* This information is required under the UK regulations governing the Control Of Substances Hazardous to Health (COSHH) and Genetically Modified Organisms (Contained Use) Regulations 1992.

8 KNOWN STABILITY OF FORMULATED TEST ARTICLE

Please provide details (vehicle, temperature, pH, duration etc).	

Where options are given please tick (√) those required or delete those not applicable

9 TRANSPORT INFORMATION

It may be that certain compounds are considered hazardous for transport by air. In order for Covance to fully comply with IATA Dangerous Goods Regulations (if applicable) the following information is required. Failure to supply relevant information may result in Covance being able to transport a compound by air.

Is the compound considered to be hazardous for shipping by air?	Yes ☐ No ✠
If Yes, please complete the following:	
Proper shipping name:	
UN Number:	
Hazard Class:	
Packing Group:	
Compound form:	
MSDS available:	

10 FOR CELL CULTURE SAMPLES

CULTURE CHARACTERISTICS

For cryopreserved cells	Please indicate in the relevant box whether cells grow as			
	A monolayer	x	A suspension culture	
	Expected viability	%		
For all cells	Preferred split ratio		Frequency of splitting (days)	

MEDIUM FOR CELL CULTURE

Name of medium:		Antibiotics and concentration:	
Please supply any other relevant information:			
Where a Specialist or Non-Standard Medium is required, please supply the following information			
Medium to be supplied by sponsor?	Yes ☐	No ☐	
If "yes" please give details of storage conditions and expiry date (last line of this table) If "no" give full details as requested below			
Name of medium:		Basal medium:	
Additives and concentration			
Antibiotics and concentration			
Storage conditions		Medium expiry date	

Where options are given please tick (√) those required or delete those not applicable

SUPERNATANT INFORMATION

Where a Cell Culture Supernatant is supplied for testing **please supply the following information**	
Age of culture from which the supernatant was taken (since last re-feed)	
Any other relevant information	
Has the supernatant been centrifuged?	Yes ☐ No ☐
If "yes", please give details	

11 REPORTING

Regulatory Authority (ies) to which the data may be submitted?		
REPORT FORMAT	Number of copies required	
	Draft Report	Final Report
Bound/Double Sided		1
Bound/Single Sided		
Unbound/Double Sided		
Unbound/Single Sided		1
Electronic (By E-mail)	1	1
Electronic (On CD)		

National Stem Cell Bank

Certificate of Analysis

Product Description	WA01 Master Cell Bank
Cell Line Provider	WiCell
MCB Lot Number	H1MCB.1
Date Vialed	20Nov06
Passage Number	P20
Culture Method	**SOP-CC-024A**
Cryopreservation Method	**SOP-CC-035A**

The following testing specifications have been met for the specified product lot:

Test Description	Test Method	Test Specification	Result
Post-Thaw Viable Cell Recovery	**SOP-CH-305A**	Viable cells recovered	Pass
Identity by STR	**SOP-CH-302A**	Positive identity	**Waiting for report**
HLA profile	**SOP-CH-303A**	Positive identity	**Waiting for report**
Identity by SNP	**SOP-CH-306A**	Positive identity	**Waiting for report**
Sterility - Direct transfer method with bacteriastasis & fungistasis	**SOP-CH-307A**	No contamination detected	**Waiting for report**
Mycoplasma - FDA PTC method	**SOP-CH-308A**	No contamination detected	**Waiting for report**
Karyotype by G-banding	**SOP-CH-003A**	Normal Karyotype	**Waiting for report**
Comparative Genome Hybridization	**SOP-CH-309A** **SOP-CH-310A**	Report Copy Number Variants	**Waiting for report**
Growth Characteristics	**SOP-CH-104A**	Report plating efficiency and doubling time	**Waiting for report**
Flow Cytometry for ESC Marker Expression	**SOP-CH-101A** **SOP-CH-102A** **SOP-CH-103A**	Report values Oct-4 > X%	**Waiting for report**
Gene Expression Profile	**SOP-CH-311A**	Report Values	**Waiting for report**
Bovine pathogens	**SOP-CH-312A**	No contamination detected	**Waiting for report**
Porcine pathogens	**SOP-CH-313A**	No contamination detected	**Waiting for report**
Murine Antibody Production (MAP)	**SOP-CH-314A**	No contamination detected	**Waiting for report**
In vitro adventitious virus	**SOP-CH-315A**	No contamination detected	**Waiting for report**

National Stem Cell Bank

Certificate of Analysis

In vivo adventitious virus	**SOP-CH-316A**	No contamination detected	**Waiting for report**
Retrovirus by thin section EM	**SOP-CH-317A**	No contamination detected	**Waiting for report**
Co-cultivation with Mus Dunni Cells and PG4 S+L- assay	**SOP-CH-319A**	No contamination detected	**Waiting for report**
HIV 1&2 by PCR	**SOP-CH-318A**	No contamination detected	**Waiting for report**
HTLV 1&2 by PCR	**SOP-CH-318A**	No contamination detected	**Waiting for report**
HBV by PCR	**SOP-CH-318A**	No contamination detected	**Waiting for report**
HCV by PCR	**SOP-CH-318A**	No contamination detected	**Waiting for report**
CMV by PCR	**SOP-CH-318A**	No contamination detected	**Waiting for report**
EBV by PCR	**SOP-CH-318A**	No contamination detected	**Waiting for report**
HHV-6 by PCR	**SOP-CH-318A**	No contamination detected	**Waiting for report**
HHV-7 by PCR	**SOP-CH-318A**	No contamination detected	**Waiting for report**
HHV-8 by PCR	**SOP-CH-318A**	No contamination detected	**Waiting for report**
HP B19 by PCR	**SOP-CH-318A**	No contamination detected	**Waiting for report**

NSCB Quality Assurance: _____ Date: 3/6/07

Preparation Test article cell lysate.

Test article labelled:
COVANCE 10ML1@ 1x10⁶cfu1 HCB.A.H1p30.24JAN07.DF
CNB 2x15ML1 centrifuge tubes with identical labelling AS 06/06/07

I confirm that the Test Article used in this assay corresponds to the Test Article Receipt Form and Test Article Safety and Pre-Study Questionnaire supplied by the sponsor.

Study Director: CH **Date:** 6/6/07

Document Authorised CH 6/6/07

Preparation of Test Article

Equipment used throughout assay

Equipment	ID Number	
Safety Cabinet	BS204	☐ N/A
Waterbath	BS 219	☐ N/A
Centrifuge	BS236	☐ N/A
Micropipette		☑ N/A
Pipetaid	BS 257	☐ N/A
Other (freezer)	BS 152	☒ N/A ① AS06/06/07
Other ( )		☑ N/A
Initials/Date	AS /06/06/07	

Steps – preparation of cell lysate	Task complete
Was removed from freezer BS 152 and thawed at 37°C.	☑
Freeze –thaw test article three times in liquid nitrogen and 37°C waterbath, making sure TA is completely frozen and completely thawed each time. Complete the table below	☑
Clarify the resulting lysate by centrifugation at 150 x "g" for 10 minutes at approx. 20°C	☑
Test article relabelled 2823-001 H1-MCB.1 TEST ARTICLE CELL LYSATE AS06/06/07	☑
X1 Aliquot the lysate and store deep frozen or keep on ice and use within 2 hours	☑ (freeze)
Initials/Date	AS /06/06/07

	1st Thaw	2nd Thaw	3rd Thaw
Thaw start time	16:01	16:31	17:20
Thaw end time	16:28	17:15	18:00
Initials/date	AS /06/06/07		

X1 Clarified lysate pooled into single volume, approx 20ml
AS06/06/07

Comments

In Vitro Evaluation of Adventitious Viruses in Cell Cultures – 28 day assay

Test Article;

2823-001 M1-MCB.1 TEST ARTICLE CELL LYSATE AS 06/06/07

I confirm that the Test Article used in this assay corresponds to the Test Article Receipt Form and Test Article Safety and Pre-Study Questionnaire supplied by the sponsor or the relevent cell banking documentation.

Study Director: ..... ⊕ **Date:** 12 June 07 Confirmed by SD CH 12/6/07

⊕ Signed in absence of SD CH 12/6/07

Document Authorised CH 11/6/07

Codes used throughout working document

NC = Negative Control
TA = Test Article
STA = Spiked Test Article
PC = Positive Control
APC = Assay Positive Control

PNC = Passaged Negative Control
PTA = Passaged Test Article
PSTA = Passaged Spiked Test Article
PPC = Passaged Positive Control

Comments

N/A CH 27/7/07

Document index

1 Day –1: Preparation of indicator cultures

Equipment

Lab 3	ID Number	
Safety Cabinet	~~BS220~~ BS207	☐ N/A
Incubator	BS220	☐ N/A
Waterbath	BS218	☐ N/A
Microscope	BS239	☐ N/A
Micropipette		☑ N/A
Pipetaid	BS189	☐ N/A
Other ( )		☑ N/A
Other ( )		☑ N/A
Initials/Date	RL 11/6/07	

Reagents

	RI code	Batch No	Exp. date
MEM/10E – seeding media	RI/184	50506/07 50601/07	11/8/07 6/09/07
D-PBS	RI/025	50584/07	04/09
HBSS	RI/024	NA	NA
TrypLE	RI/141	50578/07	09/09
Trypan blue (0.4%)	RI/044	50116/07	07/08
Other ( )		NA	
Other ( )	RL 11/6/07		
Initials/Date		RL/11/6/07	

Day –1: Preparation of indicator cultures

Steps – Seeding of MRC-5 cells.	Task complete
Cell line details. Cell concentration required **5 x 10^4 cells/ml.** **MRC-5** passage number x, C number 17, flasks confluency x.	☑
Using a sterile pipette, aspirate the medium from 7 x T 150 flasks of Cells.	☑
Wash the flasks with sufficient D-PBS to cover the monolayer.	☑
Remove washings and add 2.0 ml of TrypLE select each flask. Incubate the flask at 37 ± 1°C for approximatley 5 – 15 minutes.	☑
Gently agitate the flask to loosen the cell sheet and add 10.0 ml of complete medium. Mix the cell suspension.	☑
If multiple flasks are used pool in one sterile container. Final volume 84 ml	☑
Perform viable cell counts in the presence of Trypan Blue. Add 0.2 ml of cell suspension to 0.2 ml of Trypan blue and mix.	☑
Load the haemocytometer and count 3 of the 16 squares.	☑
Dilute cell suspension and seed 6 T75 flasks with 15ml cell suspension at 5 x 10^4 cells/ml.	☑
Incubate all flasks at 37 ± 1°C with 5-10% CO_2.	☑
Initials/Date	RL / 11/6/07

Cell Counts [x1]			
1. 8	**2.** 16	**3.** 14	**Mean** 13 **(B)**

Cell Concentration = (B x 2 x 10^4)	2.6×10^5 **Cells/ml**
Cell Concentration required	**5 x 10^4 cells/ml**
Dilution required	**1 In** 5.2
Total volume of cell suspension required (number of T75 flasks prepared x 15ml + excess)	95 **ml**
Volume of cell suspension (added to make up to required concentration)	18.3 **ml**
Volume of complete media (added to make up to required concentration)	76.7 **ml**
Calculation performed by/date	RL / 11/6/07
Calculation checked performed by/date	SB / 11 June 07

x 1 x T150 passage 6 100%, 4 x T150 passage 11/6 90% and 2 x T150 passage 12/6 40% RL 11/6/07

Page completion check initials/date TSB/6/07 page 5 of 43

Data check initials/date CH 27/7/07

x1 Cells also used for 2823-002, 0065-373, DRL 11/6/07 ~~0065~~ RL 11/6/07

Day –1: Preparation of indicator cultures

Steps – Seeding of Vero cells.	Task complete
Cell line details. Cell concentration required **5 x 10^4 cells/ml.** **Vero** passage number 24[24], C number 1, flasks confluency 100.	☑
Using a sterile pipette, aspirate the medium from 5 x T 150 flasks of Cells.	☑
Wash the flasks with sufficient D-PBS to cover the monolayer.	☑
Remove washings and add 2·0 ml of TrypLE select each flask. Incubate the flask at 37 ± 1°C for approximatley 5 – 15 minutes.	☑
Gently agitate the flask to loosen the cell sheet and add 10·0 ml of complete medium. Mix the cell suspension.	☑
If multiple flasks are used pool in one sterile container. Final volume 60 ml	☑
Perform viable cell counts in the presence of Trypan Blue. Add 0.2 ml of cell suspension to 0.2 ml of Trypan blue and mixed.	☑
Load the haemocytometer and count 3 of the 16 squares.	☑
Dilute cell suspension and seed 6 T75 flasks with 15ml cell suspension at 5 x 10^4 cells/ml.	☑
Incubate all flasks at 37 ± 1°C with 5-10% CO_2.	☑
Initials/Date	RL 11/6/07

Cell Counts ×			
1. 106	**2.** 114	**3.** 103	**Mean** 108 **(B)**

Cell Concentration = (B x 2 x 10^4)	2·16 x 10^6 **Cells/ml**
Cell Concentration required	**5 x 10^4 cells/ml**
Dilution required	1 **in** 43·2
Total volume of cell suspension required (number of T75 flasks prepared x 15ml + excess)	250 **ml**
Volume of cell suspension (added to make up to required concentration)	5·8 **ml**
Volume of complete media (added to make up to required concentration)	244·2 **ml**
Calculation performed by/date	RL 11/6/07
Calculation checked performed by/date	CM 12.6.07

× Data transcribed from 0065-373. Cells also used for 0065-373, 2823-002, 0065-379, 0065-380 + 0065-381. RL 11/6/07

Day –1: Preparation of indicator cultures

Steps – Seeding of HeLa cells.	Task complete
Cell line details. Cell concentration required **5 x 10^4 cells/ml.** **HeLa** passage number 198, C number 8, flasks confluency 100.	☑
Using a sterile pipette, aspirate the medium from 2 x T 150 flasks of Cells.	☑
Wash the flasks with sufficient D-PBS to cover the monolayer.	☑
Remove washings and add 20 ml of TrypLE select each flask. Incubate the flask at 37 ± 1°C for approximatley 5 – 15 minutes.	☑
Gently agitate the flask to loosen the cell sheet and add 10.0 ml of complete medium. Mix the cell suspension.	☑
If multiple flasks are used pool in one sterile container. Final volume 24.0 ml	☑
Perform viable cell counts in the presence of Trypan Blue. Add 0.2 ml of cell suspension to 0.2 ml of Trypan blue and mixed.	☑
Load the haemocytometer and count 3 of the 16 squares.	☑
Dilute cell suspension and seed 6 T75 flasks with 15ml cell suspension at 5 x 10^4 cells/ml.	☑
Incubate all flasks at 37 ± 1°C with 5-10% CO_2.	☑
Initials/Date	RL / 11/6/07

Cell Counts *			
1. 47	**2.** 63	**3.** 56	**Mean** 55 **(B)**

Cell Concentration = (B x 2 x 10^4)	1.10×10^6 **Cells/ml**
Cell Concentration required	**5 x 10^4 cells/ml**
Dilution required	1 **In** 22
Total volume of cell suspension required (number of T75 flasks prepared x 15ml + excess)	100 ~~4.5~~ **ml** Sec 11/6/07
Volume of cell suspension (added to make up to required concentration)	45 **ml** RL 11/6/07
Volume of complete media (added to make up to required concentration)	95.5 **ml**
Calculation performed by/date	RL / 11/6/07
Calculation checked performed by/date	AM / 11/06/07

* Cells also used for 2823-002 RL 11/6/07

2823-001

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On day 13 it was noticed that all the 3T3 cells looked unhealthy. There were many floating cells and the media had turned a yellowish-orange

It was decided to repeat the cells. Confirmed by SO CH 25/6/07

Supernatant was harvested, centrifuged at 160 x 'g' for 10 mins and supernatant was removed from cells and stored at -80 Storage location: BS 152, 3rd shelf on top of box C. x'

Cells were washed with DPBS by adding 10ml + aspirating

Batch number: 50634/07 expiry date: 5/09

Fresh medium was added:

Batch number: 50343/07 expiry date: 27.6.07

Cells were placed in incubator BS 228

Equipment list:
Safety Cabinet BS 199
Pipette aid BS 260
Centrifuge BS 040

See Envt # 231136 for further information DF 25.6.7

x' NC and TA 002 Supernatants were discarded in error DF x2 25.6.7

Page Completion check CH 27/7/07
Data check CH 27/7/07

x2 These supernatants were not required Added in response to QA audit CH 29/9/07

4 Day 13: Preparation of fresh [redacted] indicator cultures for Haemadsorption positive control.

N/A 15274/6/07

Equipment

Lab [redacted]	ID Number
Safety Cabinet	[redacted] ☐ N/A
Incubator	[redacted] ☐ N/A
Waterbath	[redacted] ☐ N/A
Microscope	[redacted] ☐ N/A
Pipetaid	[redacted] ☐ N/A
Other ([redacted])	[redacted] ☐ N/A
Other ([redacted])	[redacted] ☐ N/A
Initials/Date	[redacted] / [redacted]

Reagents

	RI code	Batch No	Exp. date	
MEM/10E – seeding media	RI/184	[redacted]	[redacted]	☐ N/A
MEM/5E – refeed media	RI/183	[redacted]	[redacted]	☐ N/A
D-PBS	RI/025	[redacted]	[redacted]	☐ N/A
HBSS	RI/024	[redacted]	[redacted]	☐ N/A
TrypLE	RI/141	[redacted]	[redacted]	☐ N/A
Trypan blue (0.4%)	RI/044	[redacted]	[redacted]	☐ N/A
Other ([redacted])	[redacted]	[redacted]	[redacted]	☐ N/A
Other ([redacted])	[redacted]	[redacted]	[redacted]	☐ N/A
Initials/Date		[redacted] / [redacted]		

X, see note 231387 15274/6/07

N/A see note 231387 TS 27/6/07

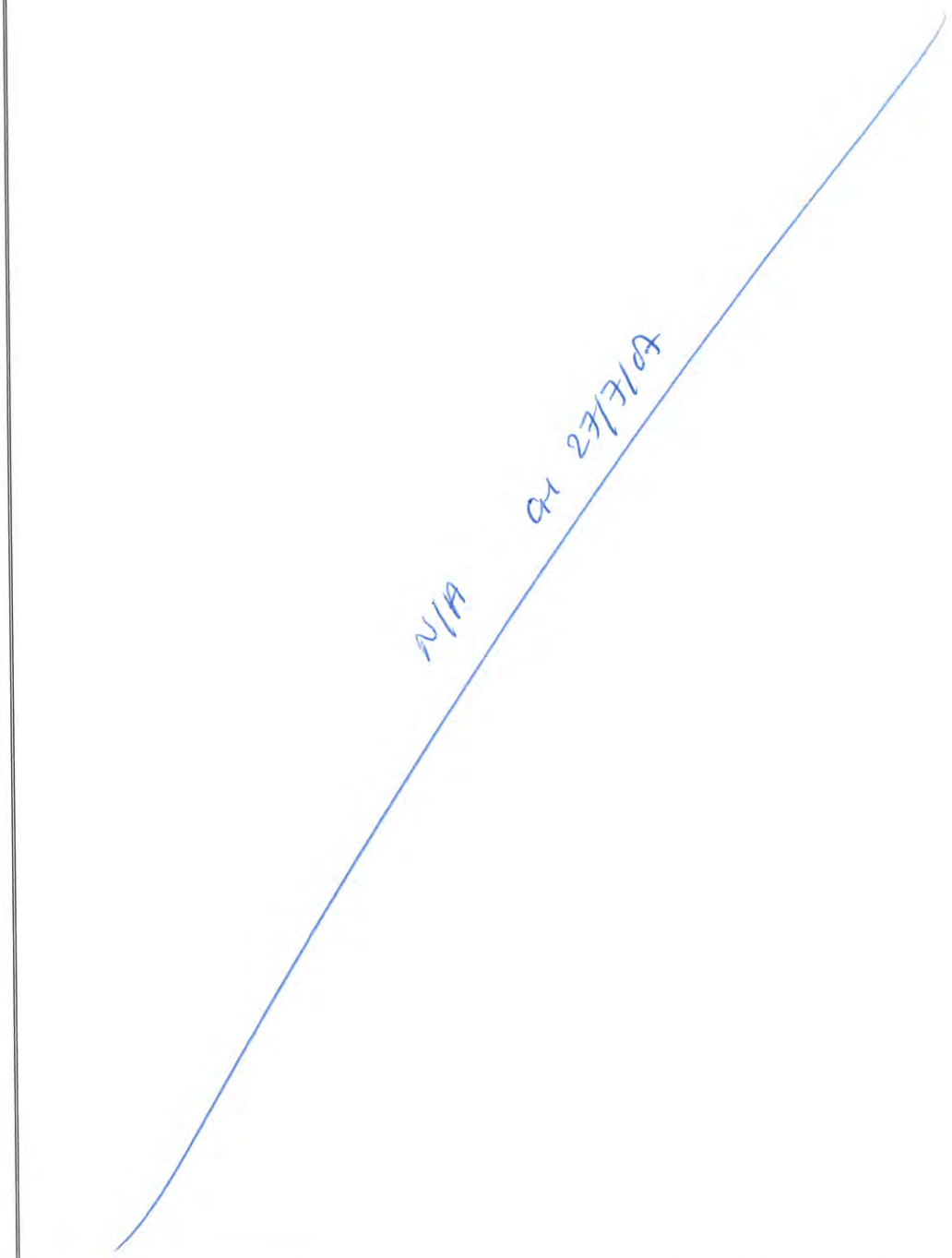
Day 13: Preparation of fresh indicator cultures for Haemadsorption positive control.

Steps – Seeding of cells.	Task complete
Cell line details. Cell concentration required **5 x 10^4 cells/ml.** **MRC-5** passage number , C number , flasks confluency .	☐
Using a sterile pipette, aspirate the medium from x T flasks of Cells.	☐
Wash the flasks with sufficient D-PBS to cover the monolayer.	☐
Remove washings and add ml of TrypLE select each flask. Incubate the flask at 37 ± 1°C for approximatley 5 – 15 minutes.	☐
Gently agitate the flask to loosen the cell sheet and add ml of complete medium. Mix the cell suspension.	☐
If multiple flasks are used pool in one sterile container. Final volume ml	☐
Perform viable cell counts in the presence of Trypan Blue. Add 0.2 ml of cell suspension to 0.2 ml of Trypan blue and mixed.	☐
Load the haemocytometer and count 3 of the 16 squares.	☐
Dilute cell suspension and seed T75 flasks with 15ml cell suspension at 5 x 10^4 cells/ml.	☐
Incubate all flasks at 37 ± 1°C with 5-10% CO_2.	☐
Initials/Date	/

Cell Counts			
1.	**2.**	**3.**	**Mean (B)**

Cell Concentration = (B x 2 x 10^4)	**Cells/ml**
Cell Concentration required	**5 x 10^4 cells/ml**
Dilution required	**In**
Total volume of cell suspension required (number of T75 flasks prepared x 15ml + excess)	**ml**
Volume of cell suspension (added to make up to required concentration)	**ml**
Volume of complete media (added to make up to required concentration)	**ml**
Calculation performed by/date	/
Calculation checked performed by/date	/

Comments



X1

5 Day 14: Inoculation of fresh indicator cultures for Haemadsorption positive control and subculture of cells.

Equipment

Lab 9	ID Number	
Safety Cabinet	BS199	☐ N/A
Incubator	BS228	☐ N/A
Waterbath	BS452	☐ N/A
Microscope	BS439	☐ N/A
Micropipette		☑ N/A
Pipetaid	BS269	☐ N/A
Other ( )		☑ N/A
Other ( )		☑ N/A
Initials/Date	RL / 26/6/07	

Reagents

	RI code	Batch No	Exp. date	
MEM/5E – refeed media	RI/183			☑ N/A
MEM/5TPB	RI/187			☑ N/A
D-PBS	RI/025	50634/07	05/09	☐ N/A
HBSS	RI/024			☑ N/A
Other (MEM/10E)	184	50601/07	6/9/07	☐ N/A
Other (DMEM/10)	190	50329/07	23/6/07	☐ N/A
Initials/Date		RL / 26/6/07		

TrypLE X RI 141 50608/07 02/09
see note 231387 AS 26/6/07

Day 14: Inoculation of fresh indicator cultures for Haemadsorption positive control and subculture of cells.

Steps - Preparation of positive control	Task completed
Thaw rapidly a frozen ampoule of PI3 virus,batch [redacted] at [redacted] $TCID_{50}$/ml in waterbath set at 37 ± 1°C. Once thawed keep ampoule on ice until required.	☐
Perform virus dilution and complete table below.	☐
Required virus concentration for positive control is **$1x10^4$TCID50/ml**	☐

Virus Suspension(ml)	Diluent (ml)	Total Volume Required (ml)	Dilution	TCID50/ml
[redacted]	[redacted]	[redacted]	1 in [redacted]	[redacted]
[redacted]	[redacted]	[redacted]	1 in [redacted]	[redacted]
[redacted]	[redacted]	[redacted]	1 in [redacted]	[redacted]
[redacted]	[redacted]	[redacted]	1 in [redacted]	[redacted]
[redacted]	[redacted]	[redacted]	1 in [redacted]	[redacted]

Calculation performed by/date	[redacted] / [redacted]
Calculation check performed by/date	[redacted] / [redacted]
Initials/Date	[redacted] / [redacted]

Steps – inoculation of positive control flask	Task completed
Aspirate medium from approx . [redacted] % confluent . [redacted] cells.	☐
Wash cells with sufficient amount of D-PBS.	☐
Inoculate one flask with 5ml of **positive control** and incubate flasks at 37 ± 1°C, 5% CO_2 for 60-90 min. Start time: [redacted] End time: [redacted]	☐ start ☐ end
After incubation aspirate the inoculum and **wash the cells with ~10mL DPBS**. Then refeed cells with 15ml appropriate refeed media and incubate flasks at 37 ± 1°C, 5% CO_2.	☐
Initials/Date	[redacted] / [redacted]

X1 see note 231387 AS 26/6/07

X1 N/A AS 27/6/07

Day 14: Subculture of Cultures

Steps	Task completed
Discard positive control flasks if show CPE. X	NA ☐ (✓,N/A) by date RL/26/6/07
Subculture	☑ (✓,N/A)
Flasks to be subcultured All 3T3 flasks 1in4 Vero NC + TA 1in4 All MRC-5 flasks 1in3 HeLa NC + TA 1in4	SD confirmed CH/26/6/07
Aspirate medium from all the flasks that show no CPE and are 100% confluent.	
Wash the flasks with sufficient D-PBS to cover the monolayer.	☑
Remove washings and add 2 ml of TrypLE select. Incubate the flask at 37 ± 1°C for approx. 5-15 minutes.	☑
Gently agitate the flask to loosen the cell sheet and add 10ml[x2] of complete medium. Mixed the cell suspension. Final volume 12ml	☑
Seed [x1] ml of cell suspension (equal to a 1 in [x1] split) into fresh T75 flasks and make up to a total flask volume of 15 ml with complete media.	☑
Flask labelled with cell line, study number and test material (eg PNC, PTA etc)	☑
Return the flask to incubators at 37 ± 1°C 5% CO_2	☑
Initials/Date	RL/26/6/07

Comments

x Positive flasks discarded previously CH 26/6/07.

x_1 3T3 0.5ml 1in4
Vero 0.5ml 1in4
HeLa 0.5ml 1in4
MRC-5 0.6ml 1in3 RL 26/6/07

x_2 10ml of media was not added but the box was ticked in error. The final volume was 2ml + the split ratio's are detailed in the x, comment. Comment added in retrospect for clarity. RL 27/7/07 x3 x3 cells looked fine the next day (see results) so it is clear that a 1in4 split was done not a 1in24 CH 27/7/07

6 Day 21 : Subculture/Refeed of Cultures

Equipment

Lab 10	ID Number	
Safety Cabinet	BS 198	☐ N/A
Incubator	BS 228	☐ N/A
Waterbath	BS 251	☐ N/A
Microscope	BS 244	☐ N/A
Pipetaid	BS 263	☐ N/A
Other ( )		☑ N/A
Other ( )		☑ N/A
Initials/Date	DF 13.7.7	

Reagents

	RI code	Batch No	Exp. date	
MEM/10E – seeding media	RI/184	5060107	06/09/07	X' ☐ N/A
MEM/5E – refeed media	RI/183			☑ N/A
D-PBS	RI/025	5064307	02/09	☐ N/A
HBSS	RI/024			☑ N/A
TrypLE	RI/141	5011507	08/08	☐ N/A
Trypan blue (0.4%)	RI/044			☑ N/A
Other (DMEM/10E)	RI/190	5060407	06/09/07	☐ N/A
Other ( )				☑ N/A
Initials/Date	DF 13.7.7			

X' See Grate 236055 DF 13.7.7

7 Day 21 : Subculture/Refeed of Cultures

Steps	Task completed
Discard positive control flasks if show CPE.	N/A ☐ (✓,N/A) by date DF 13.7.7
If cultures are 100% confluent confirm with SD how to proceed.	☑
Refeed	N/A (✓,N/A)
Flasks to be refed	SD confirmed CH 13/7/07
Aspirate medium from the flasks that show no CPE.	☐
Add 15 ml fresh refeed media.	N/A ☑
Incubate flasks at 37 ± 1°C, 5% CO_2	☐
Initials/Date	DF 13.7.7
Subculture	☑ (✓,N/A)
Flasks to be subcultured 3T3 PC, NC, STA, TA Vero NC, TA Hela NC, TA MRC-5 NC, TA, STA, PC	SD confirmed CH 13/7/07
Aspirate medium from all the flasks that show no CPE and are 100% confluent.	✓
Wash the flasks with sufficient D-PBS to cover the monolayer.	☑
Remove washings and add 2 ml of TrypLE select. Incubate the flask at 37 ± 1°C for approx. 5-15 minutes.	☑
Gently agitate the flask to loosen the cell sheet and add 10ml of complete medium. Mixed the cell suspension. Final volume 12ml	☑
Seed x1 ml of cell suspension (equal to a 1 in x' split) into fresh T75 flasks and make up to a total flask volume of 15 ml with complete media.	☑
Flask labelled with cell line, study number and test material (eg PNC, PTA etc)	☑
Return the flask to incubators at 37 ± 1°C 5% CO_2	☑
Initials/Date	~~DF 13.7.7~~ 3.7.7

CH DF 3.7.7

x' See page 25 for further details DF 3.7.7

CH 4/7/07

Comments

x1 Hela, 3T3 and Vero NC flasks had 2 ml of Trypleˣ² added with 0.5 ml of Trypleˣ² to be passed into fresh flasks. Equating to a 1 in 4 split. MRC-5 NC flasks had 1.5 ml of Trypleˣ² added with 0.5 ml of Trypleˣ² passed into fresh flasks equating to a 1 in 3 split.

Hela, 3T3, and Vero TA flasks were treated as with the Negative Controls with a 1 in 4 split performed. The MRC-5 TA flasks had a 1 in 3 split performed. (I)DF 3.7.7

STA and PC for 3T3 were Split at a 1 in 8 ratio
STA and PC for MRC-5 cells were Split at a ratio of 1 in 3 DF 3.7.7

x² Cell Suspension DF 4.7.7

2 flask change AS04/07/07

8 Day 2~~5~~ : Preparation of fresh MRC-5 indicator cultures for Haemadsorption positive control.

Equipment

Lab A	ID Number	
Safety Cabinet	BS 208	☐ N/A
Incubator	BS 210	☐ N/A
Waterbath	~~BS~~ AS04/07/07	☑ ~~N/A~~
Microscope	BS 249	☐ N/A
Pipetaid	BS 192	☐ N/A
Other ( )		☑ N/A
Other ( )		☑ N/A
Initials/Date	AS/04/07/07	

*1

Reagents

	RI code	Batch No	Exp. date	
MEM/10E – seeding media	RI/184	50601/07	06/09/07	☐ N/A
MEM/5E – refeed media	RI/183			☑ ~~N/A~~
D-PBS	RI/025	50643/07	02/2009	☐ N/A
HBSS	RI/024			☑ ~~N/A~~
TrypLE	RI/141	50652/07	02/2009	☐ N/A
Trypan blue (0.4%)	RI/044	50620/06	13/12/07	☐ N/A
Other ( )				☑ ~~N/A~~
Other ( )				☑ ~~N/A~~
Initials/Date		AS/04/07/07		

*1 waterbath needed @ 56°C, therefore used incubator to warm reagents. AS04/07/07

2 test change AS05/07/07

Day 25 : Preparation of fresh MRC-5 indicator cultures for Haemadsorption positive control.

Steps – Seeding of cells if required. ☑ (✓,N/A) 1x10⁵ x 2	**Task complete**
Cell line details. Cell concentration required ~~5 x 10⁴~~ **cells/ml.** MRC-5 passage number 11[17], C number 17, flasks confluency 100%	☑
Using a sterile pipette, aspirate the medium from 1 x T 150 flasks of Cells.	☑
Wash the flasks with sufficient D-PBS to cover the monolayer.	☑
Remove washings and add 2 ml of TrypLE select each flask. Incubate the flask at 37 ± 1°C for approximatley 5 – 15 minutes.	☑
Gently agitate the flask to loosen the cell sheet and add 10 ml of complete medium. Mix the cell suspension.	☑
If multiple flasks are used pool in one sterile container. Final volume 12 ml	☑
Perform viable cell counts in the presence of Trypan Blue. Add 0.2 ml of cell suspension to 0.2 ml of Trypan blue and mixed.	☑
Load the haemocytometer and count 3 of the 16 squares.	☑
x2 1x10⁵ Dilute cell suspension and seed 2[x1] T75 flasks with 15ml cell suspension at ~~5 x 10⁴~~ cells/ml.	☑
Incubate all flasks at 37 ± 1°C with 5-10% CO_2.	☑
Initials/Date	AS/04/07/07

Cell Counts			
1. 18	**2.** 20	**3.** 21	**Mean** 20 **(B)**

Cell Concentration = (B x 2 x 10^4)	4x10⁵ **Cells/ml**
Cell Concentration required	x2 1x10⁵ ~~5 x 10⁴~~ **cells/ml**
Dilution required	1 **In** 4
Total volume of cell suspension required (number of T75 flasks prepared x 15ml + excess)	40 **ml**
Volume of cell suspension (added to make up to required concentration)	10 **ml**
Volume of complete media (added to make up to required concentration)	30 **ml**
Calculation performed by/date	AS/04/07/07
Calculation checked performed by/date	CH 4/7/07

[x1] 2 x T75 flasks were seeded because we may inoculate at 2 concentrations of virus tomorrow AS04/07/07

Comments

N/A CH 23/7/07

3 tert change AS 05/07/07

9 Day 26: Inoculation of fresh indicator cultures for Haemadsorption positive control.

Equipment

Lab 9	ID Number	
Safety Cabinet	BS 199	☐ N/A
Incubator	BS 228	☐ N/A
Waterbath	BS 257	☐ N/A
Microscope	BS 439	☐ N/A
Micropipette		☑ N/A
Pipetaid	BS 269	☐ N/A
Other ( )		☑ N/A
Other ( )		☑ N/A
Initials/Date	AS / 05/07/07	

Reagents

	RI code	Batch No	Exp. date	
MEM/5E – refeed media	RI/183	50602/07	06/09/07	☐ N/A
MEM/5TPB	RI/187	50570/07	24/08/07	☐ N/A
D-PBS	RI/025	50643/07	02/2009	☐ N/A
HBSS	RI/024			☑ N/A
Other ( )				☑ N/A
Other ( )				☑ N/A
Initials/Date		AS / 05/07/07		

A3 text change on 27/7/07

Day 26: Inoculation of fresh indicator cultures for Haemadsorption positive control.

Steps - Preparation of positive control if required. ☑(✓,N/A)	Task completed
Thaw rapidly a frozen ampoule of PI3 virus, batch PI3 050721 at 1.1x10⁵ $TCID_{50}$/ml in waterbath set at 37 ± 1°C. Once thawed keep ampoule on ice until required.	☑
Perform virus dilution and complete table below.	☑
Required virus concentration for positive control is **1x10⁴TCID50/ml** x1	☑

Virus Suspension(ml)	Diluent (ml)	Total Volume Required (ml)	Dilution	TCID50/ml
0.5	5.0	5.5	1 in 11	1x10⁷
0.5	4.5	5.0	1 in 10	1x10⁶
1	9	10	1 in 10	1x10⁵
1	9	10	1 in 10	1x10⁴
	N/A AS 05/07/07		1 in	
Calculation performed by/date				AS 05/07/07
Calculation check performed by/date				AL 15/7/07
Initials/Date				AS 05/07/07

Steps – inoculation of positive control flask	Task completed
Aspirate medium from approx. 60 % confluent MRC-5 cells.	☑
Wash cells with sufficient amount of D-PBS.	☑
Inoculate one flask with 5ml of **positive control** and incubate flasks at 37 ± 1°C, 5% CO_2 for 60-90 min. x1 Start time: 9.43 End time: 11.12	☑ start ☑ end
After incubation aspirate the inoculum and refeed cells with 15ml appropriate refeed media and incubate flasks at 37 ± 1°C, 5% CO_2.	☑
Initials/Date	AS/05/07/07

x1 Two positive control flasks set up, 1 @ 1x10⁵ $TCID_{50}$/ml and one @ 1x10⁴ $TCID_{50}$/ml AS 05/07/07 (2) AS 05/07/07

10 Day 28: Haemadsorption Assay [x1]

Equipment

Virology Lab 4	ID Number	
Safety Cabinet	BS 199	☐ N/A
Incubator	BS 228	☐ N/A
Waterbath	BS 452	☐ N/A
Microscope	BS 439	☐ N/A
Micropipette		☑ N/A
Pipetaid	BS 261 + 269	☐ N/A
Other (Centrifuge)	BS 040	☐ N/A
Other ( )		☑ N/A
Initials/Date	MJS / 10/07/07	

Reagents

	RI code	Batch No	Exp. date
D-PBS	RI/025	50643/07	02/2009
Human O red blood cells	RI/007	50731/07	27/07/07
Guinea pig red blood cells	RI/006	50708/07	11/07/07
Adult chicken red blood cells	RI/005	50710/07	19/07/07
Other (HBSS)	RI/124	50624/07	04/09
Other ( )		N/A MJS 10/07/07	
Initials/Date		MJS / 10/07/07	

x1 Pages 31, 32, 33, 35 & 36 were transcribed from 2823-002 because the same procedure was carried out for these two studies MJS, 10/07/07

Preparation of cell culture supernatants

Steps	Task completed
Collect media from all flasks into uniquely labelled centrifuge tubes and add 10ml of D-PBS to each flask. Store flasks at 37 ± 1°C, 5% CO_2 incubator until required [x1]	☑
Centrifuge all supernatants at 1000 x 'g' for 10 minutes at 4 ±2C° and keep until required. Supernatants were stored at <-70°C in BS [x2] N/A	☒ N/A
Use cells for haemadsorption.	☐
Initials/Date	CMS / 10/07/07

Comments

x1 D-PBS was added by CMS 10.07.07

x2 Supernatants were discarded ~~as~~ at the discretion of the SP. CMS 10/07/07
ICH 20/9/07

Confirmed by SO CH 11/7/07.

Day 28: Haemadsorption Assay

Preparation of 2.0% blood solution

Steps					Task completed
Dilute the three types of blood to 2.0%.					☑
Blood type	**Orig Stock conc.%**	**Dilution**	**Total Volume Required (ml)**	**Blood (ml)**	**Chilled HBSS (ml)**
Adult Chicken	7	1 in 3.5	49.0 x2	14.0	35.0
Human O	50	1 in 25.0	50.0 x2	2.0	48.0
Guinea Pig	15	1 in 7.5	49.5 x2	6.6	42.9
Calculation performed by/date					MS 11/07/07
Calculation check performed by/date					RL 11/7/07 x3
Initials/Date					MS 11/07/07

Steps		Task completed
Centrifuge red blood cells at 160 'g' for 10 minutes at 4±2°C		☑
If the supernatant **is clear**: Aspirate supernatant and resuspend pellet in the same total volume of chilled HBBS. Keep blood on ice.	Adult Chicken	☐ (✓ N/A)
	Human O	☑ (✓ N/A)
	Guinea Pig	☑ (✓ N/A)
If the supernatant **is not clear**: Centrifuge supernatant again until it is clear. Aspirate supernatant and resuspend pellet in the same total volume of chilled HBBS. Keep blood solution on ice until required.	Adult Chicken	☑ (✓ N/A)
	Human O	☑ (✓ N/A)
	Guinea Pig	☑ (✓ N/A)
Record the number of times the blood was centrifuged to get a clear supernatant.	Adult Chicken	1
	Human O	2
	Guinea Pig	3 x1
Initials/Date		MS 11/07/07

x1 Last spin was performed @ +4°C, 800 'g' for 10 minutes. MS 10/07/07

x2 Large volumes of blood were prepared because blood was shared with 2823-002 study. Adult Chicken & Guinea Pig blood was also shared with 1889-104 + 2638-002 studies. MS 10/07/07

x3 Calculations were checked on the day of the procedure in 2823-002 study. MS 11/07/07

Comments

N/A CM 11/7/07.

Day 28: Preparation of erythrocytes for Haemadsorption

Preparation of 0.5% blood solution

Steps	Task completed
Dilute three types of blood from 2.0% to 0.5%.	☑

Blood type	Dilution	Total Volume Required (ml)	Blood solution at 2.0%(ml)	Chilled HBSS (ml) x1
Adult Chicken	1 in 4	160	40	120
Human O	1 in 4	160	40	120
Guinea Pig	1 in 4	160	40	120

Calculation performed by	[illegible] 10/07/07
Calculation check	RL 11/7/07 x2
Keep blood solutions on ice until required.	☑
Initials/Date	[illegible] 10/07/07

Comments

x1 HBSS was aliquoted by [illegible] 10.07.07

x2 Calculations were checked on the day of the procedure in 2823-002 Study. [illegible] 11/07/07

11 Day 28: Haemadsorption Assay

Steps	Task completed
Pool equal volumes of three blood type solutions at 0.5% together, enough to add 9ml of blood to each plate **twice**.	☑
Aspirate wash from flasks. X1	☑
Add 9ml of blood solution mixture at 0.5% to each flask.	☑
Refrigerate flasks in refrigerator BS166 (recorded temp. 7.6 °C*) for 30±5 minutes. Start time: 14:58 End time: 15:28	☑ Start ☑ End
After the incubation period aspirate blood from flasks and wash with 10ml DPBS	☑
Score flasks and record the results in the result table.	☑
Aspirate wash from flasks. X1	☑
Add 9ml of blood solution mixture at 0.5% to each flask.	☑
Incubate flasks in incubator or at room temperature (depending on protocol) 24.0 (recorded temp. 24.0 °C*) for 30±5 minutes. Start time: 16:13 End time: 16:43	☑ Start ☑ End
After the incubation period aspirate blood from flasks and wash with 10ml DPBS	☑
Score flasks and record the results in the result table.	☑
* All temperatures were taken at the start of the incubation period.	
Initials/Date	[illegible] 11/07/07

Comments

X1 DPBS was aspirated by [illegible] 10.07.07 [illegible]

The MRC-5 STA was also tested for haemadsorption as only early signs of CPE were noted. This could confirm whether the signs of CPE were due to viral infection or not. Added for information CH 11/7/07.

12 Result Tables

Results: Observation of CPE (Day 1-14)

Cell line:		MRC-5				Vero			
		Flasks showing CPE							
By/date	Days Post Inoculation	NC	PC	TA	STA	NC	PC	TA	STA
DF 22.6.7	1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
AS 14.6.07	2	0/1	0/1	0/1	0/1	0/1	1/1	0/1	0/1
DF 25.6.7	3	0/1	0/1	0/1	0/1	0/1	1/1	0/1	0/1
AS 18/06/07	6	0/1	0/1 x1	0/1	1/1	0/1	1/1	0/1	1/1
DF 19.6.7	7	0/1	0/1	0/1	x4 x4 /1	0/1	x4 /1	0/1	1 x2 /1
DF 20.6.7	8	0/1	0/1	0/1	1/1	0/1	N/A/1	0/1	N/A/1
AS 21/6/07	9	0/1	0/1 x5	0/1	0/1 x5	0/1	N/A/1	0/1	N/A/1
DF 22.6.7	10	0/1	0/1	0/1	0/1	0/1	N/A/1	0/1	N/A/1
DF 25.6.7	13	0/1	0/1	0/1	0/1	0/1	N/A/1	0/1	N/A/1
RL 26/6/07	14	0/1	0/1	0/1	0/1	0/1	NA/1	0/1	NA/1

x3

Comments x1 less contrast than the NC + some vacuolation observed AS 18/06/07
x2 Positive flasks discarded.
x3 All flasks were stored in BS228 DF 21.6.7
x4 Wrong Box annotated flask was not discarded DF 21.6.7
x5 Some vacuolation observed but not clearly CPE, therefore scored negative by SO CH 21/6/07.

Results: Observation of CPE (Day 1-14)

Cell line:		HeLa				NIH 3T3			
		Flasks showing CPE							
By/date	Days Post Inoculation	NC	PC	TA	STA	NC	PC	TA	STA
DF 13.6.7	1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
AS 14.6.07	2	0/1	0/1	x_1 0/1	0/1	0/1	0/1	0/1	0/1
DF 15.6.7	3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
AS 18/06/07	6	0/1 x_4	1/1	0/1 x_4	1/1	0 x_2/1 x_3	0/1	0/1	0/1
DF 19.6.7	7	0/1	x_5 1/1	0/1	x_5 x_6 1/1	0/1	0/1	0/1	0/1
DF 20.6.7	8	0/1	N/A/1	0/1	N/A/1	0/1	0/1	0/1	0/1
AS 21/6/07	9	0/1	N/A/1	0/1	N/A/1	0/1	0/1	0/1	0/1
DF 22.6.7	10	0/1	N/A/1	0/1	N/A/1	0/1	0/1	0/1	0/1
BS 25.6.7	13	0/1	N/A/1	0/1	N/A/1	0/1 x_8	0/1 x_8	0 x_8/1	0 x_8/1
RL 26/6/07	14	0/1 x_9	NA/1	0/1 x_9	NA/1	RL 26/6/07 0/1	0/1	0/1	0/1

Comments

x_1 Some floating cells AS 14/06/07
x_2 Some loose cells in media AS 18/06/07
x_3 @ AS 18/06/07
x_4 cells overconfluent - some patches + loose cells in media AS 18/6/07
x_5 (DF 19.6.7
x_6 Positive flasks Discarded DF 19.6.7
x_7 All flasks were stored in BS 228 DF 21.6.7
x_8 media yellow in colour See pg 16a for details DF 25.6.7
x_9 Some floating cells observed. RL 26/6/07

x10 Please see page 42 for comment x10 and any other comments to follow. AS 10/07/07 @ AS 10/07/07.

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Biotechnology department

Study number: 2823-001

x10 Early signs of cpe observed. AS, 10/07/07

x11 Not ~~scored~~ recorded at time but can be assumed negative as negative the following day. In response to QA audit CH 20/9/07 CH 20/9/07

Results: Observation of CPE (Day 15-28)

Cell line:		x7 MRC-5				Vero			
		Flasks showing CPE							
By/date	Days Post Inoculation	NC	PC	TA	STA	NC	PC	TA	STA
DF 27.6.7	15	0/1	0/1	0/1	0/1	0/1	N/A/1	X1 N/A/1	X1 0/1
AS 28/6/07	16	0/1	0/1	0/1	0/1	0/1	N/A/1	X1 0/1 DF 28.6.7	N/A/1
AS 29/6/07	17	0/1	0 X2/1	0 X2/1	0/1	0/1	N/A/1	0/1	N/A/1
DF 2.7.7	20	0/1	0/1	0/1	0/1	0/1	N/A/1	0/1	N/A/1
DF 3.7.7	21	0/1	0/1 X3	0/1	0/1 X3	0/1	N/A/1	0/1	N/A/1
AS/DF 04/07/07	22	0/1	0 X4/1	0/1	0/1	0/1	N/A/1	0/1	N/A/1
AS 05/07/07	23	0/1	0 X6/1	0/1	0 X5/1 (AS 6/7/07)	0/1	N/A/1	0/1	N/A
AS 06/07/07	24	0/1	X 0/7/1 X8	0/1	X 8/1 X8 11	0/1	N/A/1	0/1	N/A/1
AS 09/07/07	@ AS 9/7/07 2827	0/1	0/1 X9	0/1	0/1 X9	0/1	N/A/1	0/1	N/A/1
AS/CH 10/07/07	28	0/1	/1	0/1	0/1 X10	0/1	N/A/1	0/1	N/A/1

Comments

X1 Recorded wrong way round in error 28.6.7 DF Confirmed from previous records that STA flask has been discarded and TA flask negative DF 28.6.7

X2 Some rounded cells loosely attached to the monolayer AS 29/6/07

X3 Rounded cells, not seen in NC, but monolayer still intact CH 3/7/07

X4 See X3 comment. PPC. AS 04/07/07

X5 Alot of vacuolation, not seen in the NC AS 05/07/07

X6 See X3 comment AS 05/07/07

X7 APC flasks set up on 05/07/07 will be scored and results recorded on page 42 of this document AS 06/07/07

X8 less confluent than PNC, loose cells in media, some vacuolation. AS 06/07/07

X9 Cells are less confluent and look wider in comparison to NC & PA. Also rounded cells in light phase observed. AS 09/07/07

x13 Please see page 42 for observations made 06/07/07 onwards AS 06/07/07

x14 Please see page 42 for comment 14 & any other comments to follow. AS 09/07/07

Results: Observation of CPE (Day 15-28)

Cell line:		HeLa				NIH 3T3			
By/date	Days Post Inoculation	Flasks showing CPE							
		NC	PC	TA	STA	NC	PC	TA	STA
DK 27.6.7	15	0/1	N/A/1	0/1	N/A/1	0/1	0/1	0/1	0/1
AS 28/6/07	16	0/1	N/A/1	0/1	N/A/1	0/1 x1	0/1 x1	0/1 x1	0/1 x1
AS 29/6/07	17	0 x2/1	N/A/1	0/1	N/A/1	0/1 x3	0 x4/1	0 x5/1	0 x4/1
DK 2.7.7	20	0/1 x6	N/A/1	0/1 x6	N/A/1	0/1 x6 x7	0/1 x6 x7	0/1 x6 x7	0/1 x6 x7
DK 3.7.7	21	0/1	N/A/1	0/1	N/A/1	0/1	0/1 x9	0/1	0/1
AS/DK 04/07/07	22	0/1	N/A/1	0/1	N/A/1	0/1	0/1 x10	0/1	0 x11/1
AS 05/07/07	23	0/1	N/A/1	0/1	N/A/1	0/1	0/1	0/1	0 x12/1
AS 06/07/07	24	0/1	N/A/1	0/1	N/A/1	0/1	0 x13/1	0/1	0 x10/1
AS 09/07/07	@ AS 9/7/07 22 27	0/1 x14	N/A/1	0/1 x14	N/A/1 x @ AS 9/7/07	0/1 x5	2 @ AS 9/7/07 /1 x15	0/1 x15	0/1 x16 x15
AS / CH 10/07/07	28	0/1 x17	N/A/1	0/1 x17	N/A/1	2 x16 /1 x18	1/1 x19 x20	2 x16 /1 x19	1/1 x20

@ AS 10/07/07 @ AS 10/07/07

Comments

x1 loose cells in media PNC looks less confluent than other flasks but still look healthy AS 28/6/07 ① AS 28/6/07

x2 some loose cells in media. Cells ~ 70% confluent, look healthy AS 29/6/07

x3 loose cells in media, cells ~80% confluent, look healthy AS 29/6/07

x4 lots of loose cells in media (more than NC) and rounded cells loosely attached to the monolayer. cells look less healthy than NC but some confluency. AS 29/6/07

x5 loose cells in media, 90% confluent, healthy AS 29/6/07

x6 Lots of loose cells in media DK 2.7.7

x7 media yellow in colour therefore was refed with D-Ex/56 DK 2.7.7 x8

x8 Flasks refed with 15ml RT SO5/11/07, Supernatant stored in BS152, on top of box R221 DK 2.7.7 See Grate # 232896 for further information DK 2.7.7

x9 More rounded cells than seen in the negative control but monolayer still intact CH 3/7/07.

x10 PPC + STA AS 04/07/07 x11 A lot more loose cells in media than the PNC AS 04/07/07

x12 See x11 comment AS 05/07/07

13 Results: Haemadsorption

Result table for the incubation in refrigerator:

	MRC-5	VERO	HELA	NIH 3T3
PSA ×2	1/1	×2 MIXTURE OF BLOOD TYPES		
PNC	0/1	0/1 ×1	0/1	0/1
PTA	0/1	0/1 ×1	0/1	0/1
PPC*	1/1	N/A	N/A	N/A
APC*	2/2	N/A	N/A	N/A
Initials/Date			CH 10/07/07	

* if applicable.

Result table for the incubation at room temperature:

	MRC-5	VERO	HELA	NIH 3T3
PSA ×2	1/1	×2 MIXTURE OF BLOOD TYPES		
PNC	0/1	0/1 ×1	0/1	0/1
PTA	0/1	0/1 ×1	0/1	0/1
PPC*	1/1			
APC*	2/2		N/A 10/07/07	
Initials/Date			10/07/07	

* if applicable. text deleted CH 20/9/07
Results (~~+ = haemadsorption observed, - = no haemadsorption observed~~).
All flasks were discarded following scoring.

×1 Some non specific binding of red blood cells to the cell monolayer observed. 10/07/07

×2 Text addition. 10/07/07

Results 0/1 = 0 of 1 flask positive for haemadsorption
2/2 = 2 of 2 flasks positive for haemadsorption
Text added in response to QA audit CH 20/9/07

Comments x13 lots of loose cells in media, not more than the PNC. Cells look unhealthy, debris in media AS 06/07/07

x14 Many floating cells, gaps in monolayers possibly due to overgrowth. AS 09/07/07

x15 Many floating cells. Cells gone in patches. Media appear very yellow in colour AS 09/07/07

x16 Perhaps early signs of cpe observed. AS 09/07/07

x17 See x14 comment. AS 10/07/07

x18 Possibly due to overgrowth some of the monolayers came off. AS 10/07/07

x19 See comment x15. AS 10/07/07

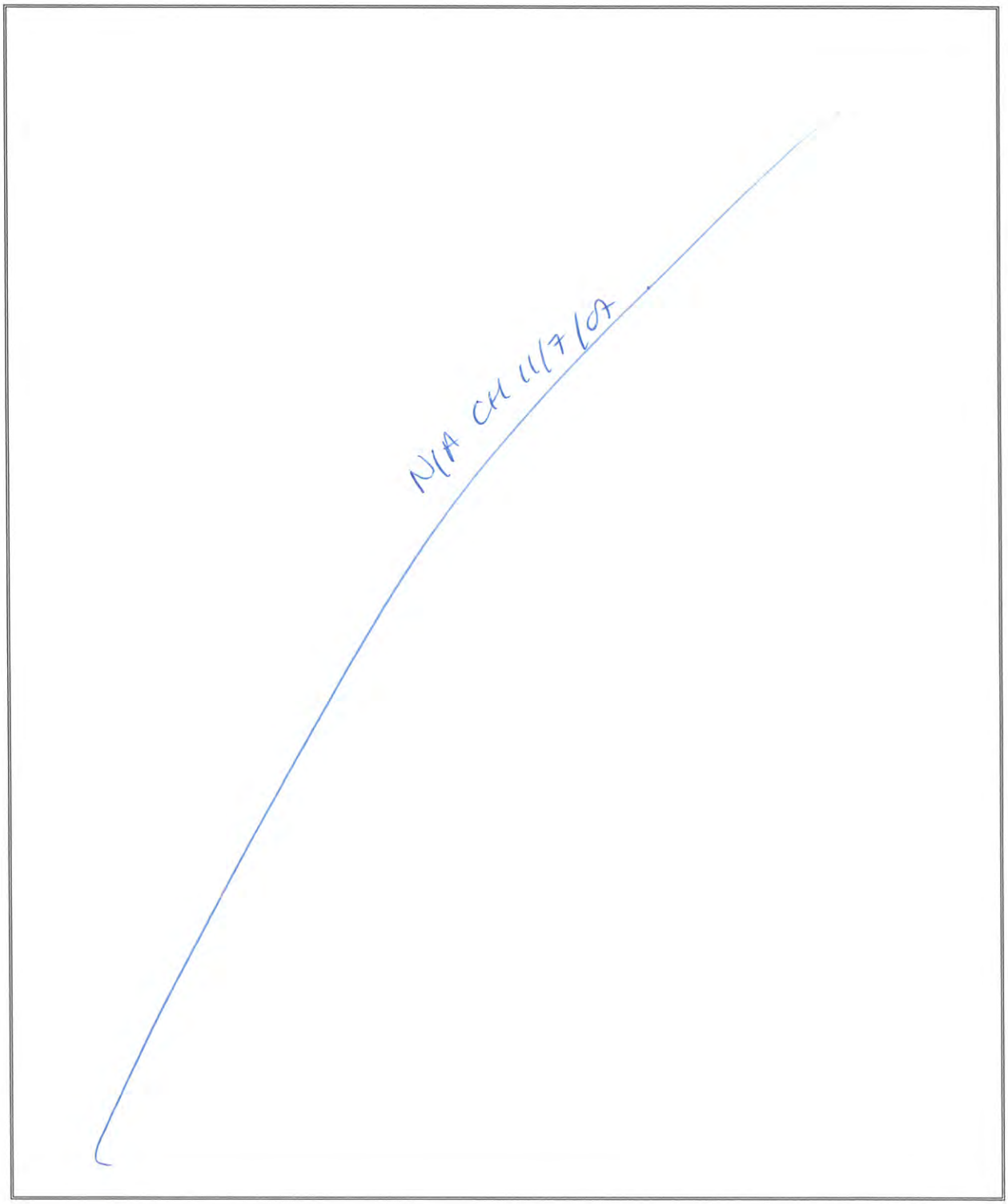
x20 Media looks very yellow in the all control flasks AS 10/07/07. X21

x21 Yellow media is a result of overgrowth of these cells CH 11/7/07.

MRC-5 APC 005 -

841 DATE	DAY	1×10^5	1×10^4
AS 06/07/07	24	0/1	0/1
AS 09/07/07	27	0/1	0/1
AS 10/07/07	28	1/1	1/1

N
H
AS 10/07/07


N/A CH 11/7/07

Day –1: Preparation of indicator cultures

Steps – Seeding of NIH 3T3 cells.	Task complete
Cell line details. Cell concentration required **1 x 10^5 cells/ml.** **NIH 3T3** passage number 112, C number 1, flasks confluency 100.	☑
Using a sterile pipette, aspirate the medium from 3 x T 150 flasks of Cells.	☑
Wash the flasks with sufficient D-PBS to cover the monolayer.	☑
Remove washings and add 20 ml of TrypLE select each flask. Incubate the flask at 37 ± 1°C for approximately 5 – 15 minutes.	☑
Gently agitate the flask to loosen the cell sheet and add 10.0 ml of complete medium. Mix the cell suspension.	☑
If multiple flasks are used pool in one sterile container. Final volume 36.0 ml	☑
Perform viable cell counts in the presence of Trypan Blue. Add 0.2 ml of cell suspension to 0.2 ml of Trypan blue and mixed.	☑
Load the haemocytometer and count 3 of the 16 squares.	☑
Dilute cell suspension and seed 6 T75 flasks with 15ml cell suspension at 1 x 10^5 cells/ml.	☑
Incubate all flasks at 37 ± 1°C with 5-10% CO_2.	☑
Initials/Date	RL 11/6/07

Cell Counts ×			
1. 22	**2.** 20	**3.** 27	**Mean** 23 **(B)**

Cell Concentration = (B x 2 x 10^4)	4.6 x 10^5 **Cells/ml**
Cell Concentration required	**1 x 10^5 cells/ml**
Dilution required	1 **In** 4.6
Total volume of cell suspension required (number of T75 flasks prepared x 15ml + excess)	100 **ml**
Volume of cell suspension (added to make up to required concentration)	21.7 **ml**
Volume of complete media (added to make up to required concentration)	78.3 **ml**
Calculation performed by/date	RL 11/6/07
Calculation checked performed by/date	AJS 11/06/07

× Cells also used for 2823-002. RL 11/6/07

Comments

N/A CH 27/7/07

2 Day 0: Inoculation of Indicator Cultures

Equipment

Lab Cat 3	ID Number	
Safety Cabinet	BS ,96	☐ N/A
Incubator	BS 225, 138	☐ N/A
Waterbath	BS 251	☐ N/A
Microscope	BS 243	☐ N/A
Micropipette		☑ N/A
Pipetaid	Clean Cat 3 pipettaid	☐ N/A
Other ( )		☑ N/A
Other ( )		☑ N/A
Initials/Date	DF 1.12.6.7	

Reagents

	RI code	Batch No	Exp. date	
MEM/5E – refeed media	RI/183	50507/07	11.8.07	☐ N/A
MEM/5TPB	RI/187	50576/07	24.8.07	☐ N/A
D-PBS	RI/025	50609/07	5/09	☐ N/A
HBSS	RI/024			☑ N/A
Other (DMEM/5E)		50663/07	6.9.7	☐ N/A
Other ( )				☑ N/A
Initials/Date		DF 1.12.6.7		

Day 0: Inoculation of Indicator Cultures

Steps - Perform the same steps for all cell lines.	Task completed
Aspirate medium from approx 30 % confluent **MRC-5** cells.	☑
Aspirate medium from approx 30 % confluent **Vero** cells.	☑
Aspirate medium from approx. 30 % confluent **HeLa** cells.	☑
Aspirate medium from approx. X² ~~30~~ % confluent **NIH 3T3** cells. 30 (DDF 18.6.7)	☑
Wash cells with sufficient amount of D-PBS	☑
Inoculate one flask per cell line with 5ml **negative control** (MEM/5TPB) and incubate flasks at 37 ± 1°C, 5% CO_2 for 60-90 min. Start time: 15.01 End time: ~~15.40~~ 16.20 (JDF 12.6.7)	☐ Start ☑ End
Inoculate one flask per cell line with 5ml **test article** and incubate flasks at 37 ± 1°C, 5% CO_2 for 60-90 min. (JDF 12.6.7) X¹ Start time: 15.10 End time: ~~15.50~~ 16.30	☐ Start ☐ End
After incubation aspirate the inoculum and **wash with ~10ml DPBS.** Then refeed cells with 15ml appropriate refeed media and incubate flasks at 37 ± 1°C, 5% CO_2.	☑
Initials/Date	JDF 12.6.07

Comments

X¹ There was only 20ml of TA cell lysate available. So the TA lysate was diluted 1 in 2 with 20ml of MEM/5TPB JDF 12.6.7

X² 30 was written, but was not clear % was re written clearer JDF 18.6.7

Day 0: Preparation of Inoculum for Positive Control

If same virus is used for all cell lines complete this page and N/A the following pages.

Steps - Preparation of positive control for MRC-5, Vero and HeLa	**Task completed**
Thaw rapidly a frozen ampoule of PI3 virus, batch 050721 at 1.1X10⁸ $TCID_{50}$/ml in waterbath set at 37 ± 1°C. Once thawed keep ampoule on ice until required.	☑
Perform virus dilution and complete the table below.	☑
Required virus concentration for spike test article control is **$5x10^5$TCID50/ml**	☑
Required virus concentration for positive control is **$1x10^4$TCID50/ml**	☑

Virus Suspension(ml)	**Diluent (ml)**	**Total Volume Required (ml)**	**Dilution**	**TCID50/ml**
0.5	5	5.5	1 in 11	1X10⁷
1	19	20	1 in 20	5X10⁵
1	49	50	1 in 50	1X10⁴
			1 in	
			1 in	

Calculation performed by/date	DC 12.6.07
Calculation check performed by/date	CH 12.6.07
Initials/Date	DC 12.6.07

Comments

N/A CH 27/7/07

Day 0: Preparation of Inoculum for Positive Control

☒ N/A

Steps - Preparation of positive control for NIH 3T3 cells	Task completed
Thaw rapidly a frozen ampoule of MVM virus, batch 00066 at 2×10^6 $TCID_{50}$/ml in waterbath set at 37 ± 1°C. Once thawed keep ampoule on ice until required.	☑
Perform virus dilution and complete table below.	☑
Required virus concentration for spike test article control is **5×10^5TCID50/ml**	☑
Required virus concentration for positive control is **1×10^4TCID50/ml**	☑

Virus Suspension(ml)	Diluent (ml)	Total Volume Required (ml)	Dilution	TCID50/ml
0.5	19.5	20	1 in 40	5×10^6
1	9	10	1 in 10	5×10^5
0.5	24.5	25	1 in 50	1×10^4
			1 in	
			1 in	

Calculated performed by/date	MK / 12.6.07
Calculation check performed by/date	AM / 12.6.07
Initials/Date	MK / 12.6.07

Comments

N/A CH 27/7/07

Day 0: Inoculation of Indicator Cultures

Steps	Task complete
Inoculate one flask per cell line with 5ml of the appropriate **spiked test article** (4.9ml test article + 0.1ml positive control) and incubate flasks at 37 ± 1°C, 5% CO_2 for 60-90 min. Start time: 15.47 End time: 17.08	☑ start ☑ end
Inoculate one flask per cell line with 5ml of the appropriate **positive control** and incubate flasks at 37 ± 1°C, 5% CO_2 for 60-90 min. Start time: 16.22 End time: 17.44	☑ start ☑ end
After incubation aspirate the inoculum and **wash with ~10ml DPBS.** Then refeed cells with 15ml appropriate refeed media and incubate flasks at 37 ± 1°C, 5% CO_2.	☑
Initials/Date	DF 15/2-6-7

Comments

N/A CH 27/7/07

3 Day 7: Refeed or Subculture of Cultures

Equipment

Lab 8 / 10 (1) DF 19.6.7	ID Number	
Safety Cabinet	BS198	☐ N/A
Incubator	BS 139, BS 228 x'	☐ N/A
Waterbath	BS 251	☐ N/A
Microscope	BS 266	☐ N/A
Pipetaid	BS 265	☐ N/A
Other ( )		☑ N/A
Other ( )		☑ N/A
Initials/Date	DF 19.6.7	

Reagents

	RI code	Batch No	Exp. date	
MEM/10E – seeding media	RI/184	5042407	17.7.07	☐ N/A
MEM/5E – refeed media	RI/183	5033107	25.6.7	☐ N/A
D-PBS	RI/025	5063407	05/09	☐ N/A
HBSS	RI/024			☑ N/A
TrypLE	RI/141	5052807	02/09	☐ N/A
Trypan blue (0.4%)	RI/044			☑ N/A
Other (DMEM/10E x²)	RI/190	5031507	21.6.7	☐ N/A
Other ( )				☑ N/A
Initials/Date		DF 19.6.7		

x' All flasks stored in BS228 after refeed / Subculture DF 21.6.7

x² See Enote# 231131 for further information DF 25.6.7

Steps	Task completed
Discard positive control flasks if show CPE. Confirmed by SD CH 19/6/07.	☑ (✓,N/A) by date DF 19.6.7
If cultures are 100% confluent confirm with SD how to proceed.	☑
Refeed	☑ (✓,N/A)
Flasks to be refed MRC-5 NC, MRC-5 TA, MRC-5 STA,	SD confirmed CH / 19/6/07 x
Aspirate medium from the flasks that show no CPE.	☑
Add 15 ml fresh refeed media.	☑
Incubate flasks at 37 ± 1°C, 5% CO_2	☑
Initials/Date	DF 19.6.7
Subculture	☑ (✓,N/A)
Flasks to be subcultured VERO NC 1 in 4, 3T3 NC 1 in 4, HeLa NC 1 in 2, VERO TA 1 in 4 3T6 SDDA 19.6.7 3T3 TA 1 in 4, HeLa TA 1 in 2, 3T3 STA 1 in 4	SD confirmed CH / 19/6/07 x
Aspirate medium from all the flasks that show no CPE and are 100% confluent.	
Wash the flasks with sufficient D-PBS to cover the monolayer.	☑
Remove washings and add 2 ml of TrypLE select. Incubate the flask at 37 ± 1°C for approx. 5-15 minutes.	☑
Gently agitate the flask to loosen the cell sheet and add 10ml of complete medium. Mixed the cell suspension. Final volume 12ml	☑
Seed N/A ml of cell suspension (equal to a 1 in N/A split) into fresh T75 flasks and make up to a total flask volume of 15 ml with complete media.	☑
Flask labelled with cell line, study number and test material (eg PNC, PTA etc)	☑
Return the flask to incubators at 37 ± 1°C 5% CO_2	☑
Initials/Date	DF 19.6.7

x SD has annotated on flask which flasks are to be refed and which are to be subcultured and at what ratio CH 19/6/07.

Page completion check initials/date KHa 19 June 07

Data check initials/date CH 27/7/07

Page 16a to follow

18/12/2007

Data Index Listings

Study Number: 2823/002

Study Title: In vitro evaluation of adventitious viruses in cell cultures - 28 day assay

Contact Name: **Study Director**

Study Type: In vitro Adventitious **Test Article:** H9-MCB.1

Report Date: 02/10/2007

Box Number:	**90118612**	Type of Data:	Archive Confirmation Form Final Report Protocol File Study File eNotes

Total Number of boxes for this study: 1

COVANCE
THE DEVELOPMENT SERVICES COMPANY

Archival Confirmation

Study Number:	2823-002	Study Director/Manager:
Department:	Biotechnology	
Study Title:	*In Vitro* Evaluation of Adventitious Viruses in Cell Cultures – 28 day assay	

Please complete one of the following, and enter any additional relevant information:-

☑ I hereby confirm that this study has been finalised. The protocol and all data including E notes for the above study are now lodged in Central Scientific Records (CSR)

☐** I hereby confirm that the protocol and all data for this study, for which a formal claim of FDA/EPA GLP compliance is to be made, has been submitted to CSR.

☐ I hereby confirm that the above study has been cancelled/aborted, and no final report is to be issued. I confirm that the necessary amendments have been made to the protocol. The protocol including E notes and any data generated during the study are now lodged in CSR, and the archive period should commence from the following date

☐* I hereby confirm that the above study was a non-regulatory study, therefore no final report was issued. All study data, protocol ,E notes and letter report (delete if not applicable) is now lodged in CSR and the archiving period should commence from the following date

☐* I hereby confirm that the above study was a non-GLP study and that all reports, protocol and all study data including E notes are now lodged in CSR. The archiving period should commence from the following date

☐* I hereby confirm that the study did not commence and an unsigned protocol and/or study correspondence including E notes are lodged in CSR, and this may now be destroyed.

☐* If any of these options are marked then the Study Director/Manager must send a copy of this form to QA

☐** Confirmed by CSR dated/Signature .....

Additional Information:

Study Director/Manager / Head of Department

Date 12/Dec/07

N.B. - This form must be signed by the Study Director/Manager, and lodged with CSR as soon as one of the above criteria has been met, but not later than 3 months after issue of the final report. Where the Study Director/Manager has left the employment of Covance, the Head of Department should sign.

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THE DEVELOPMENT SERVICES COMPANY

Archive Summary

Study Number 2823-002		Responsible Person	Department Biotechnology
Item	**Type**	**Comment**	**Packet Bar Code**
1.	Protocol File	Protocol Reading List Client Protocol	00140657
2.	Study File	TAD Working Documents	00140658

Lodged By	**Accepted By**
Signature	**Signature**
Printed Name	**Printed Name**
Date	**Date**

Box 90118612.

Final Report

Study Title	*In Vitro* Evaluation of Adventitious Viruses in Cell Cultures – 28 day assay
Test Article	H9-MCB.1
Author	
Test Facility	Covance Laboratories Ltd
Sponsor Representative	
Sponsor	
Covance Study Number	2823/002
Covance Report Number	2823/002-D5141
Report Issued	October 2007
Page Number	1 of 28

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STUDY DIRECTOR AUTHENTICATION AND GLP COMPLIANCE STATEMENT

In Vitro Evaluation of Adventitious Viruses in Cell Cultures – 28 day assay

I, the undersigned, hereby declare that the work was performed by me or under my supervision and that the findings provide a true and accurate record of the results obtained.

The study was performed in accordance with the agreed Protocol and with Covance Laboratories Limited Standard Operating Procedures, unless otherwise stated, and the study objectives were achieved.

The study was conducted in compliance with:

The United Kingdom Statutory Instrument 1999 No.3106, the Good Laboratory Practice Regulations 1999, as amended by the Good Laboratory Practice (Codification Amendments Etc.) Regulations 2004.

The Organisation for Economic Co-operation and Development (OECD) Principles on Good Laboratory Practice (revised 1997, issued January 1998). ENV/MC/CHEM(98)17.

02/Oct/07
Date

Study Director

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QUALITY ASSURANCE STATEMENT

In Vitro Evaluation of Adventitious Viruses in Cell Cultures – 28 day assay

This study has been reviewed by the Quality Assurance Unit of Covance Laboratories Ltd. and the report accurately reflects the raw data. The following inspections were conducted and findings reported to the study director (SD) and associated management.

Critical procedures, which are performed routinely in an operational area, may be audited as part of a "process" inspection programme. This can be in addition to phases scheduled on an individual study basis. Selected process inspections conducted and considered applicable to this study are included below.

In addition to the inspection programmes detailed below, a facility inspection programme is also operated. Details of this programme, which covers all areas of the facility annually (at a minimum), are set out in standard operating procedures.

Inspection Dates		Phase	Date Reported to SD and SD Management
From	To		
12 Jun 2007	12 Jun 2007	Protocol Review	12 Jun 2007
13 Aug 2007	13 Aug 2007	Draft Report and Data Review	13 Aug 2007
02 Oct 2007	02 Oct 2007	Final Report Review	02 Oct 2007

Process

Inspection Dates		Phase	Date Reported to SD and SD Management
From	To		
04 Jul 2007	04 Jul 2007	Test Article Receipt	04 Jul 2007

2 Oct 07

Date

Quality Assurance Unit

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RESPONSIBLE PERSONNEL

In Vitro Evaluation of Adventitious Viruses in Cell Cultures – 28 day assay

The following personnel were responsible for key elements of the study:

Study Director:

Study Supervisor:

STUDY SCHEDULE

The study schedule was as follows:

Study initiation date:	6th June 2007 (Date Study Director signed Client Protocol).
Assay initiation date:	6th June 2007 (Date of the first study specific data capture).
Assay completion date:	10th July 2007 (Date of final data capture).
Study completion date:	Date Study Director signed Final Report.

ARCHIVE STATEMENT

In Vitro Evaluation of Adventitious Viruses in Cell Cultures – 28 day assay

All primary data, or authenticated copies thereof, specimens and the Final Report will be retained in the Covance Laboratories Limited archives for one year after issue of the Final Report. At this time, the Sponsor will be contacted to determine whether the data should be returned, retained or destroyed on their behalf. Sponsors will be notified of the financial implications of each of these options at that time.

Specimens or samples requiring frozen storage at Covance are specifically excluded from the above. These will be retained for as long as the material permits further evaluation, up to three months after issue of the Draft Report. At this time, the Sponsor will be contacted to determine whether samples should be returned, retained or destroyed on their behalf. Any financial implications of these options will also be notified at this time. Samples will not be destroyed without prior approval of the Study Director.

CONTENTS

SUMMARY

Four indicator cell lines (Vero, MRC-5, HeLa and NIH 3T3) were inoculated with either negative control (diluent), positive control virus or test article and incubated for 28 days. All positive controls turned positive for cytopathic effect (CPE). A fresh positive control for the MRC-5 cell line was set up on day 23 and this was positive for haemadsorption, as was the original MRC-5 positive control. All test article and negative control inoculated cells were negative for CPE and haemadsorption.

INTRODUCTION AND OBJECTIVE

The objective of this study was to determine the viral status of the test article. The assay was to detect the presence of viruses that induce CPE in culture cells; furthermore, the assay was to detect viruses capable of inducing haemadsorption.

MATERIALS

Protocol Adherence

The study described in this report was carried out according to the agreed Client Protocol, see Annex for details. Minor deviations, which are deemed not to have affected the study, are presented in the Appendix.

Test Article

The test article was received at Covance Laboratories Ltd on 29th March 2007 in two 15 ml centrifuge tubes each containing approximately 11 mls of an orange frozen material. The sample was received on dry ice and stored according to Sponsor instructions until required for the assay.

Identification:	H9-MCB.1
Source:	Sponsor.
Details on Test Article Vessel:	Covance 2 x 10 ml @ $1x10^6$ c/ml MCB.A.H9p27 22 JAN07 DF

Appearance:	Orange frozen material.
Description:	Cell suspension.
Storage conditions:	< -70°C.
Sterility check performed:	No.

This study to determine the presence of extraneous agents was conducted to define the purity of the test substance therefore, information regarding the purity of the test article was not available at study initiation. Stability of the test article was not considered relevant, as the objective of the study was to test for extraneous agents (adventitious viruses) that may be present in the test material.

Test Article Preparation

Prior to the assay starting, a cell lysate was prepared by freeze-thawing the test article three times in liquid nitrogen and a waterbath set at 37°C. The test article was then clarified by centrifugation.

TEST SYSTEM

Positive control virus:	*Parainfluenza 3* (PI3) strain SF-4 used at approximately $1x10^4$ $TCID_{50}$/ml (control for Vero, HeLa and MRC-5 cells). Minute virus of mice (MVM) used at approximately $1x10^4$ $TCID_{50}$/ml (control for CPE on NIH 3T3)
Source:	Maintained as laboratory stocks, original stocks supplied by ATCC.
Negative control (virus diluent):	Minimal essential medium + 5% tryptose phosphate broth.
Source:	Minimum essential medium. Tryptose phosphate broth. Gentamycin. Fungizone. Supplied by Invitrogen.

Indicator cell lines:	MRC-5. Vero. HeLa NIH 3T3
Source:	Maintained as laboratory stocks, original stocks supplied by ATCC or ECACC.
Growth medium:	Minimal essential medium containing Earles salts, non-essential amino acids plus 10% foetal calf serum. Minimum essential medium containing Earles Salts, non-essential amino acids plus 5% foetal calf serum for the re-feed.
Source:	Minimum essential medium. Non-essential amino acids. Gentamycin. Fungizone. Supplied by Invitrogen. Foetal calf serum. Supplied by PAA.

EXPERIMENTAL PROCEDURES AND DATA ANALYSIS

The experimental procedures were performed as outlined in the Client Protocol, see Annex for details. Minor deviations deemed not to have affected the study are presented in the Appendix.

The assay acceptance and evaluation criteria as detailed in the Client Protocol were achieved, see Annex for details.

RESULTS

Four indicator cell lines (Vero, MRC-5, HeLa and NIH 3T3) were inoculated with either negative control (diluent), positive control virus, test article or test article spiked with positive control and incubated for 28 days. MRC-5 cells that were negative for CPE were refed and Vero, HeLa and NIH 3T3 cells that were negative for CPE were subcultured on day 7. On day 13 and day 20 the NIH 3T3 cells were looking unhealthy so were refed to revive them. On day 14 and day 21 all cells negative for CPE were subcultured. They were observed for CPE and haemadsorption. Positive controls and spiked test article-inoculated cells for Vero and HeLa cells were positive for CPE by day 7. The positive control and spiked test article-inoculated cells for MRC-5 and NIH 3T3 cells were positive by day 28. A fresh positive control for the MRC-5 cell line was set up on day 23 and this was positive for haemadsorption with Guinea Pig, Human O and Adult Chicken blood at 7.6°C and 24.0°C. All test article and negative control-inoculated cells were negative for CPE and haemadsorption.

TABLES

Table 1: Day 14 and 28 Observation for Cytopathic Effect using Indicator Cells Inoculated with Test Article, Spike Test Article, Positive and Negative Controls

Sample	Indicator cell lines			
	Vero	MRC-5	HeLa	NIH 3T3
Indicator Assay First 14-Day Period Observations				
Negative Control	–	–	–*[3]	–*[1]
Test Article	–	–	–*[3]	–*[1]
Spiked Test Article	+	–*	+	–*[1]
Positive Control	+	–*	+	–*[1]
Indicator Assay Second 14-Day Period Observations				
Negative Control	–	–	–*[3]	–*[1]*[3]
Test Article	–	–	–*[3]	–*[1]*[3]
Spiked Test Article	N/A	+*[2]	N/A	+
Positive Control	N/A	+*[4]	N/A	+

\+ = Some or all flasks exhibited CPE.
– = Flasks did not exhibit CPE (normal morphology observed).
N/A = Not applicable as cells were discarded in first 14 days.
* = Some vacuolation observed but not positive for CPE.
*[1] = Cells were very overgrown and starting to die so were refed on day 13 and day 20.
*[2] = Early signs of CPE observed, which was confirmed as viral in haemadsorption assay (Table 2).
*[3] = Floating cells observed due to overgrowth.
*[4] = Both original and fresh positive control (for haemadsorption assay) were positive for CPE

Table 2: Day 28 Observation for Haemadsorption using Indicator Cells Inoculated with Test Article, Spiked Test Article, Positive and Negative Controls

Sample	Indicator cell lines			
	Vero	MRC-5	HeLa	NIH 3T3
		1-10°C Incubation		
Negative Control	–*[1]	–	–	–
Test Article	–*[1]	–	–	–
Spiked Test Article	N/A	+	N/A	N/A
Positive Control	N/A	+	N/A	N/A
Fresh Positive Control	N/A	+*	N/A	N/A
		37 ± 1°C Incubation		
Negative Control	–*[1]	–	–	–
Test Article	–*[1]	–	–	–
Spiked Test Article	N/A	+	N/A	N/A
Positive Control	N/A	+	N/A	N/A
Fresh Positive Control	N/A	+*	N/A	N/A

All Indicator cell lines were tested with a mixture of Adult Chicken blood, Guinea Pig blood and Human O blood.
+ = Haemadsorption observed.
– = No haemadsorption observed.
N/A = Not applicable.
* = Two fresh positive controls were set up, one inoculated with $1x10^4$ $TCID_{50}$/ml and one with $1x10^5$ $TCID_{50}$/ml, both were positive for haemadsorption.
*[1] = Some non-specific binding observed.

CONCLUSION

The test article (H9-MCB.1) was assessed for presence of adventitious viruses that are capable of causing cytopathic effects or haemadsorption within this system. The results show that no evidence of viral contamination was observed in the test article.

APPENDIX

Minor Deviations from the Protocol

1. DMEM was used to culture the 3T3 cell line during this study. The Protocol states MEM should be used, but DMEM is the preferred medium for this cell line. This deviation should improve the growth of the cells and would therefore not impact on the outcome of the study.

2. The NIH 3T3 cells were refed on day 13 and day 20 and then subcultured on day 14 and day 21. The refeed is in deviation to the Protocol but was necessary has cells had overgrown and the media had changed colour. This is a minor deviation to the Protocol that would not affect the outcome of the study.

3. The fresh positive control for the MRC-5 cell line was set up on day 23 instead of day 14 as stated in the Protocol. This is a minor deviation to the Protocol that did not affect the outcome of the study as the cells were positive for haemadsorption on day 28.

4. Medium containing 10% FCS was used for the subculture of cells on day 21. This is a deviation to the Protocol that states 5% FCS should be used following inoculation. This is considered a minor deviation to the Protocol that did not affect the outcome of the study as the cells were at a suitable concentration of the haemadsorption on day 28.

ANNEX

The Annex consists of 13 pages, including this one, and includes:

- Client Protocol (12 pages)

CLIENT PROTOCOL

Procedure Number	49001
Version Number	00
Supersedes	N/A
Study Title	In Vitro Evaluation of Adventitious Viruses in Cell Cultures – 28 day assay
Test Facility	Covance Laboratories Ltd.,

Protocol Produced on: 29 May 2007

Prepared at Covance Laboratories by

29/May/07
Date

INTRODUCTION

Rationale

The cell lines utilised in this assay are sensitive to a wide variety of viruses. The end-points utilised, cytopathic effect (CPE) and haemadsorption with three types of erythrocytes, provide a series of methods for evaluating the viral status of the test article cells [1, 2 & 3].

Objective

The objective of this assay is to evaluate the adventitious viral status of the test article.

TEST ARTICLE

The test article will be supplied by the Sponsor and all relevant information completed on the Test Article Safety & Pre-Study Questionnaire.

This study for the presence of extraneous agents will be conducted to define the purity of the test substance; therefore, information regarding the purity of the test article is not available at study initiation. Stability of the test article is not considered relevant, as the objective of this study is to test for extraneous agents (adventitious viruses) that may be present in the test material.

TEST SYSTEM

Positive control virus:	*Parainfluenza type 3* (PI3) strain SF-4 used at approximately $1x10^4$ $TCID_{50}$/ml (control for Vero, HeLa and MRC-5 cells). *MVM virus* used at approximately $1x10^4$ $TCID_{50}$/ml (control for CPE on NIH 3T3).
Source:	Maintained as laboratory stocks, original stocks supplied by ATCC.
Negative control:	Minimal essential medium + 5% tryptose

(virus diluent)	phosphate broth.
Source:	Minimum essential medium. Tryptose phosphate broth. Gentamycin. Fungizone. Supplied by Invitrogen.
Indicator cell lines:	MRC-5. Vero. HeLa NIH 3T3.
Source:	Maintained as laboratory stocks, original MRC-5, Vero, HeLa and NIH 3T3 stocks supplied by ATCC or ECACC.
Growth medium:	Minimal essential medium containing Earles salts, non-essential amino acids plus 10% foetal calf serum for culture establishment. Minimum essential medium containing Earles Salts, non-essential amino acids plus 5% foetal calf serum will be used for growth of cultures post inoculation.
Source:	Minimum essential medium. Non-essential amino acids. Gentamycin. Fungizone. Supplied by Invitrogen. Foetal calf serum. Supplied by PAA.

EXPERIMENTAL PROCEDURES

Preparation of Test Article

The test article will be prepared according to standard methods. Where needed the inoculation volume and surface of indicator cells, as described below, may be adapted. Any alterations will be noted in study records

Adventitious Virus Assay

For each indicator cell line, the following will be carried out:

Indicator cells will be seeded into T 75 flasks (4 x T 75 flasks). A flask will be labelled as the test article, a flask as the positive control, a flask as the test article interference control and a flask as the negative control. All flasks will be labelled with the cell type, the study number, and any other relevant information. The flasks will be incubated at 37 ± 1°C in a humidified 5% CO_2 in air incubator until required for assay.

For each indicator cell type, the medium will be aspirated from sub-confluent cultures and the cell sheets will be washed with a balanced salt solution. The final wash will be aspirated from the wells and 5ml of test article will be inoculated into a flask of each indicator cell line. To determine if the test article interferes with the assays ability to detect the relevant viruses, 5ml of test article spiked with the relevant positive control at an appropriate concentration will be inoculated into a flask of each indicator cell line. Appropriate positive and negative controls will be similarly inoculated into a flask of each indicator cell line. Flasks will be incubated at 37 ± 1°C for 60-90 minutes. The inoculum will be aspirated from the flasks and cells washed in a balanced salt solution, then refed with the appropriate growth medium. Flasks will be incubated and observed for cytopathic effect over 14 days. Each culture will be refed or subcultured on approximately day 7 with the appropriate growth medium.

If the test article inoculated indicator cells give a negative result on day 14, the test article and any other cultures negative for CPE will be split and seeded appropriately into fresh T75 flask. If positive controls turn positive they will be discarded. A fresh positive control will also be set up for the MRC-5 cell line and inoculated with the appropriate positive control virus. These flasks will then be incubated and observed for cytopathic effect over a further 14 days. Each culture will be refed or subcultured on approximately day 21 with the appropriate growth medium If the positive control shows CPE too early a fresh positive control may be set up for the MRC-5 cell line inoculated with the appropriate positive control virus. On day 28, a haemadsorption assay will be

carried out on the flasks inoculated with test article, the flasks inoculated with negative control and the MRC-5 flask inoculated with positive control.

For each indicator cell type, the haemadsorption assay will be carried out as follows:

The cell sheets will be washed with Dulbecco's Phosphate Buffered Saline and after aspirating the final wash, a mix of adult chicken, guinea pig and human type O blood will be added to each flask. Firstly, the flasks will be placed in the refrigerator for 30 ± 5 minutes and then incubated at room temperature for 30 ± 5 minutes. At the end of each incubation period the cell sheets will be observed for haemadsorption and the results recorded.

All culture vessels and plates will be discarded by the end of the experiment.

DATA ANALYSIS

Assay Acceptance Criteria

The assay will be considered acceptable if:

1. The positive control and test article spiked with positive control viruses cause CPE in all the indicator cells used.

2. The positive control inoculated cells cause haemadsorption in the indicator cells used.

3. The negative control inoculated indicator cells show normal morphology.

4. The negative inoculated indicator cells show no evidence of haemadsorption.

Evaluation Criteria

1. The assay will be considered as positive if any test article inoculated indicator cells or test article cells show a cytopathic effect or haemadsorption with any of the blood types assayed.

2. The test article will be considered to have interfered with the assay for the detection of adventitious viruses if CPE is not detected in all of the indicator cell lines inoculated with test article spiked with the appropriate positive control.

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Following completion of the study a draft report will be issued. Client comments should be supplied for inclusion into a final document within six weeks of receipt of the draft document. If no client comments are received within six weeks of issue, the report will be finalised. Any further changes after this time will be addressed as an amended final report which may result in additional costs.

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United Kingdom Statutory Instrument 1999 No. 3106, The Good Laboratory Practice Regulations 1999, as amended by the Good Laboratory Practice (Codification Amendments Etc.) Regulations 2004.

OECD Principles on Good Laboratory Practice (revised 1997, Issued Jan 1998) ENV/MC/CHEM(98)17

All procedures will be performed in accordance with Covance Laboratories standard operating procedures (SOPs). The study will be subject to Quality Assurance evaluation by the Covance Laboratories Quality Assurance Department (QAD) in accordance with SOPs.

REFERENCES

[1] Jacobs J P, Magrath D I, Garrett A J and Schild G C (1981) Guidelines for the acceptability, management, and testing of serially propagated human diploid cells for the production of live virus vaccines for use in man. J Biol Standard *9*, 331-342.

[2] Schmidt N J (1979) Cell culture techniques for diagnostic virology. In: Diagnostic procedures for viral, rickettsial and chlamydial infections 5th edition. E H Lennette and N J Schmidt, editors. American Public Health Association, Washington, DC.

[3] Poiley JA (1990) Methods for the detection of adventitious viruses in cell culture used in the production of biotechnology products. In: Large-scale mammalian cell culture technology. (AS Lubiniecki, ed) Marcel Dekker Inc.

ARCHIVE STATEMENT

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The study records will be prepared to contain the following information:

Client Protocol, file note(s)*, study related correspondence*, Test Article Safety and Pre-Study Questionnaire, Test article receipt and utilisation, metrology#, records for reagents and stock solutions#, Test article cell culture records#, work sheets, indicator cell culture records#, positive control culture records#.

* Where appropriate.
# Some records held centrally.

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- Any unforeseen circumstances which may have affected the quality or integrity of the study.

The following items will be presented in the Draft Report:

- Summary.
- Results.
- Conclusion.
- The name and address of the testing facility and the dates, on which the study was initiated, assay initiation and assay completion.

The Protocol will be attached as an Annex to the Report detailing proccdures, assay acceptance criteria, evaluation criteria and references. Minor deviation(s) from the Protocol (if applicable) will be presented in the Report.

Final Report

The Final Report will be issued following Quality Assurance Department (QAD) evaluation of the Draft Report. The report will include all details described above and will include but not be limited to the following additions:

- Signed Quality Assurance statement.

- The signature of the Study Director for date of study completion and authentication of the Report.

PROTOCOL REVISION SUMMARY

Protocol Number: 49001

Protocol Title: In Vitro Evaluation of Adventitious Viruses in Cell Cultures – 28 day assay

Version Number	Revision Description	Authorisation Date
00	First issue	29 May 2007

TEST ARTICLE DETAILS AND STUDY AUTHORISATION

Test Article(s):
(As it should appear
on all documentation) H9-MCB.1

Experimental Phase

Start Date: 6th June 2007

End Date: 10th August 2007

6/June/07.
Date

Study Director

6th June 2007
Date

Covance Biotechnology Management

SPONSOR ACCEPTANCE SHEET

Sponsor Name

Title

Sponsors Company

Sponsor Address

Sponsor Contact Details

Telephone

e-mail

5/29/07
Date

Sponsor Approval

6/4/07
Date

Sponsor QA

This form must be completed, signed and returned to Biotechnology Services Administration, Covance Laboratories by post.

A faxed copy sent to, can be used for assay initiation.

THE DEVELOPMENT SERVICES COMPANY

Report Copying Request

28

Department:	**Biotechnology**	Cost Centre:	**D5141**
Report Number:	**2823-002**	Report Type:	**Final**
Study Director:		Extension:	**8335**
Study Co-ordinator:		Extension:	**8930**

Special Instructions for Amount of Copies

Client Copies (sponsor, extra, etc)	*Single or Double Sided*	*Bound or Unbound*	*Amount*
Sponsor	**Single**	**Unbound**	**1**
Sponsor	**Double**	**Bound**	**1**
SD	**Double**	**Bound**	**1**
TOX ADMIN			
QA			
CSR	**Double**	**Bound**	**1**

Extra Special Instructions for Sponsor Copies

Original Signatures to be Included in	Copied Signatures to be Included in	Paper Required	Split Volumes
All Sponsor Copies	**No Sponsor Copies**	**A4 paper**	**N/A**
Type of Binding	**Comb Strip**	Insertions	**N/A**

Is the address on the letter different to the address on the Report title page? **No**

If to be despatched with other report(s), please state study number(s): **2823-001**

Please state name and address of client for extra copies to be sent: **N/A**

CD N/A ☒	CD to Follow ☐	Admin to scan report as PDF copy onto CD ☐	CD Included ☐
Letter Included ☒	Letter to Follow ☐	Letter N/A ☐	

If the report has been sent to the sponsor via e-mail please enter the date so Admin can ensure CMS is updated correctly:

Does the client require a Customer Satisfaction Survey: **Yes**

If not then an email must be sent to say report is on it's way: **N/A**

For Japanese clients a hard copy survey should be supplied to Admin Services and CLJ are copied into emails.

Please type an email address:

Please give a telephone number of the sponsor(s) receiving the report col

Any other comments: **Please PDF and forward to**

PLEASE STATE REQUIRED DATE OF DESPATCH, ON/BY
(PLEASE GIVE SPECIFIC DATE IF NECESSARY):

COVANCE
THE DEVELOPMENT SERVICES COMPANY

Report Copying Request

Admin Use Only

Report Study Number	2823|002	Report Type	Final

Report Checks	1st Check	2nd Check
Contents page checked/headers, date and pages counted	✓	
SC/SD informed of errors	—	
Errors logged on spreadsheet	—	
Checked CD for correct study number	—	
Report Scanned as PDF file onto CD	—	
CD Labels, Trays and covers made for PDF report	—	
Printing of report on Doc90/Fiery 2101	☑	
Checked for marks	CC	
Total pages counted/Checked pages are in numerical order	CC	
Bound/Unbound (If bound see reverse for type of binding)	L.W.	
Dates and details added to CMS	☑	
Shipping labels and Shipping request form	☑	
Customer Satisfaction Survey/Email sent	☑	
PDF version of report sent electronically to sponsor with Disclaimer	—	
PDF version of report sent electronically to SD/SC	☑	

Any other comments:	
TOTAL AMOUNT OF COPIES 4	*TOTAL AMOUNT OF PAGES* 28

PLEASE STAMP DATE OF DESPATCH HERE: 04 OCT 2007

OVERALL SIGN OFF CHECK ..... MJ .....

COVANCE
THE DEVELOPMENT SERVICES COMPANY

Archive Packet Contents

Study Number	2823-001	**Responsible Person**		**Department**	Biotechnology
Item Number	1	**Type**	Protocol File		
Description				**Comments**	
Protocol Reading List Client Protocol					

Prepared by	L Brown	**Date**	11-12-07	**Checked by**	**Date** 12/12/2007

COVANCE
THE DEVELOPMENT SERVICES COMPANY

PROTOCOL READING LIST

Study Title: In vitro Evaluation of Adventitious Viruses in cell cultures - 28 day assay.

Covance Study Number: 2823-001

Name	Definitive protocol read	Amendment number read	Signature/date
	✓	N/A	AS 12/6/07
	✓	N/A	SB 18/6/07
	✓	NA	RL 18/6/07
	✓	N/A	DF 19.6.07
	✓	N/A	JHS 29/06/07
	✓	N/A	etq 4/July 07
	✓	NA	11.07.07
	✓	NA	11.07.07

Page completion check by ..CH.. date ..27/7/07..

CLIENT PROTOCOL

Procedure Number	49001
Version Number	00
Supersedes	N/A
Study Title	In Vitro Evaluation of Adventitious Viruses in Cell Cultures – 28 day assay
Test Facility	Covance Laboratories Ltd.,

Protocol Produced on: 29 May 2007

Prepared at Covance Laboratories by _____ Date 29/May/07

INTRODUCTION

Rationale

The cell lines utilised in this assay are sensitive to a wide variety of viruses. The end-points utilised, cytopathic effect (CPE) and haemadsorption with three types of erythrocytes, provide a series of methods for evaluating the viral status of the test article cells [1, 2 & 3].

Objective

The objective of this assay is to evaluate the adventitious viral status of the test article.

TEST ARTICLE

The test article will be supplied by the Sponsor and all relevant information completed on the Test Article Safety & Pre-Study Questionnaire.

This study for the presence of extraneous agents will be conducted to define the purity of the test substance; therefore, information regarding the purity of the test article is not available at study initiation. Stability of the test article is not considered relevant, as the objective of this study is to test for extraneous agents (adventitious viruses) that may be present in the test material.

TEST SYSTEM

Positive control virus:	*Parainfluenza type 3* (PI3) strain SF-4 used at approximately $1x10^4$ $TCID_{50}$/ml (control for Vero, HeLa and MRC-5 cells).
	MVM virus used at approximately $1x10^4$ $TCID_{50}$/ml (control for CPE on NIH 3T3).
Source:	Maintained as laboratory stocks, original stocks supplied by ATCC.
Negative control:	Minimal essential medium + 5% tryptose

(virus diluent)	phosphate broth.
Source:	Minimum essential medium. Tryptose phosphate broth. Gentamycin. Fungizone. Supplied by Invitrogen.
Indicator cell lines:	MRC-5. Vero. HeLa NIH 3T3.
Source:	Maintained as laboratory stocks, original MRC-5, Vero, HeLa and NIH 3T3 stocks supplied by ATCC or ECACC.
Growth medium:	Minimal essential medium containing Earles salts, non-essential amino acids plus 10% foetal calf serum for culture establishment. Minimum essential medium containing Earles Salts, non-essential amino acids plus 5% foetal calf serum will be used for growth of cultures post inoculation.
Source:	Minimum essential medium. Non-essential amino acids. Gentamycin. Fungizone. Supplied by Invitrogen. Foetal calf serum. Supplied by PAA.

EXPERIMENTAL PROCEDURES

Preparation of Test Article

The test article will be prepared according to standard methods. Where needed the inoculation volume and surface of indicator cells, as described below, may be adapted. Any alterations will be noted in study records

Adventitious Virus Assay

For each indicator cell line, the following will be carried out:

Indicator cells will be seeded into T 75 flasks (4 x T 75 flasks). A flask will be labelled as the test article, a flask as the positive control, a flask as the test article interference control and a flask as the negative control. All flasks will be labelled with the cell type, the study number, and any other relevant information. The flasks will be incubated at 37 ± 1°C in a humidified 5% CO_2 in air incubator until required for assay.

For each indicator cell type, the medium will be aspirated from sub-confluent cultures and the cell sheets will be washed with a balanced salt solution. The final wash will be aspirated from the wells and 5ml of test article will be inoculated into a flask of each indicator cell line. To determine if the test article interferes with the assays ability to detect the relevant viruses, 5ml of test article spiked with the relevant positive control at an appropriate concentration will be inoculated into a flask of each indicator cell line. Appropriate positive and negative controls will be similarly inoculated into a flask of each indicator cell line. Flasks will be incubated at 37 ± 1°C for 60-90 minutes. The inoculum will be aspirated from the flasks and cells washed in a balanced salt solution, then refed with the appropriate growth medium. Flasks will be incubated and observed for cytopathic effect over 14 days. Each culture will be refed or subcultured on approximately day 7 with the appropriate growth medium.

If the test article inoculated indicator cells give a negative result on day 14, the test article and any other cultures negative for CPE will be split and seeded appropriately into fresh T75 flask. If positive controls turn positive they will be discarded. A fresh positive control will also be set up for the MRC-5 cell line and inoculated with the appropriate positive control virus. These flasks will then be incubated and observed for cytopathic effect over a further 14 days. Each culture will be refed or subcultured on approximately day 21 with the appropriate growth medium If the positive control shows CPE too early a fresh positive control may be set up for the MRC-5 cell line inoculated with the appropriate positive control virus. On day 28, a haemadsorption assay will be

carried out on the flasks inoculated with test article, the flasks inoculated with negative control and the MRC-5 flask inoculated with positive control.

For each indicator cell type, the haemadsorption assay will be carried out as follows:

The cell sheets will be washed with Dulbecco's Phosphate Buffered Saline and after aspirating the final wash, a mix of adult chicken, guinea pig and human type O blood will be added to each flask. Firstly, the flasks will be placed in the refrigerator for 30 ± 5 minutes and then incubated at room temperature for 30 ± 5 minutes. At the end of each incubation period the cell sheets will be observed for haemadsorption and the results recorded.

All culture vessels and plates will be discarded by the end of the experiment.

DATA ANALYSIS

Assay Acceptance Criteria

The assay will be considered acceptable if:

1. The positive control and test article spiked with positive control viruses cause CPE in all the indicator cells used.

2. The positive control inoculated cells cause haemadsorption in the indicator cells used.

3. The negative control inoculated indicator cells show normal morphology.

4. The negative inoculated indicator cells show no evidence of haemadsorption.

Evaluation Criteria

1. The assay will be considered as positive if any test article inoculated indicator cells or test article cells show a cytopathic effect or haemadsorption with any of the blood types assayed.

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PROTOCOL REVISION SUMMARY

Protocol Number: 49001

Protocol Title: In Vitro Evaluation of Adventitious Viruses in Cell Cultures – 28 day assay

Version Number	Revision Description	Authorisation Date
00	First issue	29 May 2007

TEST ARTICLE DETAILS AND STUDY AUTHORISATION

Test Article(s):
(As it should appear
on all documentation) H1-MCB.1

Experimental Phase

Start Date: 6th June 2007

End Date: 10th August 2007

6th June 2007
Date

Study Director

6th Jun. 2007
Date

Covance Biotechnology Management

SPONSOR ACCEPTANCE SHEET

Sponsor Name

Title

Sponsors Company

Sponsor Address

Sponsor Contact Details

Telephone

e-mail

5/29/07
Date

Sponsor Approval

6/4/07
Date

Sponsor QA

This form must be completed, signed and returned to Biotechnology Services Administration, Covance Laboratories by post.

A faxed copy sent to, can be used for assay initiation.

COVANCE
THE DEVELOPMENT SERVICES COMPANY

eNotes Version: 2.5.6 Printed by on 29 Jul 2008, 04:16 PM (GMT +1)

Study Number: 2823-001
Category: Protocol Deviation

Tracking Id	Created Date
236055	13 Jul 2007

Comment Text

Corrective Action: Documentation of deviation serves as the corrective action.

Comment Text

Impact: No impact to study integrity apparent at this time.

Comment Text

The cell confluence at day 28 was suitable for haemadsorption so this deviation did not affect the outcome of the study.

Comment Text

MEM/10 was used as opposed to MEM/5 for cell splits on day 21.

Tracking Id	Created Date
232896	02 Jul 2007

Comment Text

Corrective Action: Documentation of deviation serves as the corrective action.

Comment Text

Impact: No impact to study integrity apparent at this time.

Comment Text

This colour change in the medium is believed to be due to overgrowth of the cells.

Comment Text

3T3 cells were refed on day 20 with DMEM/5E as the media was yellow and the cells were looking unhealthy although a monolayer was still present.

Tracking Id	Created Date
231387	26 Jun 2007

Comment Text

Corrective Action: Documentation of deviation serves as the corrective action.

Comment Text

Impact: No impact to study integrity apparent at this time.

Comment Text

Please note that the day 14 fresh positive control was not set up on day 13 or inoculated on day 14 in error. However the original positive control flask is still in use with a good monolayer. A fresh positive control will be set up on approximatly day 21 AS 26 June o7

Tracking Id 231136 Created Date 25 Jun 2007

Comment Text

Corrective Action: Documentation of deviation serves as the corrective action.

Comment Text

Impact: No impact to study integrity apparent at this time.

Comment Text

On observing the cells there were a lot of floating cells and gaps in the monolayer. This was probably caused by the cells overgrowing over the weekend and starting to die and come off the surface of the flask. This overgrowth can cause the medium to turn yellow due to the metabolism of the cells.

Comment Text

3T3 cell media was yellow on day 13, cells were refed with fresh media.

Tracking Id 231131 Created Date 25 Jun 2007

Comment Text

Corrective Action: Documentation of deviation serves as the corrective action.

Comment Text

Impact: No impact to study integrity apparent at this time.

Comment Text

DMEM was used as the growth media for the 3T3 cell line as opposed to MEM as stated in the protocol as this is the preferential growth media for this cell line.

COVANCE
THE DEVELOPMENT SERVICES COMPANY

Archive Packet Contents

Study Number	2823-002	**Responsible Person**		**Department**	Biotechnology
Item Number	2	**Type**	Study File		

Description	**Comments**
TAD Working Documents	

Prepared by		**Date**	11-12-07	**Checked by**		**Date**	12 / 12 / 07

J7U04229

COVANCE
THE DEVELOPMENT SERVICES COMPANY

CLE Study No. 2823-002

BIOTECHNOLOGY DEPT.

Test Article Receipt Form

Test article name: H9-MCB-1

Identification on vessel: COVANCE 2x 10mL @ 1x10⁶ c/mL
MCB.A.H9 p27 22 JAN 07 DF

Date received: 29/3/07 Received by:

How received: By courier on dry ice

Physical description: 2x orange capped 15 mL centrifuge tubes containing approx. 1/2 of frozen orange material

Storage location: BS115 -80°C freezer (Box D) x1

Logging in checked by: Date: 29/Mar/07

Date used	Material used	Material remaining	By	CLE Study No.
06/06/07	All	used to make cell lysate x2 (20ml)	AS	2823-002
12.6.7	All	NONE	DF	2823-002
		N/A		

x3

Remaining test article transferred to:
CLE Study number: By: Date:

Remaining test article disposed:
By Covance method: By: Date:

Remaining test article returned to Sponsor:
By: Date: How:
Returned to:

Comments:
x1 Moved to BS152 3rd shelf box on LHS AS05Jun07
x2 entire sample from 2 tubes used to make cell lysate - cell lysate pooled into 1 tube following clarification.
x3 cell lysate labelling: (2823-002 H9-MCB-1 test Article cell lysate AS06/06/07)

Abbreviated name: Subsequent records refer to cell lysate usage AS06/06/07
AS06/06/07

2714 (February 2001)

Test Article Safety and Pre-Study Questionnaire Biotechnology Based Compounds

Confidential

To be completed by the Study Sponsor and returned to Covance Laboratories

Test Facility

Covance Laboratories Ltd
Otley Road, Harrogate
North Yorkshire HG3 1PY
UK

Reception Telephone: +44-(0)1423 500011
General Fax: +44-(0)1423 569595
Business Development Fax: +44-(0)1423 501999

This information is required to comply with UK Health & Safety regulations, IATA regulations (for transport), GLP compliance guidelines, the Animals (Scientific Procedures) Act 1986 and to assist with study design.

Covance Laboratories (Europe) will not accept contracts to conduct *in vivo* experiments on cosmetic products or substances intended to have primary use as an ingredient of a cosmetic product. Under Article 1 of Directive 76/768/EEC a cosmetic product is any substance or preparation intended to be placed in contact with the various external parts of the human body (epidermis, hair system, nails, lips) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance and/or correcting body odours and/or protecting them in good condition.

Please note: Where it is determined that insufficient information is provided to enable Covance Laboratories Ltd fulfil its legal obligations, the commencement of studies/compound development appraisal may be delayed until such time as adequate information is obtained.

Authorised by:
(please sign your name and type or print your company status underneath your signature)

Signature:	Erika Mitchen
Name and Position:	Erika Mitchen, Quality Assurance Manager
Date:	2/21/07

Where options are given please tick (√) those required or delete those not applicable

1 COMPANY REFERENCE

Name and Address of Company (as it should appear on all documents)	Name and Address of person to whom all financial correspondence should be addressed (if different)
WiCell Research Institute	WiCell Accounts Payable

Name(s) of Study Monitor(s) and contact details			
Name		Name	
Telephone		Telephone	
Fax		Fax	
E-mail		E-mail	

2 TEST ARTICLE INFORMATION

Supplier's Name and Address (if different from above)			
Test article name as it should appear in all documents		H9-MCB.1	
Exact details as presented on the test article vessel		H9-MCB.1	
Batch/Lot Number	H9-MCB.1	Quantity Supplied	2
Appearance (e.g. solid, liquid, lyophilised powder)	liquid	Concentration	$10x10^6$ cells
Despatch Date		Vial size (cryopreserved cells)	15ml conical tube

Where options are given please tick (√) those required or delete those not applicable

3 TEST ARTICLE TYPE

Please indicate type of Test Article in boxes below				
Virus		Vaccine (specify)		
DNA plasmid		Peptide		
Protein		Cells	Cryopreserved	Yes, but no longer viable. Human Embryonic Stem Cells
			Live	
Oligonucleotide		Cell culture Supernatant		

4 STERILITY

Has compound been sterility checked	No-in progress
If known please specify bioburden	

5 STORAGE AND DISPOSAL

Storage temperature:							
Liquid nitrogen (<-150°C)		<-50°C	-70C freezer	<-10°C		1 to 10°C	
Storage conditions (please specify):							
Under Nitrogen		Desiccated		Other	-70C freezer	Other	
Expiry date (if stored under the above conditions) none							
Disposal of unused Test Article	Incinerate: Yes ☒ No ☐		Return to Sponsor: Yes ☐ No ☒		Identify Recipient of Returned Test Article:		

6 FORMULATION

Please indicate optimum formulation conditions:-

Vial composition (e.g. polypropylene, siliconised glass)	n/a
Diluent	n/a
Method of mixing if applicable	n/a
Method of sterilisation (e.g. 0.2µm filter)	n/a

Where options are given please tick (√) those required or delete those not applicable

7 HEALTH AND SAFETY

Please indicate known or suspected bazards. (Do not leave blanks, state "Not Known" if no data available). State LD_{50} and species if known, state "Not known" if this is the case.

Does the test article contain any chemical which may be hazardous, e.g. DMSO	Yes ☐ No ☒
If "yes", please give details Medium contains DMEM-F12, Knock out Serum Replacer, Amino Acids. None of these items are hazardous. Does not contain antibiotics or DMSO.	
Specific handling precautions (please state)	Standard PPE
Known antidote and/or First Aid procedures	Not known
Contact for Safety Information (Name & Telephone Number)	

A Certificate of Analysis confirming the identity and purity of the test article is a requirement of UK GLP, please indicate correct option below:

Certificate of Analysis:	Is attached	Will accompany sample	Will follow later	Is not available
		x		

Cell lines for GMP cell banking and cell banks for GMP storage will be required to meet minimum acceptance testing criteria before being handled/stored in GMP facilities. Requirements will be advised separately:

FOR VIRAL COMPOUNDS AND CELL LINES:

Has the test article been classified as a "Genetically Modified Organism" (EC Directive 90/219/EEC and 94/51/EC)	Yes ☐ No ☒
If "yes", please supply **full** Risk Assessment including whether the test article is classified as group 1 or group 2.	

* This information is required under the UK regulations governing the Control Of Substances Hazardous to Health (COSHH) and Genetically Modified Organisms (Contained Use) Regulations 1992.

8 KNOWN STABILITY OF FORMULATED TEST ARTICLE

Please provide details (vehicle, temperature, pH, duration etc).	

Where options are given please tick (√) those required or delete those not applicable

9 TRANSPORT INFORMATION

It may be that certain compounds are considered hazardous for transport by air. In order for Covance to fully comply with IATA Dangerous Goods Regulations (if applicable) the following information is required. Failure to supply relevant information may result in Covance being able to transport a compound by air.

Is the compound considered to be hazardous for shipping by air?	Yes ☐ No ✠
If Yes, please complete the following:	
Proper shipping name:	
UN Number:	
Hazard Class:	
Packing Group:	
Compound form:	
MSDS available:	

10 FOR CELL CULTURE SAMPLES

CULTURE CHARACTERISTICS

For cryopreserved cells	Please indicate in the relevant box whether cells grow as			
	A monolayer	x	A suspension culture	
	Expected viability	%		
For all cells	Preferred split ratio		Frequency of splitting (days)	

MEDIUM FOR CELL CULTURE

Name of medium:		Antibiotics and concentration:	
Please supply any other relevant information:			
Where a Specialist or Non-Standard Medium is required, please supply the following information			
Medium to be supplied by sponsor?	Yes ☐	No ☐	
If "yes" please give details of storage conditions and expiry date (last line of this table) If "no" give full details as requested below			
Name of medium:		Basal medium:	
Additives and concentration			
Antibiotics and concentration			
Storage conditions		Medium expiry date	

Where options are given please tick (√) those required or delete those not applicable

SUPERNATANT INFORMATION

Where a Cell Culture Supernatant is supplied for testing **please supply the following information**	
Age of culture from which the supernatant was taken (since last re-feed)	
Any other relevant information	
Has the supernatant been centrifuged?	Yes ☐ No ☐
If "yes", please give details	

11 REPORTING

Regulatory Authority (ies) to which the data may be submitted?		
REPORT FORMAT	Number of copies required	
	Draft Report	Final Report
Bound/Double Sided		1
Bound/Single Sided		
Unbound/Double Sided		
Unbound/Single Sided		1
Electronic (By E-mail)	1	1
Electronic (On CD)		

National Stem Cell Bank

Certificate of Analysis

Product Description	WA09 Master Cell Bank
Cell Line Provider	WiCell
MCB Lot Number	H9MCB.1
Date Vialed	16Nov06
Passage Number	P17
Culture Method	SOP-CC-024A
Cryopreservation Method	SOP-CC-035A

The following testing specifications have been met for the specified product lot:

Test Description	Test Method	Test Specification	Result
Post-Thaw Viable Cell Recovery	SOP-CH-305A	Viable cells recovered	Pass
Identity by STR	SOP-CH-302A	Positive identity	Waiting for report
HLA profile	SOP-CH-303A	Positive identity	Waiting for report
Identity by SNP	SOP-CH-306A	Positive identity	Waiting for report
Sterility - Direct transfer method with bacteriastasis & fungistasis	SOP-CH-307A	No contamination detected	Waiting for report
Mycoplasma - FDA PTC method	SOP-CH-308A	No contamination detected	Waiting for report
Karyotype by G-banding	SOP-CH-003A	Normal Karyotype	Waiting for report
Comparative Genome Hybridization	SOP-CH-309A SOP-CH-310A	Report Copy Number Variants	Waiting for report
Growth Characteristics	SOP-CH-104A	Report plating efficiency and doubling time	Waiting for report
Flow Cytometry for ESC Marker Expression	SOP-CH-101A SOP-CH-102A SOP-CH-103A	Report values Oct-4 > X%	Waiting for report
Gene Expression Profile	SOP-CH-311A	Report Values	Waiting for report
Bovine pathogens	SOP-CH-312A	No contamination detected	Waiting for report
Porcine pathogens	SOP-CH-313A	No contamination detected	Waiting for report
Murine Antibody Production (MAP)	SOP-CH-314A	No contamination detected	Waiting for report
In vitro adventitious virus	SOP-CH-315A	No contamination detected	Waiting for report

National Stem Cell Bank

Certificate of Analysis

In vivo adventitious virus	**SOP-CH-316A**	No contamination detected	**Waiting for report**
Retrovirus by thin section EM	**SOP-CH-317A**	No contamination detected	**Waiting for report**
Co-cultivation with Mus Dunni Cells and PG4 S+L- assay	**SOP-CH-319A**	No contamination detected	**Waiting for report**
HIV 1&2 by PCR	**SOP-CH-318A**	No contamination detected	**Waiting for report**
HTLV 1&2 by PCR	**SOP-CH-318A**	No contamination detected	**Waiting for report**
HBV by PCR	**SOP-CH-318A**	No contamination detected	**Waiting for report**
HCV by PCR	**SOP-CH-318A**	No contamination detected	**Waiting for report**
CMV by PCR	**SOP-CH-318A**	No contamination detected	**Waiting for report**
EBV by PCR	**SOP-CH-318A**	No contamination detected	**Waiting for report**
HHV-6 by PCR	**SOP-CH-318A**	No contamination detected	**Waiting for report**
HHV-7 by PCR	**SOP-CH-318A**	No contamination detected	**Waiting for report**
HHV-8 by PCR	**SOP-CH-318A**	No contamination detected	**Waiting for report**
HP B19 by PCR	**SOP-CH-318A**	No contamination detected	**Waiting for report**

NSCB Quality Assurance: _____ Date: 3/6/07

WiCell Research Institute
P.O. Box 7365
Madison, WI 53707-7365
USA

Invoice
Date: 03/27/07

Covance Laboratories Ltd

Invoice No. 032707A

Contents:

Quantity	Item
8	Frozen cell pellet. Human cell lines H1MCB.1, H9MCB.1
4	10ml frozen human cells. Cell lines H1MCB.1, H9MCB.1

Description:
Package contains frozen noninfectious human cells. For laboratory research purposes only. The cells show no detectable signs of contamination. The cells were produced, isolated, and cultured in the US. Cells are shipped in dry ice. Total package weight is 20 lb.

Commercial value: US$1.00

We certify that this invoice shows the full value of the goods and that no further invoice shall be issued.

Signed.

Laboratory Manager - Distribution

WiCell Research Institute

Packing Slip

P.O. Box 7365
Madison, WI 53707-7365
Phone: (608) 441-2719 Fax: (608) 441-2766

Sent to:

Date:

03/27/07

Covance Laboratories Ltd

Contents - Description	Location
2 x 10ml @ 1x10^6 cells/ml H1MCB.1p30	-80
2 x 10ml @ 1x10^6 cells/ml H9MCB.1p27	
4 pellets @ 1x10^6 cells/pellet H1MCB.1p30	
4 pellets @ 1x10^6 cells/pellet H9MCB.1p27	

Preparation Test article cell lysate.

Test article labelled:

X1 [illegible]

X1 2x15ml Centrifuge tubes with identical labeling AS06/06/07

I confirm that the Test Article used in this assay corresponds to the Test Article Receipt Form and Test Article Safety and Pre-Study Questionnaire supplied by the sponsor.

Study Director: CH **Date:** 6/6/09

Document Authorised CH 6/6/09

Preparation of Test Article

Equipment used throughout assay

Equipment	ID Number	
Safety Cabinet	BS 204	☐ N/A
Waterbath	BS 219	☐ N/A
Centrifuge	BS 236	☐ N/A
Micropipette		☑ N/A
Pipetaid	BS 257	☐ N/A
Other (freezer)	BS 152	☑ N/A (2) AS 06/06/07
Other ( )		☑ N/A
Initials/Date	AS / 06/06/07	

Steps – preparation of cell lysate	Task complete
Was removed from freezer BS 152 and thawed at 37°C.	☑
Freeze –thaw test article three times in liquid nitrogen and 37°C waterbath, making sure TA is completely frozen and completely thawed each time. Complete the table below	☑
Clarify the resulting lysate by centrifugation at 150 x "g" for 10 minutes at approx. 20°C	☑
Test article relabelled 2823-002 M1 mL 1 TEST ARTICLE CELL LYSATE AS 06/06/07	☑
X1 Aliquot the lysate and store deep frozen or keep on ice and use within 2 hours	☑ (freeze)
Initials/Date	AS/06/06/07

	1st Thaw	2nd Thaw	3rd Thaw
Thaw start time	16:01	16:31	17:20
Thaw end time	16:28	17:15	18:00
Initials/date	AS / 06/06/07		

X1 Clarified lysate pooled into single volume, approx 20ml
AS 06/06/07

Comments

In Vitro Evaluation of Adventitious Viruses in Cell Cultures – 28 day assay

Test Article;

I confirm that the Test Article used in this assay corresponds to the Test Article Receipt Form and Test Article Safety and Pre-Study Questionnaire supplied by the sponsor or the relevent cell banking documentation.

Study Director:.....EAH.@ **Date:**.....12 June 07 Confirmed by SO CH 12/6/07

@ Signed in absence of SD CH 12/6/07

Document Authorised CH 11/6/07.

Codes used throughout working document

NC = Negative Control

TA = Test Article

STA = Spiked Test Article

PC = Positive Control

APC = Assay Positive Control

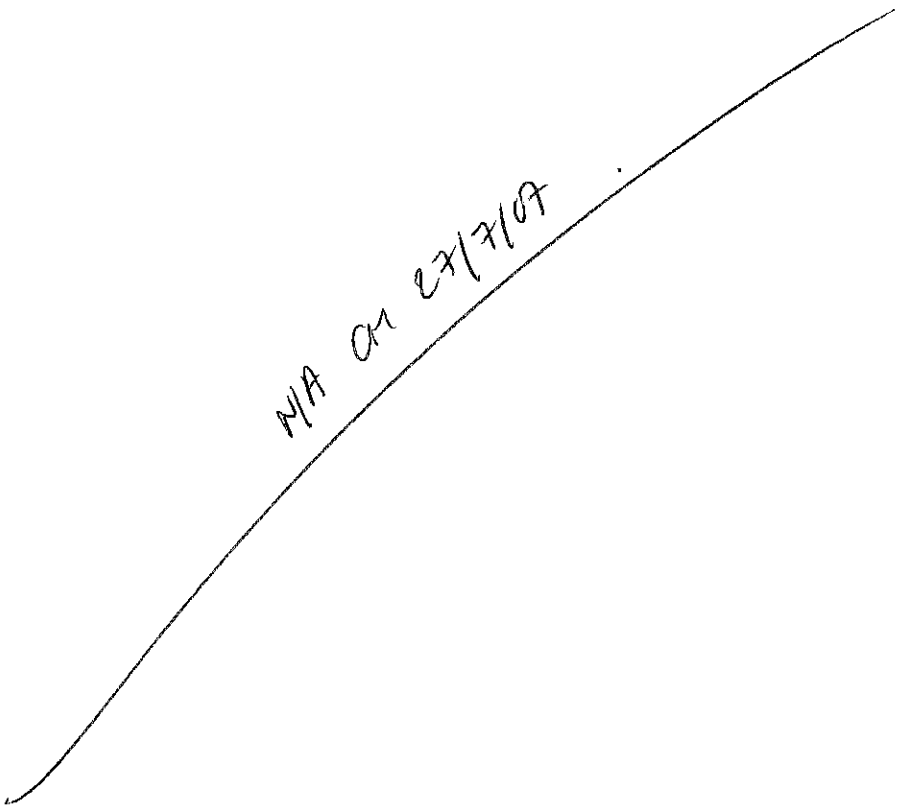
PNC = Passaged Negative Control

PTA = Passaged Test Article

PSTA = Passaged Spiked Test Article

PPC = Passaged Positive Control

Comments



Document index

1 Day –1: Preparation of indicator cultures

Equipment

Lab 3	ID Number	
Safety Cabinet	BS207	☐ N/A
Incubator	BS220	☐ N/A
Waterbath	BS218	☐ N/A
Microscope	BS239	☐ N/A
Micropipette		☑ N/A
Pipetaid	BS159	☐ N/A
Other ( )		☑ N/A
Other ( )		☑ N/A
Initials/Date	RL / 11/6/07	

Reagents

	RI code	Batch No	Exp. date
MEM/10E – seeding media	RI/184	50506107 50601107	11/8/07 6/9/07
D-PBS	RI/025	50584107	04/09
HBSS	RI/024	NA	NA
TrypLE	RI/141	50578107	09/09
Trypan blue (0.4%)	RI/044	50116107	07/08
Other ( )		NA	
Other ( )		RL 11/6/07	
Initials/Date		RL / 11/6/07	

Day –1: Preparation of indicator cultures

Steps – Seeding of MRC-5 cells.	Task complete
Cell line details. Cell concentration required **5 x 10^4 cells/ml.** **MRC-5** passage number x, C number 17 x2; flasks confluency x.	☑
Using a sterile pipette, aspirate the medium from 7 x T 150 flasks of Cells.	☑
Wash the flasks with sufficient D-PBS to cover the monolayer.	☑
Remove washings and add 20 ml of TrypLE select each flask. Incubate the flask at 37 ± 1°C for approximatley 5 – 15 minutes.	☑
Gently agitate the flask to loosen the cell sheet and add 10.0 ml of complete medium. Mix the cell suspension.	☑
If multiple flasks are used pool in one sterile container. Final volume 184 ml RL 11/6/07	☑
Perform viable cell counts in the presence of Trypan Blue. Add 0.2 ml of cell suspension to 0.2 ml of Trypan blue and mix.	☑
Load the haemocytometer and count 3 of the 16 squares.	☑
Dilute cell suspension and seed 6 T75 flasks with 15ml cell suspension at 5 x 10^4 cells/ml.	☑
Incubate all flasks at 37 ± 1°C with 5-10% CO_2.	☑
Initials/Date	RL / 11/6/07

Cell Counts x1			
1. 8	**2.** 16	**3.** 14	**Mean** 13 **(B)**

Cell Concentration = (B x 2 x 10^4)	2.6 x 10^5 **Cells/ml**
Cell Concentration required	**5 x 10^4 cells/ml**
Dilution required	1 In 5.2
Total volume of cell suspension required (number of T75 flasks prepared x 15ml + excess)	95 **ml**
Volume of cell suspension (added to make up to required concentration)	18.3 **ml**
Volume of complete media (added to make up to required concentration)	76.7 **ml**
Calculation performed by/date	RL / 11/6/07
Calculation checked performed by/date	CH / 12.6.07

x1 x T150 passage 6 100%, 4xT150 passage 11/6 90% and 2xT150 passage 12/6 40% RL 11/6/07

x. Cells also used for 2823-001, 0065-373. Data transcribed from 2823-002. RL 11/6/07

x2 C Number was omitted in error at time by analyst but has been transcribed from 2823-001 p50 of 43. Comment added in retrospect. RL 18/6/07 Transcription checked CH 20/9/07

x3 Should read 2823-001 as 27/7/07

Day –1: Preparation of indicator cultures

Steps – Seeding of Vero cells.	Task complete
Cell line details. Cell concentration required **5 x 10^4 cells/ml.** **Vero** passage number 24 [24], C number 1 [x1], flasks confluency 100.	☑
Using a sterile pipette, aspirate the medium from 5 x T 150 flasks of Cells.	☑
Wash the flasks with sufficient D-PBS to cover the monolayer.	☑
Remove washings and add 20 ml of TrypLE select each flask. Incubate the flask at 37 ± 1°C for approximatley 5 – 15 minutes.	☑
Gently agitate the flask to loosen the cell sheet and add 100 ml of complete medium. Mix the cell suspension.	☑
If multiple flasks are used pool in one sterile container. Final volume 60 ml	☑
Perform viable cell counts in the presence of Trypan Blue. Add 0.2 ml of cell suspension to 0.2 ml of Trypan blue and mixed.	☑
Load the haemocytometer and count 3 of the 16 squares.	☑
Dilute cell suspension and seed 6 T75 flasks with 15ml cell suspension at 5 x 10^4 cells/ml.	☑
Incubate all flasks at 37 ± 1°C with 5-10% CO_2.	☑
Initials/Date	RL 11/6/07

Cell Counts [x]			
1. 106	2. 114	3. 103	**Mean** 108 **(B)**

Cell Concentration = (B x 2 x 10^4)	2.16 x 10^6 **Cells/ml**
Cell Concentration required	**5 x 10^4 cells/ml**
Dilution required	1 **in** 43.2
Total volume of cell suspension required (number of T75 flasks prepared x 15ml + excess)	250 **ml**
Volume of cell suspension (added to make up to required concentration)	5.8 **ml**
Volume of complete media (added to make up to required concentration)	244.2 **ml**
Calculation performed by/date	RL 11/6/07
Calculation checked performed by/date	AS 18/6/07 [x2]

[x] Data transcribed from 0065-373. Cells also used for 0065-373, 2823-001, 0065-379, 0065-380 + 0065-381 RL 11/6/07

[x2] checked in retrospect AS 18/6/07

Transcription checked CH 20/9/07

[x1] C Number was omitted in error at time by analyst. Can be confirmed by 2823-002 [x3] p6 of 43. Comment added in retrospect for clarity. RL 18/6/07

Day –1: Preparation of indicator cultures

Steps – Seeding of HeLa cells.	Task complete
Cell line details. Cell concentration required **5 x 10^4 cells/ml.** **HeLa** passage number 198, C number 8 x1, flasks confluency 100.	☑
Using a sterile pipette, aspirate the medium from 2 x T 150 flasks of Cells.	☑
Wash the flasks with sufficient D-PBS to cover the monolayer.	☑
Remove washings and add 2.0 ml of TrypLE select each flask. Incubate the flask at 37 ± 1°C for approximatley 5 – 15 minutes.	☑
Gently agitate the flask to loosen the cell sheet and add 10.0 ml of complete medium. Mix the cell suspension.	☑
If multiple flasks are used pool in one sterile container. Final volume 24.0 ml	☑
Perform viable cell counts in the presence of Trypan Blue. Add 0.2 ml of cell suspension to 0.2 ml of Trypan blue and mixed.	☑
Load the haemocytometer and count 3 of the 16 squares.	☑
Dilute cell suspension and seed 6 T75 flasks with 15ml cell suspension at 5 x 10^4 cells/ml.	☑
Incubate all flasks at 37 ± 1°C with 5-10% CO_2.	☑
Initials/Date	RL 11/6/07

Cell Counts x			
1. 47	2. 63	3. 56	**Mean** 55 **(B)**

Cell Concentration = (B x 2 x 10^4)	1.1 x 10^6 **Cells/ml**
Cell Concentration required	**5 x 10^4 cells/ml**
Dilution required	1 **In** 22
Total volume of cell suspension required **(number of T75 flasks prepared x 15ml + excess)**	100 **ml**
Volume of cell suspension **(added to make up to required concentration)**	45 **ml**
Volume of complete media **(added to make up to required concentration)**	RL 11/6/07 ~~95~~ **ml** 95.5
Calculation performed by/date	RL / 11/6/07
Calculation checked performed by/date	MJS / 11/06/07

x Data transcribed from 2823-001. Cells also used for 2823-001 RL 11/6/07
Transcription checked CH 20/9/07

x1 C Number was omitted at time in error. Can be confirmed by 2823-002 p79 & 43. Comment added in retrospect for clarity. RL 18/6/07 x2 x2 should read 2823-001 CH 27/7/07

Day –1: Preparation of indicator cultures

Steps – Seeding of NIH 3T3 cells.	Task complete
Cell line details. Cell concentration required **1 x 10^5 cells/ml.** **NIH 3T3** passage number 112, C number 1, flasks confluency 100	☑
Using a sterile pipette, aspirate the medium from 3 x T 150 flasks of Cells.	☑
Wash the flasks with sufficient D-PBS to cover the monolayer.	☑
Remove washings and add 20 ml of TrypLE select each flask. Incubate the flask at 37 ± 1°C for approximately 5 – 15 minutes.	☑
Gently agitate the flask to loosen the cell sheet and add 10.0 ml of complete medium. Mix the cell suspension.	☑
If multiple flasks are used pool in one sterile container. Final volume 360 ml	☑
Perform viable cell counts in the presence of Trypan Blue. Add 0.2 ml of cell suspension to 0.2 ml of Trypan blue and mixed.	☑
Load the haemocytometer and count 3 of the 16 squares.	☑
Dilute cell suspension and seed 6 T75 flasks with 15ml cell suspension at 1 x 10^5 cells/ml.	☑
Incubate all flasks at 37 ± 1°C with 5-10% CO_2.	☑
Initials/Date	RL / 11/6/07

Cell Counts K			
1. 22	2. 20	3. 27	**Mean** 23 **(B)**

Cell Concentration = (B x 2 x 10^4)	4.6x10^5 **Cells/ml**
Cell Concentration required	**1 x 10^5 cells/ml**
Dilution required	**1 In** 4.6
Total volume of cell suspension required (number of T75 flasks prepared x 15ml + excess)	100 **ml**
Volume of cell suspension (added to make up to required concentration)	21.7 **ml**
Volume of complete media (added to make up to required concentration)	78.3 **ml**
Calculation performed by/date	RL / 11/6/07
Calculation checked performed by/date	MS / 11/06/07

K Cells also used for 2823-001. Data transcribed from 2823-001. RL 11/6/07

Transcription checked

Comments

N/A CH 2717/09

2 Day 0: Inoculation of Indicator Cultures

Equipment

Lab Cat 3	ID Number	
Safety Cabinet	BS196	☐ N/A
Incubator	BS225, BS138	☐ N/A
Waterbath	BS251	☐ N/A
Microscope	BS263	☐ N/A
Micropipette		☑ N/A
Pipetaid	Clear Cat 3 pipette aid	☐ N/A
Other ( )		☑ N/A
Other ( )		☑ N/A
Initials/Date	BK 1 12 6 7	

Reagents

	RI code	Batch No	Exp. date	
MEM/5E – refeed media	RI/183	505e7/07	11. 8. 07	☐ N/A
MEM/5TPB	RI/187	5e57a/07	2.8.7	☐ N/A
D-PBS	RI/025	506019a	5/09	☐ N/A
HBSS	RI/024			☑ N/A
Other (Dnase/5E)		506030/07	6.19.7	☐ N/A
Other ( )				☑ N/A
Initials/Date		BK 1 12 6. 7		

Day 0: Inoculation of Indicator Cultures

Steps - Perform the same steps for all cell lines.	Task completed
Aspirate medium from approx 30 % confluent **MRC-5** cells.	☑
Aspirate medium from approx 30 % confluent **Vero** cells.	☑
Aspirate medium from approx. 30 % confluent **HeLa** cells.	☑
Aspirate medium from approx. 30 % confluent **NIH 3T3** cells.	☑
Wash cells with sufficient amount of D-PBS	☑
Inoculate one flask per cell line with 5ml **negative control** (MEM/5TPB) and incubate flasks at 37 ± 1°C, 5% CO_2 for 60-90 min. Start time: 1501 End time: ~~1520~~ 16.20 CH 27/7/07 x2	☑ Start ☑ End
Inoculate one flask per cell line with 5ml **test article** and incubate flasks at 37 ± 1°C, 5% CO_2 for 60-90 min. x1 Start time: 1510 End time: ~~1530~~ 16.30 CH 27/7/07 x2	☑ Start ☑ End
After incubation aspirate the inoculum and **wash with ~10ml DPBS.** Then refeed cells with 15ml appropriate refeed media and incubate flasks at 37 ± 1°C, 5% CO_2.	☑
Initials/Date	DF 12.6.07

Comments

x1 As there was only 20ml of TA, The TA was Diluted 1 in 2 with 20ml of MEM/5TPB. DF 12.6.07

x2 Inscription error made in retrospect during data check. Can be confirmed by study 2823-001 p 11 of 43, where inscription errors were made at time CH 27/7/07 x3

x3 Both studies were inoculated at the same time Added for clarity in response to QA audit CH 20/9/07

Day 0: Preparation of Inoculum for Positive Control

If same virus is used for all cell lines complete this page and N/A the following pages.

Steps - Preparation of positive control for MRC-5, Vero and HeLa	**Task completed**
Thaw rapidly a frozen ampoule of PI3 virus, batch 050721 at 1.1x10⁸ $TCID_{50}$/ml in waterbath set at 37 ± 1°C. Once thawed keep ampoule on ice until required.	☑
Perform virus dilution and complete the table below.	☑
Required virus concentration for spike test article control is **$5x10^5$TCID50/ml**	☑
Required virus concentration for positive control is **$1x10^4$TCID50/ml**	☑

Virus Suspension(ml)	**Diluent (ml)**	**Total Volume Required (ml)**	**Dilution**	**TCID50/ml**
0.5	5 TCID 12.6.7	5.5	1 in 11	1X10⁷
1	19	20	1 in 20	5X10⁵
1	49	50	1 in 50	1X10⁴
			1 in	
		N/A	1 in 12.6.7	
Calculation performed by/date				RF 12.6.7
Calculation check performed by/date				AM 12.6.7
Initials/Date				RF 12.6.7

Comments

N/A AM 27/7/07

Day 0: Preparation of Inoculum for Positive Control

☑ N/A

Steps - Preparation of positive control for NIH 3T3 cells	Task completed
Thaw rapidly a frozen ampoule of MVM virus, batch 0606 at 2X10⁶ $TCID_{50}$/ml in waterbath set at 37 ± 1°C. Once thawed keep ampoule on ice until required.	☑
Perform virus dilution and complete table below.	☑
Required virus concentration for spike test article control is **5x10⁵TCID50/ml**	☑
Required virus concentration for positive control is **1x10⁴TCID50/ml**	☑

Virus Suspension(ml)	Diluent (ml)	Total Volume Required (ml)	Dilution	TCID50/ml
0.5	19.5	20	1 in 40	5X10⁶
1	9	10	1 in 10	5X10⁵
0.5	24.5	25	1 in 50	1X10⁴
			1 in	
		N/A	1 in	

Calculated performed by/date	RM 12-6-07
Calculation check performed by/date	CH 12.6.07
Initials/Date	RM 12-6-07

Comments

N/A CH 27/7/07

Day 0: Inoculation of Indicator Cultures

Steps	Task complete
Inoculate one flask per cell line with 5ml of the appropriate **spiked test article** (4.9ml test article + 0.1ml positive control) and incubate flasks at 37 ± 1°C, 5% CO_2 for 60-90 min. Start time: 15:40 End time: 17:00	☑ start ☑ end
Inoculate one flask per cell line with 5ml of the appropriate **positive control** and incubate flasks at 37 ± 1°C, 5% CO_2 for 60-90 min. Start time: 16:22 End time: 17:44	☑ start ☑ end
After incubation aspirate the inoculum and **wash with ~10ml DPBS.** Then refeed cells with 15ml appropriate refeed media and incubate flasks at 37 ± 1°C, 5% CO_2.	☑
Initials/Date	[illegible] 12 Jun 07

Comments

N/A

3 Day 7: Refeed or Subculture of Cultures

Equipment

Lab 10	ID Number	
Safety Cabinet	BS198	☒ N/A
Incubator	BS159, BS228 x1	☐ N/A
Waterbath	BS251	☐ N/A
Microscope	BS244	☒ N/A
Pipetaid	BS263	☐ N/A
Other ( )		☑ N/A
Other ( )		☐ N/A
Initials/Date	DF 19.6.7	

Reagents

	RI code	Batch No	Exp. date	
MEM/10E – seeding media	RI/184	5040457	17.7.7	☒ N/A
MEM/5E – refeed media	RI/183	5031/07	23.6.7	☐ N/A
D-PBS	RI/025	5063607	05/09	☐ N/A
HBSS	RI/024			☑ N/A
TrypLE	RI/141	5052807	02/09	☐ N/A
Trypan blue (0.4%)	RI/044			☑ N/A
Other (DMEM/10E x2) x1 CD DF 25.6.7	RI/190	5031507	21.6.7	☐ N/A
Other ( )				☑ N/A
Initials/Date	DF 19.6.7			

ID DF 19.6.7

x1 All flasks were put in BS228 after refeed / Subculture DF 21.6.7 CD DF 26.6.7

x2 See Encore #231132 for further information DF 25.6.7 CD DF 25.6.7

Steps	Task completed
Discard positive control flasks if show CPE. Confirmed by SD CH 19/6/07.	☑ (✓,N/A) by date DC 19.6.07
If cultures are 100% confluent confirm with SD how to proceed.	☑
Refeed	☑ (✓,N/A)
Flasks to be refed MRC-5 NC, MRC-5 TA, MRC-5 STA,	SD confirmed CH 19/6/07 ×
Aspirate medium from the flasks that show no CPE.	☑
Add 15 ml fresh refeed media.	☑
Incubate flasks at 37 ± 1°C, 5% CO_2	☑
Initials/Date	DC 19.6.07
Subculture	☑ (✓,N/A)
Flasks to be subcultured Vero NC Lin 4, STJ NC Lin 4, Hela NC Lin 2, Vero TA Lin 4, STJ TA Lin 4, Hela TA Lin 2, STA STJ Lin 4,	SD confirmed CH 19/6/07 ×
Aspirate medium from all the flasks that show no CPE and are 100% confluent.	✓
Wash the flasks with sufficient D-PBS to cover the monolayer.	☑
Remove washings and add 2 ml of TrypLE select. Incubate the flask at 37 ± 1°C for approx. 5-15 minutes.	☑
Gently agitate the flask to loosen the cell sheet and add 10ml of complete medium. Mixed the cell suspension. Final volume 12ml	☑
Seed N/A ml of cell suspension (equal to a 1 in N/A split) into fresh T75 flasks and make up to a total flask volume of 15 ml with complete media.	☑
Flask labelled with cell line, study number and test material (eg PNC, PTA etc)	☑
Return the flask to incubators at 37 ± 1°C 5% CO_2	☑
Initials/Date	DC 19.6.07

× SD has confirmed + annotated on flask which flasks are to be refed and which are to be subcultured and at what rate CH 19/6/07.

Page 16a to follow.

2823 002 ; 823 002 2823-001

COVANCE
THE DEVELOPMENT SERVICES COMPANY

AUTHENTICATED PHOTOCOPY
Date. 29/9/10 Signature
Original in: 2823-001

Covance Laboratories Ltd.
Otley Road
Harrogate
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On day 13 it was noticed that all the 3T3 cells looked unhealthy. There were many floating cells and the media had turned a yellowish-orange

It was decided to reseed the cells. Confirmed by SO CH 25/6/07

Supernatant was harvested, centrifuged at 160 x g for 10m and supernatant was removed from cells and stored at -80 Storage location: BS 152, 3rd shelf on top of box C. x1

Cells were washed with DPBS by adding 10ml + aspirating

Batch number: 50634/07 expiry date: 5/09

Fresh medium was added:

Batch number: 50343/07 expiry date: 27-6-07

Cells were placed in incubator BS 228

Equipment list:
Safety Cabinet BS 149
Pipette aid BS 260
Centrifuge BS 060

See Envt # 231136 for further information BK 25.6.7

x1 NC and TA 002 Supernatants were discarded in error BK x2 25.6.07

Page Completion Check [illegible] 27/7/07
Data Check [illegible] 27/7/07

x2 These supernatants were not required. Added in response to QA audit [illegible] 29/7/07

X 1

4 Day 13: Preparation of fresh [redacted] indicator cultures for Haemadsorption positive control.

Equipment

Lab [redacted]	ID Number	
Safety Cabinet	[redacted]	☐ N/A
Incubator	[redacted]	☐ N/A
Waterbath	[redacted]	☐ N/A
Microscope	[redacted]	☐ N/A
Pipetaid	[redacted]	☐ N/A
Other ([redacted])	[redacted]	☐ N/A
Other ([redacted])	[redacted]	☐ N/A
Initials/Date	[redacted] / [redacted]	

N/A AS 26/6/07

Reagents

	RI code	Batch No	Exp. date	
MEM/10E – seeding media	RI/184	[redacted]	[redacted]	☐ N/A
MEM/5E – refeed media	RI/183	[redacted]	[redacted]	☐ N/A
D-PBS	RI/025	[redacted]	[redacted]	☐ N/A
HBSS	RI/024	[redacted]	[redacted]	☐ N/A
TrypLE	RI/141	[redacted]	[redacted]	☐ N/A
Trypan blue (0.4%)	RI/044	[redacted]	[redacted]	☐ N/A
Other ([redacted])	[redacted]	[redacted]	[redacted]	☐ N/A
Other ([redacted])	[redacted]	[redacted]	[redacted]	☐ N/A
Initials/Date		[redacted] / [redacted]		

X1 see enote 231388 AS 26/6/07

Day 13: Preparation of fresh [redacted] indicator cultures for Haemadsorption positive control.

Steps – Seeding of cells.	Task complete
Cell line details. Cell concentration required **5 x 10^4 cells/ml.** **MRC-5** passage number [redacted], C number [redacted], flasks confluency [redacted].	☐
Using a sterile pipette, aspirate the medium from [redacted] x T [redacted] flasks of Cells.	☐
Wash the flasks with sufficient D-PBS to cover the monolayer.	☐
Remove washings and add [redacted] ml of TrypLE select each flask. Incubate the flask at 37 ± 1°C for approximatley 5 – 15 minutes.	☐
Gently agitate the flask to loosen the cell sheet and add [redacted] ml of complete medium. Mix the cell suspension.	☐
If multiple flasks are used pool in one sterile container. Final volume [redacted] ml	☐
Perform viable cell counts in the presence of Trypan Blue. Add 0.2 ml of cell suspension to 0.2 ml of Trypan blue and mixed.	☐
Load the haemocytometer and count 3 of the 16 squares.	☐
Dilute cell suspension and seed [redacted] T75 flasks with 15ml cell suspension at 5 x 10^4 cells/ml.	☐
Incubate all flasks at 37 ± 1°C with 5-10% CO_2.	☐
Initials/Date	[redacted] / [redacted]

Cell Counts			
1. [redacted]	**2.** [redacted]	**3.** [redacted]	**Mean** [redacted] **(B)**

Cell Concentration = (B x 2 x 10^4)	[redacted] **Cells/ml**
Cell Concentration required	**5 x 10^4 cells/ml**
Dilution required	[redacted] **In**
Total volume of cell suspension required **(number of T75 flasks prepared x 15ml + excess)**	[redacted] **ml**
Volume of cell suspension **(added to make up to required concentration)**	[redacted] **ml**
Volume of complete media **(added to make up to required concentration)**	[redacted] **ml**
Calculation performed by/date	[redacted] / [redacted]
Calculation checked performed by/date	[redacted] / [redacted]

Comments

N/A CH 27/7/07

5 Day 14: Inoculation of fresh indicator cultures for Haemadsorption positive control and subculture of cells.

Equipment

Lab 9	ID Number	
Safety Cabinet	BS199	☐ N/A
Incubator	BS228	☐ N/A
Waterbath	BS452	☐ N/A
Microscope	BS439	☐ N/A
Micropipette		☑ N/A
Pipetaid	BS269	☐ N/A
Other ( )		☑ N/A
Other ( )		☑ N/A
Initials/Date	RL /26/6/07	

Reagents

	RI code	Batch No	Exp. date	
MEM/5E – refeed media	RI/183			☑ N/A
MEM/5TPB	RI/187			☑ N/A
D-PBS	RI/025	50634/07	05/09	☐ N/A
HBSS	RI/024			☑ N/A
Other (MEM/10E)	184	50601/07	6/9/07	☐ N/A
Other (DMEM/10)	190	50329/07	23/6/07	☐ N/A
Initials/Date		RL/26/6/07		

TrypLE RI 141 50608/07 02/09 RL 26/6/07

Day 14: Inoculation of fresh indicator cultures for Haemadsorption positive control and subculture of cells.

Steps - Preparation of positive control	Task completed
Thaw rapidly a frozen ampoule of PI3 virus,batch [redacted] at [redacted] $TCID_{50}$/ml in waterbath set at 37 ± 1°C. Once thawed keep ampoule on ice until required.	☐
Perform virus dilution and complete table below.	☐
Required virus concentration for positive control is **$1x10^4$TCID50/ml**	☐

Virus Suspension(ml)	Diluent (ml)	Total Volume Required (ml)	Dilution	TCID50/ml
[redacted]	[redacted]	[redacted]	1 in [redacted]	[redacted]
[redacted]	[redacted]	[redacted]	1 in [redacted]	[redacted]
[redacted]	[redacted]	[redacted]	1 in [redacted]	[redacted]
[redacted]	[redacted]	[redacted]	1 in [redacted]	[redacted]
[redacted]	[redacted]	[redacted]	1 in [redacted]	[redacted]
Calculation performed by/date				[redacted]
Calculation check performed by/date				[redacted]
Initials/Date				[redacted]

Steps – inoculation of positive control flask	Task completed
Aspirate medium from approx [redacted] % confluent [redacted] cells.	☐
Wash cells with sufficient amount of D-PBS.	☐
Inoculate one flask with 5ml of **positive control** and incubate flasks at 37 ± 1°C, 5% CO_2 for 60-90 min. Start time: [redacted] End time: [redacted]	☐ start ☐ end
After incubation aspirate the inoculum and **wash the cells with ~10mL DPBS.** Then refeed cells with 15ml appropriate refeed media and incubate flasks at 37 ± 1°C, 5% CO_2.	☐
Initials/Date	[redacted]

X, NIA AS 27/6/07

X, See note 231388 AS 27/6/07

Day 14: Subculture of Cultures

Steps	Task completed
Discard positive control flasks if show CPE. X	N A ☐ (✓,N/A) by date RL / 26/6/07
Subculture	☑ (✓,N/A)
Flasks to be subcultured All 3T3 flasks 1in4 Vero TM + NC 1in4 All MRC-5 flasks 1in3 Hela TM + NC 1in4	SD confirmed CH / 26/6/07
Aspirate medium from all the flasks that show no CPE and are 100% confluent.	☑
Wash the flasks with sufficient D-PBS to cover the monolayer.	☑
Remove washings and add 2 ml of TrypLE select. Incubate the flask at 37 ± 1°C for approx. 5-15 minutes.	☑
Gently agitate the flask to loosen the cell sheet and add 10ml of complete medium. Mixed the cell suspension. Final volume 12ml	☑
Seed X1 ml of cell suspension (equal to a 1 in X1 split) into fresh T75 flasks and make up to a total flask volume of 15 ml with complete media.	☑
Flask labelled with cell line, study number and test material (eg PNC, PTA etc)	☑
Return the flask to incubators at 37 ± 1°C 5% CO_2	☑
Initials/Date	RL / 26/6/07

Comments

X Positive flasks discarded previously CH 26/6/07

X1 3T3 0.5ml 1in4
Vero 0.5ml 1in4
Hela 0.5ml 1in4
MRC-5 0.6ml 1in3 RL 26/6/07

X2 10ml of complete medium was not added + the box was ticked in error. The final volume was 2ml ~~WATER~~ + the split ratio's are detailed below in the X1 comment. Comment added in retrospect for clarity. RL 27/7/07. X3

X3 Cells looked fine the next day (see results) so it is clear that a 1in4 split was done not a 1in24 CH 27/7/07

6 Day 21 : Subculture/Refeed of Cultures

Equipment

Lab 10	ID Number	
Safety Cabinet	BS148	☐ N/A
Incubator	BS228	☐ N/A
Waterbath	BS251	☐ N/A
Microscope	BS244	☐ N/A
Pipetaid	BS263	☐ N/A
Other ( )		☑ N/A
Other ( )		☑ N/A
Initials/Date	DF 13.7.7	

Reagents

	RI code	Batch No	Exp. date	
MEM/10E – seeding media	RI/184	5C601/07	06/09/07	X1 ☐ N/A
MEM/5E – refeed media	RI/183			☑ N/A
D-PBS	RI/025	5C609/07	02/09	☐ N/A
HBSS	RI/024			☑ N/A
TrypLE	RI/141	5C115/07	08/08	☐ N/A
Trypan blue (0.4%)	RI/044			☑ N/A
Other (DMEM/10E)	RI/190	5C604/07	06/09/07	☐ N/A
Other ( )				☑ N/A
Initials/Date	DF 13.7.7			

X1 See Note 28 ~~DF 236~~ DDF 13-7-7 DDF 13-7-7
236057
DF 13.7.7

7 Day 21 : Subculture/Refeed of Cultures

Steps	Task completed
Discard positive control flasks if show CPE.	N/A ☐ (✓,N/A) by date DF 13.7.7
If cultures are 100% confluent confirm with SD how to proceed.	☑
Refeed	N/A (✓,N/A)
Flasks to be refed	SD confirmed CH 13/7/07
Aspirate medium from the flasks that show no CPE.	☐
Add 15 ml fresh refeed media.	N/A ☑
Incubate flasks at 37 ± 1°C, 5% CO_2	☐
Initials/Date	DF 13.7.7
Subculture	☑ (✓,N/A)
Flasks to be subcultured 3T3 NC, PC, TA, STA Vero NC, TA HeLa NC, TA MRC5 PC, STA, TA, NC	SD confirmed CH 13/7/07
Aspirate medium from all the flasks that show no CPE and are 100% confluent.	✓
Wash the flasks with sufficient D-PBS to cover the monolayer.	☑
Remove washings and add 2 ml of TrypLE select. Incubate the flask at 37 ± 1°C for approx. 5-15 minutes.	☑
Gently agitate the flask to loosen the cell sheet and add 10ml of complete medium. Mixed the cell suspension. Final volume 12ml	☑
Seed X' ml of cell suspension (equal to a 1 in X' split) into fresh T75 flasks and make up to a total flask volume of 15 ml with complete media.	☑
Flask labelled with cell line, study number and test material (eg PNC, PTA etc)	☑
Return the flask to incubators at 37 ± 1°C 5% CO_2	☑
Initials/Date	DF 13.7.7

X' See page 25 for frontier details DF 3.7.7

Comments

X[1] Hela, 3T3 and Vero VC flasks had 2ml of TrypLE added with 0.5ml of TrypLE to be passed from the flasks to fresh flasks equating to a 1 in 4 cell split. MRC-5 VC flasks had a 1 in 3 split performed.

Hela, 3T3 and Vero TA flasks were treated in the same manner as the negative Control flasks to give a 1 in 4 split and the MRC-5 TA flasks a 1 in 3 split.

STA and PC for MRC-5 were split at a ratio of 1 in 3. STA and PC for 3T3 were split at a ratio of 1 in 4 (Ref 3.7.7)

X[2] cell Suspension (Ref 4.7.7) X3

X3 This is a text addition Added for clarity CH 20/9/07.

2 level change AS04/07/07

8 Day 2~~5~~ : Preparation of fresh MRC-5 indicator cultures for Haemadsorption positive control.

Equipment

Lab	ID Number	
Safety Cabinet	BS208	☐ N/A
Incubator	BS210	☐ N/A
Waterbath		☑ N/A
Microscope	BS249	☐ N/A
Pipetaid	BS 192	☐ N/A
Other ( )		☑ N/A
Other ( )		☑ N/A
Initials/Date	AS / 04/07/07	

X1

Reagents

② AS04/07/07

	RI code	Batch No	Exp. date	
MEM/10E – seeding media	RI/184	50601/07	06/07/07	☐ N/A
MEM/5E – refeed media	RI/183			☐ N/A
D-PBS	RI/025	50643/07	02/2009	☐ N/A
HBSS	RI/024			☑ N/A
TrypLE	RI/141	50652/07	02/2009	☐ N/A
Trypan blue (0.4%)	RI/044	50620/07	X2 13/12/07	☐ N/A
Other ( )				☑ N/A
Other ( )				☑ N/A
Initials/Date		AS/04/07/07		

X1 waterbath needed 56°C, therefore used incubator to warm reagents AS04/07/07

X2 ② AS04/07/07

22 Text change CH 20/9/07

Day ~~25~~ : Preparation of fresh MRC-5 indicator cultures for Haemadsorption positive control.

Steps – Seeding of cells if required. ☑ (✓,N/A) – x2	Task complete
Cell line details. Cell concentration required ~~5 x 10⁴~~ 1x10⁵ cells/ml. MRC-5 passage number 11 17, C number 17, flasks confluency 100%.	☑
Using a sterile pipette, aspirate the medium from 1 x T 150 flasks of Cells.	☑
Wash the flasks with sufficient D-PBS to cover the monolayer.	☑
Remove washings and add 2 ml of TrypLE select each flask. Incubate the flask at 37 ± 1°C for approximatley 5 – 15 minutes.	☑
Gently agitate the flask to loosen the cell sheet and add 10 ml of complete medium. Mix the cell suspension.	☑
If multiple flasks are used pool in one sterile container. Final volume 12 ml	☑
Perform viable cell counts in the presence of Trypan Blue. Add 0.2 ml of cell suspension to 0.2 ml of Trypan blue and mixed.	☑
Load the haemocytometer and count 3 of the 16 squares.	☑
Dilute cell suspension and seed 2 x 1 T75 flasks with 15ml cell suspension at ~~5 x 10⁴~~ cells/ml. (x2 1x10⁵)	☑
Incubate all flasks at 37 ± 1°C with 5-10% CO_2.	☑
Initials/Date	AS 04/07/07

Cell Counts			
1. 16	2. 20	3. 21	Mean 20 (B)

Cell Concentration = (B x 2 x 10^4)	4 x 10 5 Cells/ml
Cell Concentration required	1x10 x2 5 ~~5 x 10⁴~~ cells/ml
Dilution required	1 In 4
Total volume of cell suspension required (number of T75 flasks prepared x 15ml + excess)	40 ml
Volume of cell suspension (added to make up to required concentration)	10 ml
Volume of complete media (added to make up to required concentration)	30 ml
Calculation performed by/date	AS 04/07/07
Calculation checked performed by/date	CH 16/7/07

x, two T75 flasks seeded because we may inoculate at 2 VIMS concentrations tomorrow AS04/07/07

x2 text change AS 04/07/07

Comments

N/A CM 27/7/10

3/ Text change CH 27/7/07 Text change made in retrospect but it is clear from the date that this was day 23 CH 27/7/07

9 Day 26: Inoculation of fresh indicator cultures for Haemadsorption positive control.

Equipment

Lab 9	ID Number	
Safety Cabinet	BS 199	☐ N/A
Incubator	BS 228	☐ N/A
Waterbath	BS 257	☐ N/A
Microscope	BS 439	☐ N/A
Micropipette		☑ N/A
Pipetaid	BS 269	☐ N/A
Other ( )		☑ N/A
Other ( )		☑ N/A
Initials/Date	AS/05/07/07	

Reagents

	RI code	Batch No	Exp. date	
MEM/5E – refeed media	RI/183	50602/07	06/09/07	☐ N/A
MEM/5TPB	RI/187	50570/07	24/08/07	☐ N/A
D-PBS	RI/025	50643/07	02/2009	☐ N/A
HBSS	RI/024			☑ N/A
Other ( )				☑ N/A
Other ( )				☑ N/A
Initials/Date		AS/05/07/07		

3 Text change CH 20/9/07

Day 2[6]: Inoculation of fresh indicator cultures for Haemadsorption positive control.

Steps - Preparation of positive control if required. ☑ (✓,N/A)	**Task completed**
Thaw rapidly a frozen ampoule of PI3 virus, batch P13050721 at 1.1x10[8] $TCID_{50}$/ml in waterbath set at 37 ± 1°C. Once thawed keep ampoule on ice until required.	☑
Perform virus dilution and complete table below.	☑
Required virus concentration for positive control is **1x10[4]TCID50/ml** * 1	☑

Virus Suspension(ml)	Diluent (ml)	Total Volume Required (ml)	Dilution	TCID50/ml
0.5	5.0	AS 05/10/07 5.5	1 in 11	1x10[7]
0.5	4.5	5.0	1 in 10	1x10[6]
1	9	10	1 in 10	1x10[5]
1	9	10	1 in 10	1x10[4]
	N/A AS 05/10/07		1 in	

Calculation performed by/date	AS 05/10/07
Calculation check performed by/date	R 15/10/07
Initials/Date	AS 05/10/07

Steps – inoculation of positive control flask	**Task completed**
Aspirate medium from approx 60 % confluent MRC-5 cells.	☑
Wash cells with sufficient amount of D-PBS.	☑
Inoculate one flask with 5ml of **positive control** and incubate flasks at 37 ± 1°C, 5% CO_2 for 60-90 min. * 1 Start time: 09.45 End time: 11.12	☑ start ☑ end
After incubation aspirate the inoculum and refeed cells with 15ml appropriate refeed media and incubate flasks at 37 ± 1°C, 5% CO_2.	☑
Initials/Date	AS/05/10/07

* 1 Two positive control flasks set up @ 1x10[5] and 1x10[4] TCID50/ml AS 05/10/07

10 Day 28: Haemadsorption Assay [x1]

Equipment

Virology Lab 9	ID Number	
Safety Cabinet	BS 199	☐ N/A
Incubator	BS 228	☐ N/A
Waterbath	BS 452	☐ N/A
Microscope	BS 935	☐ N/A
Micropipette		☑ N/A
Pipetaid	BS 261 + 269	☐ N/A
Other (Centrifuge)	BS 040	☐ N/A
Other ( )		☑ N/A
Initials/Date	MJS / 10/07/10	

Reagents

	RI code	Batch No	Exp. date
D-PBS	RI/025	SD643107	02/2009
Human O red blood cells	RI/007	S0731/0	27/7/10
Guinea pig red blood cells	RI/006	S0708/0	19/8/10
Adult chicken red blood cells	RI/005	S0710/0	19/8/10
Other (MEM)	RI/524	S0624/0	04/2009
Other ( )		N/A MJS 10/07/10	
Initials/Date		MJS / 10/07/10	

x1 Pages 31, 32, 33, 35, 36 were transcribed to 2823-002 because the same procedure was carried out for these two studies. MJS 10/07/10

Preparation of cell culture supernatants

Steps	Task completed
Collect media from all flasks into uniquely labelled centrifuge tubes and add[x1] 10ml of D-PBS to each flask. Store flasks at 37 ± 1°C, 5% CO_2 incubator until required	☑
Centrifuge all supernatants at 1000 x 'g' for 10 minutes at 4 ±2C° and keep[x2] until required. Supernatants were stored at <-70°C in BS N/A	☑/N/A
Use cells for haemadsorption.	☑
Initials/Date	MD 10/07/07

Comments

X' DPBS was added by MD. 10.07.07

x2 Supernatants were discarded at the discretion of the SO MD 10/07/07

Confirmed by SO CH 11/7/07.

Day 28: Haemadsorption Assay

Preparation of 2.0% blood solution

Steps					Task completed
Dilute the three types of blood to 2.0%.					☑
Blood type	**Orig Stock conc.%**	**Dilution**	**Total Volume Required (ml)**	**Blood (ml)**	**Chilled HBSS (ml)**
Adult Chicken	7	1 in 3.5	49.0 x2	14.0	35.0
Human O	50	1 in 25.0	50.0 x2	2x 0	48.0 25.0
Guinea Pig	15	1 in 7.5	49.5 x2	6.6	42.9
Calculation performed by/date					MS / 10/07/10
Calculation check performed by/date					MS / 10/07/10
Initials/Date					MS / 10/07/10

Steps		Task completed
Centrifuge red blood cells at 160 'g' for 10 minutes at 4±2°C		☑
If the supernatant **is clear**: Aspirate supernatant and resuspend pellet in the same total volume of chilled HBBS. Keep blood on ice.	Adult Chicken	☑ (✓ N/A)
	Human O	N/A (✓ N/A)
	Guinea Pig	N/A (✓ N/A)
If the supernatant **is not clear**: Centrifuge supernatant again until it is clear. Aspirate supernatant and resuspend pellet in the same total volume of chilled HBBS. Keep blood solution on ice until required.	Adult Chicken	N/A (✓ N/A)
	Human O	☑ (✓ N/A)
	Guinea Pig	☑ (✓ N/A)
Record the number of times the blood was centrifuged to get a clear supernatant.	Adult Chicken	1
	Human O	2
	Guinea Pig	3 x1
Initials/Date		MS 10/07/10

x1 Last spin was performed @ +4°C, 500'g' for 10 minutes. MS 10/07/10

x2 Large volumes of blood were prepared because blood was shared with 2823-001 study. Adult Chicken and Guinea Pig blood was also shared with 1889-104 & 2638-002 studies. MS 10/07/10

Comments

N/A CH 11/7/07

Day 28: Preparation of erythrocytes for Haemadsorption

Preparation of 0.5% blood solution

Steps				Task completed
Dilute three types of blood from 2.0% to 0.5%.				☑
Blood type	**Dilution**	**Total Volume Required (ml)**	**Blood solution at 2.0%(ml)**	**Chilled HBSS x1 (ml)**
Adult Chicken	1 in 4	160	40	120
Human O	1 in 4	160	40	120
Guinea Pig	1 in 4	160	40	120
Calculation performed by				~~MD~~ 10/7/07 MA
Calculation check				CH 10/7/07
Keep blood solutions on ice until required.				☑
Initials/Date				~~MD~~ 10/7/07

Comments

x1 HBSS was added by MA. 10.07.07 MA

11 Day 28: Haemadsorption Assay

Steps	Task completed
Pool equal volumes of three blood type solutions at 0.5% together, enough to add 9ml of blood to each plate **twice**.	☑
Aspirate wash from flasks.[x1]	☑
Add 9ml of blood solution mixture at 0.5% to each flask.	☑
Refrigerate flasks in refrigerator BS166 (recorded temp. 7.6 °C*) for 30±5 minutes. Start time: 14:58 End time: 15:26	☑ Start ☑ End
After the incubation period aspirate blood from flasks and wash with 10ml DPBS	☑
Score flasks and record the results in the result table.	☑
Aspirate wash from flasks.[x1]	☑
Add 9ml of blood solution mixture at 0.5% to each flask.	☑
Incubate flasks in incubator or at room temperature (depending on protocol) 24.0° (recorded temp. 24.0 °C*) for 30±5 minutes. Start time: 16:13 End time: 16:43	☑ Start ☑ End
After the incubation period aspirate blood from flasks and wash with 10ml DPBS	☑
Score flasks and record the results in the result table.	☑
* All temperatures were taken at the start of the incubation period.	
Initials/Date	MD 10/07/07

Comments

x1 Wash was aspirated by A. 10.07.07

The MRC-5 STA was also tested for haemadsorption as only early signs of CPE were noted. This could confirm whether the signs of CPE were due to viral infection or not Added for information CM 11/7/07.

12 Result Tables

Results: Observation of CPE (Day 1-14)

Cell line:		MRC-5				Vero			
		Flasks showing CPE							
By/date	Days Post Inoculation	NC	PC	TA	STA	NC	PC	TA	STA
DF 13.6.7	1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
TS 14.6.7	2	0/1	0/1	0/1	0/1	0/1	1/1	0/1	0/1
DF 15.6.7	3	0/1	0/1	0/1	0/1	0/1	1/1	0/1	0/1
TS 18/06/07	6	0/1	0[x]/1[1]	0/1	0[x]/1[1]	0/1	1/1	0/1	1/1
DF 19.6.7	7	0/1	0/1	0/1	0/1	0/1	[1/2]/1 x2	0/1	1/1 x2
DF 20.6.7	8	0/1	0/1	0/1	0/1	0/1	0[1] DF 20.6.7 x5	0/1	N/A/1
TS 21/6/07	9	0/1	0/1 x4	0/1	0/1 x4	0/1	N/A/1	0/1	N/A/1
DF 22.6.7	10	0/1	0/1	0/1	0/1	0/1	N/A/1	0/1	N/A/1
DF 25.6.7	13	0/1	0/1	0/1	0/1	0/1	N/A/1	0/1	N/A/1
TS 26/6/07	14	0/1	0/1	0/1	0/1	0/1	NA/1	0/1	NA/1

CIDF 21.6.7 x3 x4

Comments

x1 less confluent than the NC - Some vacuolation observed TS 18/06/07

x2 Positive flasks discarded DF 19.6.7

x3 All flasks were stored in BS 228 DF 21.6.7

x4 Some vacuolation observed but not clearly CPE, therefore stored negative by SD CH 21/6/07.

x5 written in error flask also discarded day before See x2 for further details DF 21.6.7

Results: Observation of CPE (Day 1-14)

Cell line:		HeLa				NIH 3T3			
		Flasks showing CPE							
By/date	Days Post Inoculation	NC	PC	TA	STA	NC	PC	TA	STA
DF 13.6.7	1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
AS 14.6.07	2	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
DF 15.6.7	3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
AS 18/06/07	10	0/1	1/1 X1	0/1 X1	1/1	0/1	0/1	0/1	0 X2 /1
DF 19.6.7	7	0/1	1 X3 /1	0/1	1/1 X3	0/1	0/1	0/1	0/1
DF 20.6.7	8	0/1	N/A/1	0/1	N/A/1	0/1	0/1	0/1	0/1
AS 21/6/07	9	0/1	N/A/1	0/1	N/A/1	0/1	0/1	0/1	0/1
DF 22.6.7	10	0/1	N/A/1	0/1	N/A/1	0/1	0/1	0/1	0/1
DF 25.6.7	13	0/1	N/A/1	0/1	N/A/1	X5 /1	X5 /1	X5 /1	X5 /1
RL 26/6/07	14	0/1 X6	NA/1	0/1 X6	NA/1	0/1	0/1	0/1	0/1

X4

Comments X1 cells are confluent - some patches + loose cells in media AS 18/06/07

X2 some loose cells in media AS 18/06/07

X3 Positive flasks discarded DF 19.6.7

X4 All flasks were stored in BS228 DF 21.6.7

X5 media yellow in colour See pg 16a for details. DF 25.6.7

X6 some floating cells observed. RL 26/6/07

Results: Observation of CPE (Day 15-28)

Cell line:		MRC-5 X_6				Vero			
		Flasks showing CPE							
By/date	Days Post Inoculation	NC	PC	TA	STA	NC	PC	TA	STA
27.6.7	15	0 /1	0 /1	0 /1	0 /1	0 /1	N/A 1	0 /1	N/A 1
AS 28/6/07	16	0 /1	0 /1	0 /1	0 /1	0 /1	N/A /1	0 /1	N/A 1
AS 29/6/07	17	0 /1	0^{X_1} /1	0 /1	0^{X_1} /1	0 /1	N/A /1	0 /1	N/A 1
3.7.7	20	0 /1	0 /1	0 /1	0 /1	0 /1	N/A /1	0 /1	N/A /1
3.7.7	21	0 /1	0 /1 x_2	0 /1	0 /1 x_2	0 /1	N/A /1	0 /1	N/A 1
AS 04/07/07	22	0 /1	0 /1 ^{X_3} $_{X_4}$	0 /1	0^{X_4} /1	0 /1	N/A /1	0 /1	N/A 1
AS 05/07/07	23	0 /1	0^{X_5} /1	0 /1	0^{X_5} /1	0 /1	N/A 1	0 /1	N/A
AS 06/07/07	24	0 /1	0 /1 ^{X_6} $_{X_7}$	0 /1	0^{X_7} /1	0 /1	N/A /1	0 /1	N/A
AS 09/07/07	27	0 /1	0 /1 $_{x8}$	0 /1	0 /1	0 /1	N/A /1	0 /1	N/A /1
AS / CH 10/07/07	28	0 /1	1 /1	0 /1	0 /1 $_{x9}$	0 /1	N/A /1	0 /1	N/A /1

Comments X_1 some rounded cells loosely attached to the monolayer

X_2 some rounded cells (not seen in NC) but monolayer AS 29/6/07 still intact CH 3/7/07

X_3 PPC AS04/7/07 X_4 See X_2 comment AS04/07/07 @AS04/07/07

X_5 see X_2 comment AS05/07/07

X_6 observations for the AMC flasks inoculated 05/07/07 will be recorded on page 42 of this @AS06/07/07 document AS06/07/07

X_7 less confluent than the PNC, loose cells in media, some vacuolation AS 06/07/07

X_8 Cells are less confluent and look wider in comparison to NC 7/7. Also rounded cells in higher phase observed. AS 09/07/07

X_9 Early signs of cpe observed. AS 10/07/07

Page completion check initials/date ...CH. 11/7/07

Data check initials/date ....CH. 23/7/07

x10 Please see page 42 for comment x10 and all other comments to follow. AS 09/8/07

COVANCE
THE DEVELOPMENT SERVICES COMPANY
Biotechnology department

Study number: 2823-002

Results: Observation of CPE (Day 15-28)

Cell line:		HeLa				NIH 3T3			
		Flasks showing CPE							
By/date	Days Post Inoculation	NC	PC	TA	STA	NC	PC	TA	STA
DF 27.6.7	DF 15	0/1	N/A/1	0/1	N/A/1	0/1	0/1	0/1	0/1
AS 28/6/07	16	0/1	N/A/1	0/1	N/A/1	0/1 x1	0/1 x1	0/1 x1	0/1 x1
AS 29/6/07	17	0 x2/1	N/A/1	x2 @AS29/6/07 0/1	N/A/1	0/1 x3	0 x4/1	0 x5/1 x11 29/6/07	0 x4/1
DF 2.7.7	20	0/1	N/A/1	0/1	N/A/1	0/1 x6	x6 0/1	x 0/1 x6 x19	0 x6/1
DF 3.7.7	21	0 x8/1	N/A/1	0/1 x8	N/A/1	0/1	0/1 x8	0/1	0/1
AS DF 04/07/07	22	0/1	N/A/1	0/1	N/A/1	0/1	0/1 x9	0/1	0/1 x9
AS 05/07/07	23	0/1	N/A/1	0/1	N/A/1	0/1	0/1	0/1	0/1
AS 06/07/07	24	0/1	N/A/1	0/1	N/A/1	0/1	0 x10/1	0/1	0 x10/1
AS 07/07/07	27	0/1 x10	N/A/1	0/1 x10	N/A/1	0/1 x11	1/1 x11	0/1 x11	0/1 x12 x11
AS CH 10/07/07	28	0/1 x13	N/A/1	0/1	N/A/1 x18 x13	0/1 x16 x14	1/1 x16 x15	0/1 x16 x18	1/1 x16

Comments

x1 loose cells in media PNC less confluent than other flasks AS 28/6/07 NB PNC still looks healthy @AS28/6/07 AS 28/6/07

x2 Some loose cells in media. Cells ~70% confluent, look healthy. AS 29/6/07

x3 cells ~80% confluent, look healthy. Loose cells in media AS 29/6/07

x4 More loose cells in media than the NC but same confluency. Loose rounded cells loosely attached to monolayer. Cells look less healthy than the NC AS 29/6/07 @AS 29/6/07

x5 loose cells in media, 90% confluent, look healthy AS 29/6/07

x8 Cells floating in media, really yellow so cells were refed with DMEM/SE DF 2.7.7

x7 Flasks refed with 15ml DMEM/SE RT 5/07/11/07 Supernatant stored in BS152 on top of box 2H 2221 See Grate# 232899 for further information (I)DF 2.7.7 CJDF 2.7.7 DF 2.7.7. CJDF 2.7.7

x8 Some rounded cells but monolayer still intact CH 8/7/07

x9 PSTA AS 04/07/07 + PPC

x10 AS07 More loose cells + cell debris than the PNC, cells look unhealthy AS 06/07/07

13 Results: Haemadsorption

Result table for the incubation in refrigerator:

	MRC-5	VERO	HELA	NIH 3T3
PSM [x2]	0/1/1	[x2] MIXTURE OF BLOOD TYPES		
PNC	0/1	0/1 [x1]	0/1	0/1
PTA	0/1	0/1 [x1]	0/1	0/1
PPC*	1/1	N/A	N/A	N/A
APC*	2/2	N/A	N/A	N/A
Initials/Date			CH JMS 10/07/07	

* if applicable.

Result table for the incubation at room temperature:

	MRC-5	VERO	HELA	NIH 3T3
PSM [x2]	1/1	[x2] MIXTURE OF BLOOD TYPES		
PNC	0/1	0/1 [x1]	0/1	0/1
PTA	0/1	0/1 [x1]	0/1	0/1
PPC*	1/1		N/A JMS 10/07/07	
APC*	2/2			
Initials/Date			JMS 10/07/07	

* if applicable.
Results ~~(+ = haemadsorption observed, - = no haemadsorption observed).~~ Text deleted CH 20/9/07
All flasks were discarded following scoring.

[x1] There was some non specific binding of red blood cells to the monolayer observed. JMS 10/07/07

[x2] Text addition. JMS 10/07/07

Results: 0/1 0 flasks out of 1 positive for haemadsorption
2/2 2 flasks out of 2 positive for haemadsorption
Text added CH 20/9/07

Comments

x10 Many floating cells and gaps in monolayer possibly due to overgrowth. [illegible] 09/07/07

x11 Many floating cells. Cells grow in patches. Media appears very yellow. [illegible] 09/07/07

x12 Possibly early signs of cpe. observed. [illegible] 09/07/07

x13 See x10 comment. [illegible] 10/07/07

x14 Possibly due to overgrowth some monolayer came off. [illegible] 10/07/07

x15 See x11 comment [illegible] 10/07/07

x16 Media looks very yellow in all control flasks. [illegible] 10/07/07 x17 CH 11/7/07.

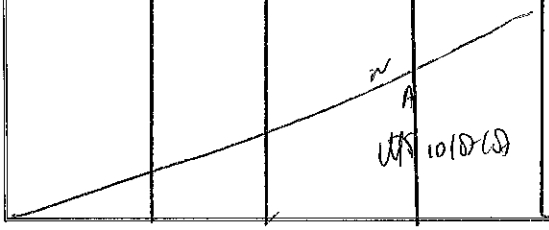
x17 & Yellow media is a result of overgrowth of these cells CH 11/7/07

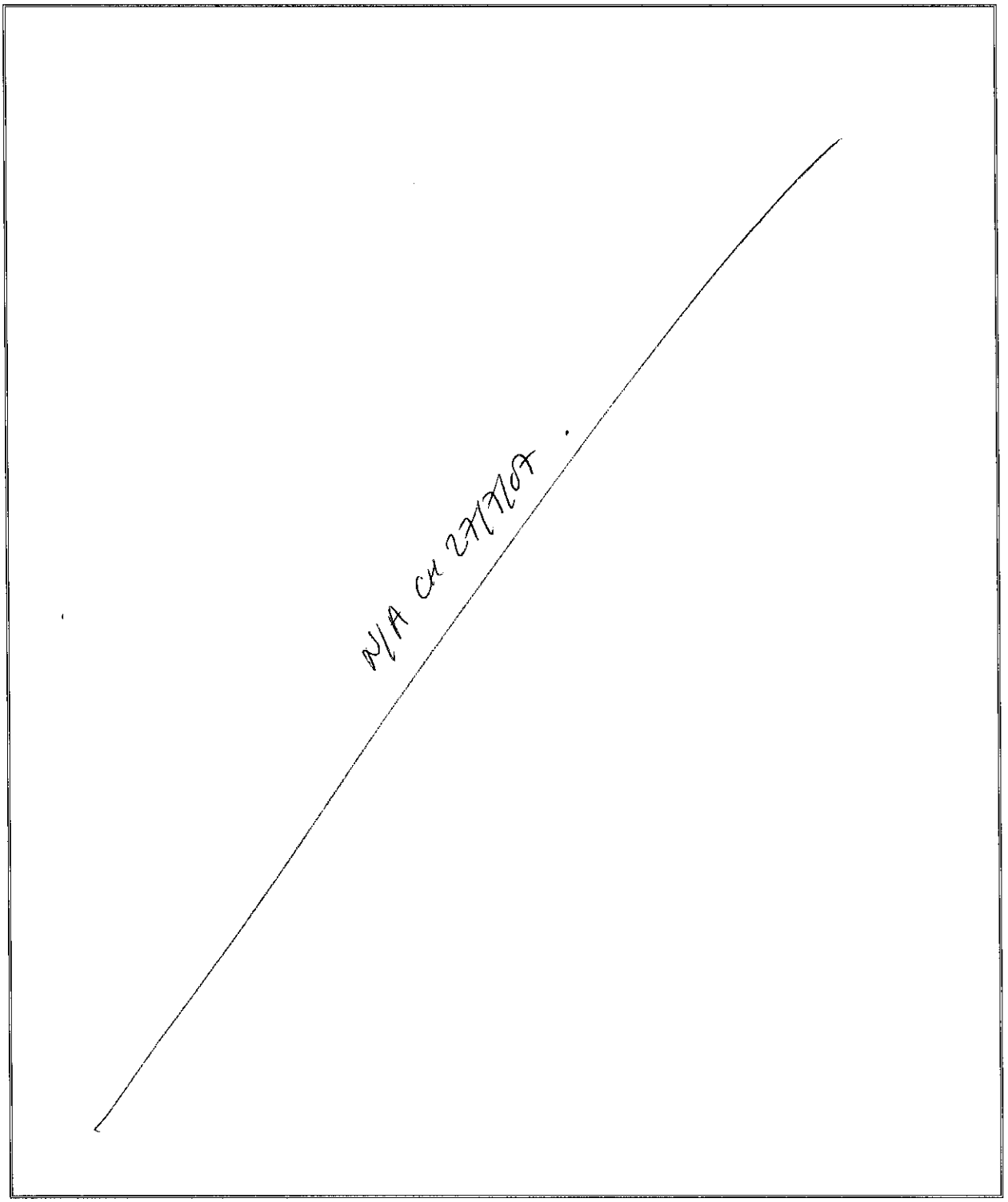
x18 x13 comment should have been written in the TA column as it refers to x10. However comment will not be changed in retrospect CH 20/9/07

x19 x6 comment added in retrospect but it is clear from the data that cells were reJed x comment had been added in error CH 20/9/07

MRC-5 APC obs

BY/ DATE	DAY	1×10^5	1×10^4
AS 06/07/07	24	0/1	0/1
[illegible] 9/07/07	27	0/1	0/1
[illegible] 10/07/07	28	1/1	1/1




N/A CM 27/7/09

Final Report

POLYMERASE CHAIN REACTION ASSAY FOR THE DETECTION OF HUMAN T-CELL LYMPHOTROPIC VIRUS TYPES I AND II (HTLV-I/II) IN BIOLOGICAL SAMPLES

Study Number: AC08DP.105013.BSV

Test Article ID: H1 MCB.1

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

One-half (0.5) μg of DNA (representing approximately 7.5 x 10^4 cells) isolated from test article H1 MCB.1 was analyzed for the presence of human T-cell lymphotropic virus types I and II (HTLV-I/II) proviral DNA by the polymerase chain reaction (PCR)[1] technique. The assay can detect 100 copies of HTLV-I/II proviral DNA in the presence of 0.5 μg of genomic DNA.

The results presented herein indicate that the test article tested negative for the presence of HTLV-I/II DNA.

---

1 The Polymerase Chain Reaction (PCR) process is covered by U.S. patents Nos. 4,683,195 and 4,683,202 owned by Roche Molecular Systems, Inc. and F. Hoffman-LaRoche Ltd.

STUDY INFORMATION

Test Article: H1 MCB.1 was received by BioReliance on 09/25/2007. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor. Retention of reserve sample from each batch of test article is the responsibility of the sponsor.

Testing Facility: BioReliance

Schedule:

Study Initiation: 09/26/2007

Lab Initiation: 10/02/2007

Lab Completion: 10/04/2007

Study Completion: See Study Director's signature date in "Approval" Section.

Study Director:

Archives: All raw data, the protocol, and a copy of the final report will be maintained according to Standard Operating Procedure OPQP3040 by the BioReliance Quality Assurance Unit headquartered at:

BioReliance

OBJECTIVE

The objective of the study is to detect the presence of HTLV-I/II proviral sequences in the test article by PCR amplification, an *in vitro* procedure that can generate millions of copies of a sequence from very low levels of template DNA.

TEST SYSTEM

PCR amplification is performed on test article DNA using HTLV-specific primers. In the presence of HTLV-I/II proviral DNA, these primers produce a 158 bp amplification product. The amplification products are analyzed by agarose gel electrophoresis in the presence of ethidium bromide. Positive results are confirmed and discrimination between HTLV-I and HTLV-II sequences is achieved by restriction endonuclease treatment of the PCR product and analysis of the restriction pattern obtained. The following controls are included in each assay:

Negative Control: Genomic DNA from MRC5 human fetal lung fibroblasts
Source: BioReliance

Positive Controls:

HTLV-I: Negative control DNA spiked with 100 copies of pH750, a plasmid containing a 752 bp fragment from the HTLV-I tax/rex gene
Source: BioReliance

HTLV-II: Negative control DNA spiked with 100 copies of pMAHTII, a plasmid containing a 552 bp fragment from the HTLV-II tax/rex gene
Source: BioReliance

No DNA Control: Nuclease-free water
Source: USB or other commercial supplier

Spiked Control: The spiked controls (amplification suitability controls) verify the absence of PCR inhibitors in the test article DNA.

HTLV-I: Test article extract spiked with 100 copies of pH750.

HTLV-II: Test article extract spiked with 100 copies of pMAHTII.

METHODS

Sample Preparation

The test article was received at BioReliance and provided to the laboratory for testing. DNA was isolated using the Easy DNA™ Kit (Invitrogen) as outlined in the kit procedure and SOP BPBT0920.

DNA Amplification

Each PCR amplification was performed on 0.5 µg of test article DNA and on the assay controls, using primers HT-OS and HT-OA, specific for the tax/rex region of HTLV-I/II, employing conditions optimized to achieve detection of 100 copies of proviral DNA. Aliquots of the amplification products obtained from the test article and from the control samples were analyzed by agarose gel electrophoresis in the presence of ethidium bromide. Amplification products were visualized on a UV transilluminator and photographed using Polaroid 667 film (OPBT0932).

RESULTS

Test article DNA (0.5 µg), representing approximately 7.5×10^4 test article cells, was analyzed for the presence of HTLV-I/II proviral DNA by PCR amplification and agarose gel electrophoresis in the presence of ethidium bromide. The results are shown in Figure 1. The No DNA control (NO) and Negative control (NC) showed no bands at 158 bp. The positive controls (PC-I and PC-II) produced a 158 bp band. The test article spiked with 100 copies of either pH750 (TAS-I) or pMAHTII (TAS-II) produced a 158 bp band, demonstrating that the test article did not inhibit the PCR reaction. The test article (TA) produced no bands at 158 bp.

These results provide evidence that the test article H1 MCB.1 tested negative for the presence of HTLV-I/II proviral DNA.

APPROVAL

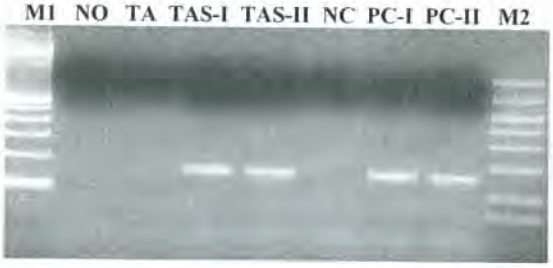
I accept responsibility for the conduct of this study which was performed in compliance with the U.S. FDA Good Laboratory Practice regulations (21 CFR 58), the UK GLP Regulations, the Japanese GLP Standard and the Organization for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice.

23 Oct 07

Date

Study Director

FIGURE 1



Detection of HTLV-I/II proviral sequences in test article H1 MCB.1 utilizing agarose gel electrophoresis in the presence of ethidium bromide. The arrow indicates specific amplification products.

M1:	100 bp DNA ladder
NO:	No DNA control
TA:	Test Article
TAS-I:	Test article spiked with 100 copies of pH750
TAS-II:	Test article spiked with 100 copies of pMAHTII
NC:	Negative control (MRC5 genomic DNA)
PC-I:	Positive control for HTLV-I (negative control DNA spiked with 100 copies pH750)
PC-II:	Positive control for HTLV-II (negative control DNA spiked with 100 copies pMAHTII)
M2:	Biomarker low DNA size marker.

Quality Assurance Statement

Study Title: POLYMERASE CHAIN REACTION ASSAY FOR THE DETECTION OF HUMAN T-CELL LYMPHOTROPIC VIRUS TYPES I AND II (HTLV-I/II) IN BIOLOGICAL SAMPLES

Study Number: AC08DP.105013.BSV

Study Director:

Quality Assurance performed the inspections listed below for this study. Verification of the study protocol was also performed and documented by QA. Procedures, documentation, equipment records, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. FDA Good Manufacturing Practices (21 CFR 210 and 211), the UK GLP Regulations, the Japanese GLP Standard, and the OECD Principles of Good Laboratory Practice and to assure that the study is conducted according to the protocol and relevant Standard Operating Procedures.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

**	Inspect On	23-Oct-07 - 23-Oct-07 To Study Dir 23-Oct-07 To Mgmt 23-Oct-07
	Phase	Final Report and data audit
*	Inspect On	24-Sep-07 - 25-Sep-07 To Study Dir 24-Sep-07 To Mgmt 24-Sep-07
	Phase	Systems Inspection - Administration of Test Substance to Test System
*	Inspect On	24-Sep-07 - 25-Sep-07 To Study Dir 25-Sep-07 To Mgmt 25-Sep-07
	Phase	Systems Inspection - Manipulation of Test System
*	Inspect On	24-Sep-07 - 25-Sep-07 To Study Dir 25-Sep-07 To Mgmt 25-Sep-07
	Phase	Systems Inspection - Observation of Test System/Data Collection and/or Analysis
*	Inspect On	24-Sep-07 - 25-Sep-07 To Study Dir 25-Sep-07 To Mgmt 25-Sep-07
	Phase	Systems Inspection - Test System Preparation

** Inspection specific for this study
* Systems Inspection

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

23Oct07

DATE

QUALITY ASSURANCE

Final Report

POLYMERASE CHAIN REACTION ASSAY FOR THE DETECTION OF CYTOMEGALOVIRUS (CMV) IN BIOLOGICAL SAMPLES

Study Number: AC08DP.105012.BSV

Test Article ID: H1 MCB.1

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

One-half (0.5) µg of DNA isolated from test article H1 MCB.1 (representing approximately 7.5 x 10^4 cells) was analyzed for the presence of human cytomegalovirus (CMV) DNA by the polymerase chain reaction (PCR)[1] technique. The assay can detect 100 copies of CMV in the presence of 0.5 µg of genomic DNA.

The results presented herein indicate that the test article tested negative for the presence of CMV DNA.

---

[1] The Polymerase Chain Reaction (PCR) process is covered by U.S. patents Nos. 4,683,195 and 4,683,202 owned by Roche Molecular Systems, Inc. and F. Hoffman-LaRoche Ltd.

STUDY INFORMATION

Test Article: H1 MCB.1 was received by BioReliance on 09/25/2007. Determination of the stability, purity, and concentration of the test article is the responsibility of the sponsor. Retention of reserve sample from each batch of test article is the responsibility of the sponsor.

Testing Facility: BioReliance

Schedule:

Study Initiation: 09/26/2007

Lab Initiation: 10/02/2007

Lab Completion: 10/08/2007

Study Completion: See Study Director's signature date in "Approval" Section.

Study Director:

Archives: All raw data, the protocol, and a copy of the final report will be maintained according to Standard Operating Procedure OPQP3040 by the BioReliance Quality Assurance Unit headquartered at:

BioReliance

OBJECTIVE

The objective of the study is to detect the presence of CMV sequences in the test article by PCR amplification, an *in vitro* procedure that can generate millions of copies of a sequence from very low levels of template DNA.

Test System:

PCR amplification is performed on test article DNA using CMV-specific primers. In the presence of CMV, these primers produce a 363 bp amplification product. The amplification products are analyzed by agarose gel electrophoresis in the presence of ethidium bromide. The following controls are included in the assay:

Negative Control:	Genomic DNA from MRC5 human fetal lung fibroblast line Source: BioReliance
Positive Control:	Negative control DNA spiked with 100 copies of pCMVpol, a plasmid containing a 552 bp fragment from the CMV polymerase gene Source: BioReliance
No DNA Control:	Nuclease-free water Source: USB or other commercial source
Spiked Control:	Test article extract spiked with 100 copies of pCMVpol, to verify the absence of PCR inhibitors in the test article DNA (amplification suitability control)

METHODS

Sample Preparation

The test article was received at BioReliance and provided to the laboratory for testing. DNA was isolated from the test article sample using the Easy DNA™ Kit (Invitrogen) as outlined in the kit procedure and SOP BPBT0920.

DNA Amplification

PCR amplification was performed on 0.5µg of test article DNA using primers CMV100 and CMV150 specific for the polymerase region of CMV, employing conditions optimized to achieve detection of 100 copies of CMV. Aliquots of the amplification products obtained from the test article and from the control samples were analyzed by agarose gel electrophoresis in the presence of ethidium bromide. Amplification products were visualized on a UV transilluminator and photographed using Polaroid 667 film (SOP OPBT0935).

REPEATS

The first performance of the PCR assay, testing 0.5µg amount of test article DNA, provided an invalid test. The positive control (PC) failed to produce a 363 bp band (results not presented). The PCR assay was repeated, using the same amount of test article DNA. The repeated assay provided a valid test with a negative result (results are presented in Results section below).

RESULTS

Test article DNA (0.5µg), representing approximately 7.5×10^4 test article cells, was analyzed for the presence of CMV DNA by PCR amplification and agarose gel electrophoresis in the presence of ethidium bromide. The results are shown in Figure 1. The No DNA control (NO) showed no bands and the Negative control (NC) showed no bands at 363 bp. The positive control (PC) produced a 363 bp band. The test article spiked with 100 copies of pCMVpol (TAS) produced a 363 bp band, demonstrating that the test article did not inhibit the PCR reaction. The test article (TA) produced no bands at 363 bp.

These results provide evidence that the test article H1 MCB.1 tested negative for the presence of CMV DNA.

APPROVAL

I accept responsibility for the conduct of this study which was performed in compliance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the UK GLP Regulations, the Japanese GLP Standard, and the Organization for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice.

23 Oct 07

Date

Study Director

FIGURE 1

Detection of CMV specific sequences in the test article H1 MCB.1 utilizing agarose gel electrophoresis in the presence of ethidium bromide

M1: 100 bp DNA ladder
NO: No DNA control
TA: Test Article
TAS: Test article spiked with 100 copies pCMVpol
NC: Negative control (MRC5 DNA)
PC: Positive control (MRC5 DNA spiked with 100 copies pCMVpol)
M2: Biomarker low, a DNA size marker

Arrow indicates the specific amplification product.

Quality Assurance Statement

Study Title: POLYMERASE CHAIN REACTION ASSAY FOR THE DETECTION OF CYTOMEGALOVIRUS (CMV) IN BIOLOGICAL SAMPLES

Study Number: AC08DP.105012.BSV

Study Director:

Quality Assurance performed the inspections listed below for this study. Verification of the study protocol was also performed and documented by QA. Procedures, documentation, equipment records, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. FDA Good Manufacturing Practices (21 CFR 210 and 211), the UK GLP Regulations, the Japanese GLP Standard, and the OECD Principles of Good Laboratory Practice and to assure that the study is conducted according to the protocol and relevant Standard Operating Procedures.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

**	Inspect On	23-Oct-07 - 23-Oct-07 To Study Dir 23-Oct-07 To Mgmt 23-Oct-07
	Phase	Final Report and data audit
*	Inspect On	24-Sep-07 - 25-Sep-07 To Study Dir 24-Sep-07 To Mgmt 24-Sep-07
	Phase	Systems Inspection - Administration of Test Substance to Test System
*	Inspect On	24-Sep-07 - 25-Sep-07 To Study Dir 25-Sep-07 To Mgmt 25-Sep-07
	Phase	Systems Inspection - Manipulation of Test System
*	Inspect On	24-Sep-07 - 25-Sep-07 To Study Dir 25-Sep-07 To Mgmt 25-Sep-07
	Phase	Systems Inspection - Observation of Test System/Data Collection and/or Analysis
*	Inspect On	24-Sep-07 - 25-Sep-07 To Study Dir 25-Sep-07 To Mgmt 25-Sep-07
	Phase	Systems Inspection - Test System Preparation

** Inspection specific for this study
* Systems Inspection

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

23 Oct 07

DATE

QUALITY ASSURANCE

Final Report

POLYMERASE CHAIN REACTION ASSAY FOR THE DETECTION OF HUMAN HERPESVIRUS 6 (HHV-6) IN BIOLOGICAL SAMPLES

Study Number: AC08DP.105020.BSV

Test Article ID: H1 MCB.1

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

One-half (0.5) µg of DNA (representing approximately 7.5 x 10^4 cells) isolated from test article H1 MCB.1 was analyzed for the presence of human herpesvirus 6 (HHV-6) viral DNA by the polymerase chain reaction (PCR)[1] technique. The assay can detect 100 copies of HHV-6 (variants A and B) viral DNA in the presence of 0.5 µg of genomic DNA.

The results presented herein indicate that the test article tested negative for the presence of HHV-6 DNA.

---

1 The Polymerase Chain Reaction (PCR) is covered by U.S. patents Nos. 4,683,195 and 4,683,202 owned by Hoffmann-LaRoche, Inc., licensed by BioReliance from Perkin-Elmer Cetus Instruments.

STUDY INFORMATION

Test Article: H1 MCB.1 was received by BioReliance on 09/25/2007. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor. Retention of reserve sample from each batch of test article is the responsibility of the sponsor.

Testing Facility: BioReliance

Schedule:

Study Initiation: 09/26/2007

Lab Initiation: 10/02/2007

Lab Completion: 10/04/2007

Study Completion: See Study Director's signature date in "Approval" Section.

Study Director:

Archives: All raw data, the protocol, and a copy of the final report will be maintained according to Standard Operating Procedure OPQP3040 by the BioReliance Quality Assurance Unit headquartered at:

BioReliance

OBJECTIVE

The objective of the study is to detect the presence of HHV-6 viral sequences in the test article by PCR amplification, an *in vitro* procedure that can generate millions of copies of a sequence from very low levels of template DNA.

TEST SYSTEM

For detection of HHV-6 viral DNA, PCR amplification is performed on test article DNA using HHV-6-specific primers according to SOP BPBT0924. In the presence of HHV-6A viral sequences, these primers produce a 328 bp amplification product, while the HHV-6A positive control plasmid (pU1102MOD) generates a 299 bp amplification product. In the presence of HHV-6B viral sequences, the primers produce a 553 bp amplification product, while the HHV-6B positive control plasmid (pZ29MOD) generates a 524 bp amplification product. The following controls are included in the assay:

Negative Control: Genomic DNA from MRC5 human fetal lung fibroblasts
Source: BioReliance

Positive controls:

HHV-6A: Negative control DNA spiked with 100 copies of plasmid pU1102MOD. Plasmid pU1102MOD contains a 2.3 Kb region from the HHV-6A (strain U1102) genome. The 2.3 Kb region contains a 29 bp internal deletion to distinguish it from the wild type HHV-6A sequence.
Source: BioReliance

HHV-6B: Negative control DNA spiked with 100 copies of plasmid pZ29MOD. Plasmid pZ29MOD contains a 2.3 Kb region from the HHV-6B (strain Z29) genome. The 2.3 Kb region contains a 29 bp internal deletion to distinguish it from the wild type HHV-6B sequence.
Source: BioReliance

No DNA Control: Nuclease free water
Source: USB or other commercial supplier

Spiked Controls: The spiked controls (amplification suitability controls) verify the absence of PCR inhibitors in the test article DNA.

HHV-6A: Test article spiked with 100 copies of plasmid pU1102MOD

HHV-6B: Test article spiked with 100 copies of plasmid pZ29MOD.

Following amplification, samples will be run on a 1.5 - 2.5% Metaphor or Agarose gel containing ethidium bromide and visualized by photography under ultraviolet light.

METHODS

Sample Preparation

The test article was received at BioReliance, and provided to the laboratory for testing. DNA was isolated using the Easy DNA™ Kit (Invitrogen) as outlined in the kit procedure and SOP OPBT0920.

DNA Amplification

PCR amplification was performed on 0.5 µg of test article DNA and on the assay controls using primers HHV-6F and HHV-6R, specific for the immediate-early region of HHV-6, employing conditions optimized to achieve detection of 100 copies of viral DNA. Aliquots of the amplification products obtained from the test article and from the control samples were analyzed by agarose gel electrophoresis in the presence of ethidium bromide. Amplification products were visualized on a UV transilluminator and photographed using Polaroid 667 film.

RESULTS

Test article DNA (0.5 µg), representing approximately 7.5×10^4 test article cells, was analyzed for the presence of HHV-6 viral DNA by PCR amplification and agarose gel electrophoresis in the presence of ethidium bromide. The results are shown in Figure 1. The following results provide evidence that the assay was valid and free of contamination:

a. the No DNA control (NO) showed no amplification bands
b. the Negative control (NC) showed no bands at 553, 524, 328 or 299 bp
c. the positive control (PC-1) produced a band at 299 bp
d. the positive control (PC-2) produced a band at 524 bp
e. the test article showed no bands at 524 or 299 bp.

The test article spiked with 100 copies of pU1102MOD (TAS-1) produced a 299 bp band and the test article spiked with 100 copies of pZ29MOD (TAS-2) produced a 524 bp band, demonstrating that the test article did not inhibit the PCR reaction.

The test article (TA) produced no bands at 553 or 328 bp.

These results provide evidence that the test article H1 MCB.1 tested negative for the presence of HHV-6 (variants A and B) viral DNA.

APPROVAL

I accept responsibility for the conduct of this study which was performed in compliance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the UK GLP Regulations, the Japanese GLP Standard and the Organization for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice.

23 Oct 07
Date

Study Director

FIGURE 1

Detection of HHV-6 (variants A and B) viral sequences in test article H1 MCB.1 utilizing agarose gel electrophoresis in the presence of ethidium bromide. Arrows indicate specific amplification products.

M1: 100 bp DNA ladder
NO: No DNA control
TA: Test Article
TAS-1: Test article spiked with 100 copies of pU1102MOD
TAS-2: Test article spiked with 100 copies of pZ29MOD
NC: Negative control (MRC5 genomic DNA)
PC-1: Positive control (negative control DNA spiked with 100 copies of pU1102MOD)
PC-2: Positive control (negative control DNA spiked with 100 copies of pZ29MOD)
M2: Biomarker low DNA size marker.

Quality Assurance Statement

Study Title: POLYMERASE CHAIN REACTION ASSAY FOR THE DETECTION OF HUMAN HERPESVIRUS 6 (HHV-6) IN BIOLOGICAL SAMPLES

Study Number: AC08DP.105020.BSV

Study Director:

Quality Assurance performed the inspections listed below for this study. Verification of the study protocol was also performed and documented by QA. Procedures, documentation, equipment records, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. FDA Good Manufacturing Practices (21 CFR 210 and 211), the UK GLP Regulations, the Japanese GLP Standard, and the OECD Principles of Good Laboratory Practice and to assure that the study is conducted according to the protocol and relevant Standard Operating Procedures.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

**	Inspect On	23-Oct-07 - 23-Oct-07 To Study Dir 23-Oct-07 To Mgmt 23-Oct-07
	Phase	Final Report and data audit
*	Inspect On	24-Sep-07 - 25-Sep-07 To Study Dir 24-Sep-07 To Mgmt 24-Sep-07
	Phase	Systems Inspection - Administration of Test Substance to Test System
*	Inspect On	24-Sep-07 - 25-Sep-07 To Study Dir 25-Sep-07 To Mgmt 25-Sep-07
	Phase	Systems Inspection - Manipulation of Test System
*	Inspect On	24-Sep-07 - 25-Sep-07 To Study Dir 25-Sep-07 To Mgmt 25-Sep-07
	Phase	Systems Inspection - Observation of Test System/Data Collection and/or Analysis
*	Inspect On	24-Sep-07 - 25-Sep-07 To Study Dir 25-Sep-07 To Mgmt 25-Sep-07
	Phase	Systems Inspection - Test System Preparation

** Inspection specific for this study
* Systems Inspection

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

23Oct07

DATE

QUALITY ASSURANCE

Final Report

POLYMERASE CHAIN REACTION ASSAY FOR THE DETECTION OF HUMAN HERPESVIRUS 7 (HHV-7) IN BIOLOGICAL SAMPLES

Study Number: AC08DP.105029.BSV

Test Article ID: H1 MCB.1

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

One-tenth (0.1) µg of DNA isolated from test article H1 MCB.1 (representing approximately 1.5 x 10^4 cells) was analyzed for the presence of Human Herpesvirus 7 (HHV-7) DNA by the polymerase chain reaction (PCR)[1] technique. The assay can detect 100 copies of HHV-7 in the presence of 0.5 µg of genomic DNA.

The results presented herein indicate that the test article tested negative for the presence of HHV-7 DNA.

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1 The Polymerase Chain Reaction (PCR) process is covered by U.S. patents Nos. 4,683,195 and 4,683,202 owned by Roche Molecular Systems, Inc. and F. Hoffman-LaRoche Ltd.

STUDY INFORMATION

Test Article: H1 MCB.1 samples were received by BioReliance on 10/17/2007 and 10/30/2007. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor. Retention of reserve sample from each batch of test article is the responsibility of the sponsor.

Testing Facility: BioReliance

Schedule:

Study Initiation: 10/18/2007

Lab Initiation: 10/19/2007

Lab Completion: 11/07/2007

Study Completion: See Study Director's signature date in "Approval" Section.

Study Director:

Archives: All raw data, the protocol, and a copy of the final report will be maintained according to Standard Operating Procedure OPQP3040 by the BioReliance Quality Assurance Unit headquartered at:

BioReliance

OBJECTIVE

The objective of the study is to detect HHV-7 DNA in the test article by PCR amplification, an *in vitro* procedure that can generate millions of copies of a sequence from very low levels of template DNA.

Test System:

PCR amplification is performed on test article DNA using HHV-7-specific primers. In the presence of HHV-7 DNA, these primers produce a 353 bp amplification product. The amplification products are analyzed by high resolution agarose gel electrophoresis in the presence of ethidium bromide. The following controls are included in the assay:

Negative Control:	Genomic DNA from MRC5 human fetal lung fibroblasts Source: BioReliance
Positive Control:	Negative control DNA spiked with 100 copies of pHH7, a plasmid containing a 1.2 Kb fragment of the HHV-7 genome. Source: BioReliance.
No DNA Control:	Nuclease-free water Source: USB or other commercial source.
Spiked Control:	Test article extract spiked with 100 copies of pHH7, to verify the absence of PCR inhibitors in the test article DNA, (amplification suitability control)

METHODS

Sample Preparation

The test article was received at BioReliance, and provided to the laboratory for testing. DNA was isolated from the test article sample using the Easy DNA™ kit (Invitrogen) as outlined in the kit procedure and SOP BPBT0920. The DNA yield from the first extraction was very low; therefore, DNA was re-extracted from the a new test article sample using the QIAamp® Blood Kit (Qiagen) as outlined in the kit procedure and SOP BPBT0917.

DNA Amplification

PCR amplification was performed on 0.1 µg of test article DNA using primers HHV7F and HHV7IR specific for sequences common to the capsid protein gene regions in the HHV-7 genome, employing conditions optimized to achieve detection of 100 copies of HHV-7 DNA. Aliquots of the amplification products obtained from the test article and from the control samples were analyzed by high resolution agarose gel electrophoresis in the presence of ethidium bromide. Amplification products were visualized on a UV transilluminator and photographed using Polaroid 667 film (SOP OPBT0929).

RETESTS

The first performance of the PCR assay, testing 0.5 µg of test article DNA, provided a non-informative result, as the test article spiked with 100 copies of pHH7 failed to produce sufficient amplification (results not presented). The assay was repeated using a reduced amount of 0.1 µg of extracted test article DNA, which provided a valid test with a negative result (presented in Results section below).

RESULTS

Test article DNA (0.1 µg), representing approximately 1.5×10^4 test article cells, was analyzed for the presence of HHV-7 DNA by PCR amplification and high resolution agarose gel electrophoresis in the presence of ethidium bromide. The results are shown in Figure 1. The No DNA control (NO) showed no amplification bands. The Negative Control (NC) showed no bands at 353 bp. The Positive Control (PC) produced a 353 bp band. The test article spiked with 100 copies of pHH7 (TAS) produced a 353 bp band, demonstrating that the test article did not inhibit the PCR reaction. The test article (TA) produced no bands at 353 bp.

These results provide evidence that the test article H1 MCB.1 tested negative for the presence of HHV-7 DNA.

APPROVAL

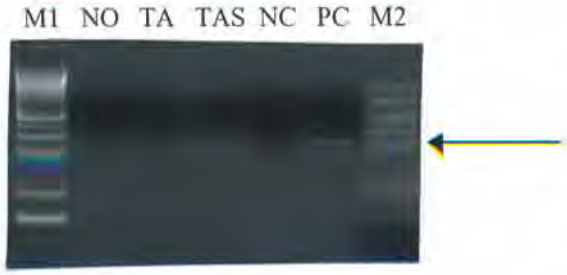
I accept responsibility for the conduct of this study which was performed in compliance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the UK GLP Regulations, the Japanese GLP Standard and the Organization for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice.

14Nov07

Date

Study Director

FIGURE 1



Detection of HHV-7 specific sequences in the test article H1 MCB.1 utilizing agarose gel electrophoresis in the presence of ethidium bromide. Arrow indicates the 353 bp amplification product.

M1: 100 bp DNA ladder.
NO: No DNA control.
TA: Test Article.
TAS: Test article spiked with 100 copies pHH7.
NC: Negative control (MRC5 genomic DNA).
PC: Positive control (negative control DNA spiked with 100 copies pHH7).
M2: Biomarker low DNA size marker.

Quality Assurance Statement

Study Title: POLYMERASE CHAIN REACTION ASSAY FOR THE DETECTION OF HUMAN HERPESVIRUS (HHV-7) IN BIOLOGICAL SAMPLES

Study Number: AC08DP.105029.BSV

Study Director:

Quality Assurance performed the inspections listed below for this study. Verification of the study protocol was also performed and documented by QA. Procedures, documentation, equipment records, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. FDA Good Manufacturing Practices (21 CFR 210 and 211), the UK GLP Regulations, the Japanese GLP Standard, and the OECD Principles of Good Laboratory Practice and to assure that the study is conducted according to the protocol and relevant Standard Operating Procedures.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

**	Inspect On	31-Oct-07 - 05-Nov-07 To Study Dir 05-Nov-07 To Mgmt 09-Nov-07
	Phase	Data Audit
**	Inspect On	14-Nov-07 - 14-Nov-07 To Study Dir 14-Nov-07 To Mgmt 14-Nov-07
	Phase	Final Report and data audit
*	Inspect On	24-Sep-07 - 25-Sep-07 To Study Dir 24-Sep-07 To Mgmt 24-Sep-07
	Phase	Systems Inspection - Administration of Test Substance to Test System
*	Inspect On	24-Sep-07 - 25-Sep-07 To Study Dir 25-Sep-07 To Mgmt 25-Sep-07
	Phase	Systems Inspection - Manipulation of Test System
*	Inspect On	24-Sep-07 - 25-Sep-07 To Study Dir 25-Sep-07 To Mgmt 25-Sep-07
	Phase	Systems Inspection - Observation of Test System/Data Collection and/or Analysis
*	Inspect On	24-Sep-07 - 25-Sep-07 To Study Dir 25-Sep-07 To Mgmt 25-Sep-07
	Phase	Systems Inspection - Test System Preparation

** Inspection specific for this study
* Systems Inspection

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

14/Nov07

DATE

QUALITY ASSURANCE

WiCell Cytogenetics Report: 000167 NSCB9592

Report Date: *3/7/2010*

Case Details:

Cell Line: *WA01-p37 (Male)*

Reference: *Male Promega*

Investigator: *National Stem Cell Bank*

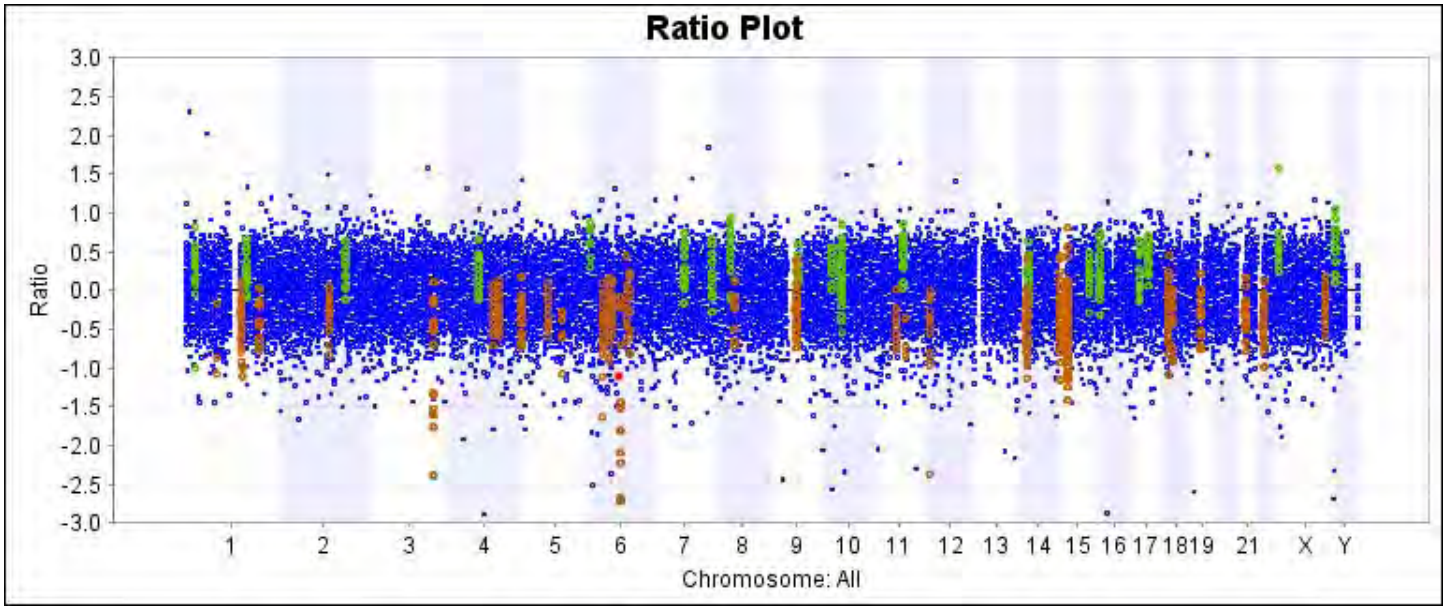
Specimen: *hESC on MEF feeder*

Date of Sample: *9/12/2007*

Reason for Testing: *Confirm normal karyotype, NSCB #9592*

GEO Accession #: *GSM476496*

aCGH Results:
Results are given in the attached Excel spreadsheet labeled "report." There were 53 copy number gains and losses identified by modified circular binary segmentation[1]. The analysis summary is depicted in the ratio plot below with copy number gains shown in green and losses in red. This data was generated using OneClickCGH™ software.



Interpretation: *The data shown in the table below are derived from the attached Excel spreadsheet labeled "select". These copy number changes are measures of sensitivity[2, 3] or may be related to differential gene expression that is monitored in the NSCB characterization protocol and the ISCI study[4]. Changes associated with karyotype abnormalities and/or previously reported publications[2, 5] are also listed. Copy number changes designated by an * in "select" report indicate inconsistency with the reference standard.*

X-chromosome Gains or Losses at Pseudoautosomal Loci[3]	*0 of 2 (none expected)*
Published Copy Number Changes[5,6]	*1 of 8*
Reference DNA Copy Number Changes[2]	*6 of 8*
Select Differentially Expressed Genes	*2 of 88 (MYH6, MYH7)*

These results are consistent with karyotype results [46,XY] as reported in 000217-091207 9592-KAR.

Test sample gain or loss is consistent with same gender reference standard. Additional analysis of this data was performed using different ratio settings and different window averaging.

Results Completed By: *CG(ASCP)CM*
Reviewed and Interpreted By: *, PhD, FACMG*

***aCGH Specifications**:*

- *Platform: NimbleGen 385K array (HG18 CGH 385K WG Tiling v2)*
- *Relative copy number is determined by competitive differential hybridization of labeled genomic DNA to the 385,000 oligonucleotide whole genome tiling array*
- *Probe length = 50-75mers for v1 and 60mers for v2, spanning non-repetitive regions of the human genome*
- *Median probe spacing = 6270bp for v1 and 7073bp for v2*
- *Analysis software: NimbleScan™ , SignalMap™, OneClickCGH (RBS v1.0)™, OneClickFusion (RBS v1.0)™*
- *Array design, genomic position, genes and chromosome banding are based on HG18.*
- *Analysis is based on examination of unaveraged and/or 60Kbp (10X) averaged data tracks as noted. Settings for data analysis in Infoquant include an average log-ratio threshold of 0.2 and no minimum aberration length.*
- *Raw data is deposited in GEO, accession number shown above.*
- *Reported gains and losses are based on test to reference ratios within OneClickCGH™, size of aberration, 8-9 probes per gene, and coverage of at least one reported gene or overlap with the DGV.*

***Limitations:** This assay will detect aneuploidy, deletions, duplications of represented loci, but will not detect balanced alterations (reciprocal translocations, Robertsonian translocations, inversions, and insertions), point mutations, uniparental disomy or imbalances less than 30kb in size. Copy number variants can be attributable to the test or reference samples used. Exact limits of detectable mosaicism have not been determined, but >20% mosaicism is reported to be visualized by aCGH. Actual chromosomal localization of copy number change is not determined by this assay. Other mapping procedures are required for determining chromosomal localization.*

Literature Sources:

1. *Olshen, A., Venkatraman, E., Lucito, R., Wigler, M. (2004). Circular binary segmentation for the analysis of array-based DNA copy number data. Biostatistics, 5, 4, 557-572.*
2. *Internal Data, Unpublished.*
3. *Mumm, S., Molini, B., Terrell, J., Srivastava, A., Schlessinger, D. (1997). Evolutionary Features of the 4-Mb Xq21.3 XY Homology Region Revealed by a Map at 60-kb Resolution. Genome Research, 7, 307-314.*
4. *Adewumi, O., Aflatoonian A., Ahrlund-Richter L., Amit M., Andrews P., Beighton G., et al. (2007). Characterization of human embryonic stem cell lines by the International Stem Cell Initiative. Nature Biotechnology, 25, 803-816.*
5. *Werbowetski-Ogilvie, T., Bosse, M., Stewart, M., Schnerch, A., Ramos-Mejia, V., Rouleau A., et al. (2008). Characterization of human embryonic stem cells with features of neoplastic progression. Nature Biotechnology, 27, 91-97.*
6. *Wu, H., Kim, K., Mehta, K., Paxia, S., Sundstrom, A., Anantharaman, T., et al. (2008). Copy number variant analysis of human embryonic stem cells. Stem Cells, 26, 1484-1489.*

***Recommendations:** For relevant findings, confirmation and localization is recommended. Contact cytogenetics@wicell.org to request further testing.*

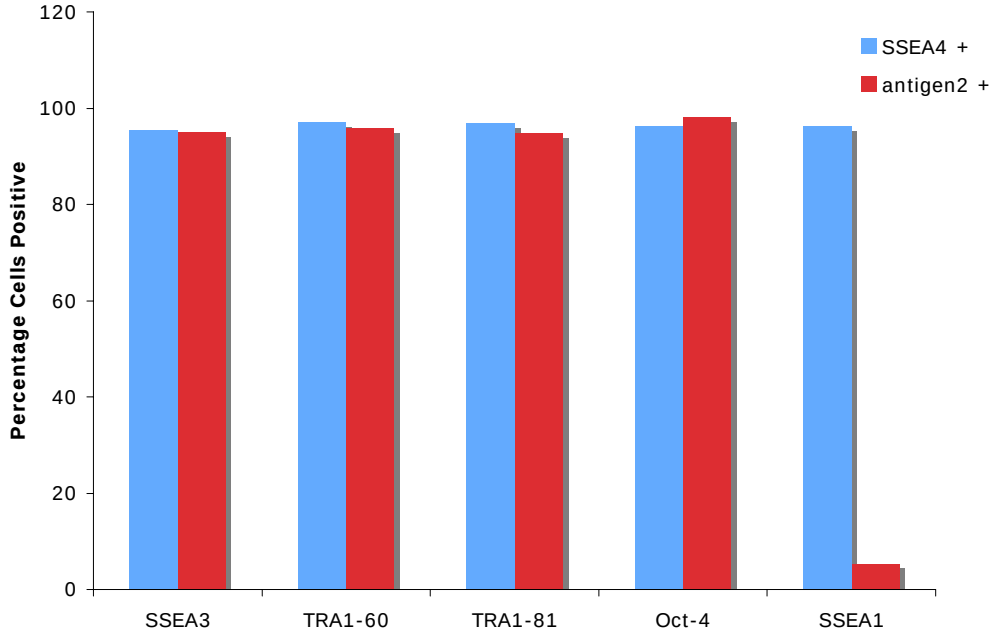
Results Transmitted by Fax / Email / Post **Date:_____**
Sent By:_____ **Sent To:_____**

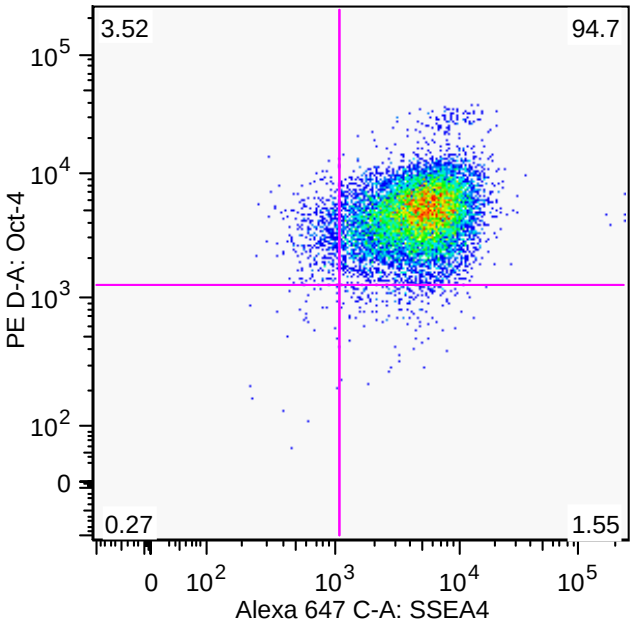
Characterization Report-
Flow Cytometry (FACS)

SOP-CH-101 B
SOP-CH-102 B
SOP-CH-103 B
SOP-CH-105 B

Cell Line: H1	**Date of**: *(mm/dd/yy)*
Passage: p37	Acquision: 9/14/07
Sample ID: 9592-FAC	File Creation: 9/14/07
File created by: EP	File Submission: 9/25/07

antigen2:	SSEA4 - antigen2 +	SSEA4 + antigen2 +	SSEA4 + antigen2 -	SSEA4 - antigen2 -	ALL SSEA4 +	ALL antigen2 +
SSEA3	1.97	93.1	2.57	2.34	95.67	95.07
TRA1-60	1.16	94.8	2.29	1.71	97.09	95.96
TRA1-81	2.03	92.9	4.13	0.93	97.03	94.93
Oct-4	3.52	94.7	1.55	0.27	96.25	98.22
SSEA1	0.25	5.12	91.2	3.47	96.32	5.37





Sample RNA: 9592	Reference DNA:	Date of report: 10-01-2008
Sample Cell Line: WA01	Reference Cell Line: H1	Report prepared by: CY
Passage: p37	Passage:	QA Reviewed: 10/9/08 EM
Lot #: WA01-MCB-1		Date sent to Genomic Center: 081008
Sample ID:		GEO accession #:GSM325739

1. Chip design: 2007-06-15_WiCell_HG18_p14_exp.ndf
2. Sample labeling:
   Cy5: WA01 2ug;
   Cy3: Unsonicated H1 gDNA 4.5ug;

3. QC comments:
   Box plots and distribution graphs are within acceptable range.

4. Expression of ES markers:

Gene Symbol	Accession	Ratio	Expression
Core ES markers			
GABRB3	NM_000814	2.787072243	Y
POU5F1	NM_002701	35.44672897	Y
TDGF1	NM_003212	29.13366337	Y
DNMT3B	NM_006892	29.27272727	Y
GDF3	NM_020634	3.454924875	Y
NANOG	NM_024865	13.01133391	Y
non-core ES markers			
PODXL	NM_001018111	32.21308411	Y
GRB7	NM_001030002	1.347118644	Y
CD9	NM_001769	14.65519253	Y
FGF4	NM_002007	0.25334608	N
SOX2	NM_003106	16.44760479	Y
LEFTY2	NM_003240	7.207194245	Y
UTF1	NM_003577	0.23730872	N
IFITM1	NM_003641	14.19598394	Y
FOXD3	NM_012183	0.368965517	Y
GAL	NM_015973	40.32815534	Y
NODAL	NM_018055	2.840858623	Y
BXDC2	NM_018321	26.56896552	Y

LEFTY1	NM_020997	8.199752628	Y
LIN28	NM_024674	7.882191781	Y
TERT	NM_198254	0.20400859	N

5. Expression of differentiation markers:

Gene Symbol	Accession	Ratio	Expression
COL1A1	NM_000088	0.633814783	Y
IPF1	NM_000209	0.074313409	N
PAX6	NM_000280	0.479360852	Y
TNNI3	NM_000363	2.705882353	Y
CGB	NM_000737	0.07079906	N
AFP	NM_001134	1.087959343	Y
CDX2	NM_001265	0.116242038	N
COL2A1	NM_001844	0.519463087	Y
FLT1	NM_002019	0.285271318	Y
GATA4	NM_002052	0.170716113	N
NEUROD1	NM_002500	0.087415946	N
SYP	NM_003179	0.094231616	N
PDHX	NM_003477	3.564766839	Y
GCM1	NM_003643	0.086124402	N
NKX2-5	NM_004387	0.057471264	N
ACTC	NM_005159	19.79487179	Y
GATA6	NM_005257	0.334590009	Y
EOMES	NM_005442	0.38292011	Y
LAMA1	NM_005559	1.68872549	Y
FOXA2	NM_021784	0.203196347	N
SOX17	NM_022454	0.295302013	Y
FN1	NM_054034	0.165234002	N

Blood Services

American Red Cross

Together, we can save a life

10/25/07

SAMPLES: DNA from Cell Lines: Date received: 09/28/07

NSCB **1590** (TS07-0459) ES03
NSCB **6185** (TS07-0460) H9
NSCB **9592** (TS07-0461) H1
NSCB **5456** (TS07-0462) HSF1

INSTITUTION: WiCell Research Institute

TESTING REQUESTED: Genotype for *ABO* and *RH*

DNA TESTING PERFORMED: ***RH:*** PCR-multiplex analysis for *RHD* exons 4, 7, the inactivating *RHD* pseudogene and C/c genotyping. AS-PCR for *RHD-CE-D* exon 3 (455A>C). PCR-RFLP for E/e. ***ABO:*** Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) testing for nucleotide positions 261 (O^1), 467 (A^2), 703 (B), and 1096 (B and O^2).

DNA MOLECULAR RESULTS:

	Genotype	Predicted Phenotype
ES03-MCB-1 NSCB **1590:**	*ABO*O^{1}O^1; RHD; RHCE*Ce/Ce*	NSCB **1590:** Group O; RhD+, C+, c-, E-, e+
H9-MCB-1 NSCB **6185:**	*ABO*A^{1}O^1; RHD; RHCE*cE/ce*	NSCB **6185:** Group A; RhD+, C-, c+, E+, e+
H1-MCB-1 NSCB **9592:**	*ABO*O^1/O^1; RHD; RHCE*Ce/Ce*	NSCB **9592:** Group O; RhD+, C+, c-, E-, e+
HSF1-MCB-1 NSCB **5456:**	*ABO*O^1/O^1; RHD; RHCE*Ce/ce*	NSCB **5456:** Group O; RhD+, C+, c+, E-, e+

COMMENTS: All samples were negative for the *RHD*-inactivating pseudogene and the *RHD-CE-D* hybrid which cause a D- phenotype and are common in African Black ethnic groups.

Scientific Director Molecular Biologist

THE MOLECULAR TEST METHODS WERE DEVELOPED, AND THEIR PERFORMANCE CHARACTERISTICS DETERMINED BY THE MOLECULAR RED CELL AND PLATELET TESTING LABORATORY AT THE AMERICAN RED CROSS PENN-JERSEY REGION. THE FDA HAS NOT REVIEWED OR APPROVED THE REAGENTS USED. THESE RESULTS ARE NOT INTENDED AS THE SOLE MEANS FOR CLINICAL DIAGNOSIS OR PATIENT MANAGEMENT DECISIONS. LIMITATIONS: The genotype may not always reflect the red cell phenotype. New mutations that inactivate gene expression or rare new variant alleles may not be identified in these assays.

Please Give Blood.