Certificate of Analysis - Amended

Product Description	WA09	
Cell Line Provider	WiCell Research Institute	
MCB Lot Number	WA09-MCB-01	
Date Vialed	16-November-2006	
Passage Number	p17	
Culture Platform	Feeder Dependent	
	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	Consistent with known profile	Pass
HLA profile	UW Molecular Diagnostics Laboratory	AlleleSEQR Kits by Abbott	Consistent with known profile	Pass
Sterility - Direct Transfer Method	WuXi Apptec	30744	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
Mouse 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	Negative	Pass
HTLV 2 PCR HTLV 1&2 by PCR	Covance BioReliance	Not Available 105013	Negative	Pass
HBV by PCR	Covance	Not Available	Negative	Pass
HCV by PCR	Covance	Not Available	Negative	Pass
CMV by PCR	BioReliance	105012	Negative	Pass
EBV by PCR	Covance	Not Available	Negative	Pass
HHV-6 by PCR	BioReliance	105020	Negative	Pass
HHV-7 by PCR	Covance	Not Available	Negative	Pass

Certificate of Analysis - Amended

HHV-8 by PCR	Covance	Not Available	Negative	Pass
HP B19 by PCR	Covance	Not Available	Negative	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	A+

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.

Amendment(s):

Reason for Amendment	Date
CoA updated to include copyright information.	See signature
CoA updated for clarification of test specifications, test description, corrected sterility – direct transfer method test method, corrected test description for Covance HTLV, and removed text regarding technical services and distribution of MCBs	23-September-2010
CoA updated for format changes, clarification of test specifications, test method, addition of test provider, culture platform, and electronic signature, and reference to WiCell instead of the NSCB	06-August-2010
Original CoA	20-November-2007

Date of Lot Release	Quality Assurance Approval
20-November-2007	9/30/2013 X AMC AMC Quality Assurance Signed by: [redacted]

H9-MCB-1 LOA Release

10/17/07 EM

UW Health

University of Wisconsin Hospital and Clinics

Histocompatibility/Molecular Diagnostics Laboratory

Short Tandem Repeat Analysis*

Sample Report: NSCB# 6185 UW HLA#: 56749 Sample Date: 07/26/07 Received Date: 07/27/07

Requestor: WiCell Research Institute

Test Date: 08/02/07 File Name: 070803 Report Date: 08/07/07

Sample Name: (label on tube)
NSCB# 6185 WiCell DNA037

Description: DNA Extracted by WiCell

180 ug/mL; 260/280 = 2.1

Locus	Repeat #	STR Genotype
D16S539	5, 8-15	12,13
D7S820	6-14	9,11
D13S317	7-15	9,9
D5S818	7-15	11,12
CSF1PO	6-15	11,11
TPOX	6-13	10,11
Amelogenin	NA	X,X
TH01	5-11	9.3,9.3
vWA	11, 13-21	17,17

Comments: Based on the NSCB# 1590 DNA submitted by WI Cell dated 07/26/07 and received on 07/27/07, this sample (UW HLA# 56747) matches exactly the STR profile of the human stem cell line H9 comprising 12 allelic polymorphisms across the 8 STR loci analyzed. No STR polymorphisms other than those corresponding to the human H9 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. These results suggest that the DNA sample submitted corresponds to the H9 stem cell line and it was not contaminated with any other human stell 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 both the Cytogenetics Department and of WI Cell Research Institute on Friday, August 10, 2007.

8-12-07
Date
HLA/Molecular Diagnostics Laboratory

08/10/07
Date
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.

File: Final STR Report

UW Health
University of Wisconsin Hospital and Clinics

Histocompatibility/Molecular Diagnostics Laboratory

Date: 08/16/2007 10:19:28

To: 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# 6185-HLA	**DQB SSP**		0201/24/26/34/90	3503/13	0401/09N	1501				
56749 /	**A,B,C Seq**	08/01/2007	0301/7/8/9/17	4427/12	0704/11	1601				
08/01/2007	**DRB Seq**	08/01/2007								

Manager
HLA/Molecular Diagnostics Laboratory

Date

PhD, Director
HLA/Molecular Diagnostics Laboratory

Date

WiCell Research Institute

April 24, 2007
P.O. #:

STERILITY TEST REPORT

Sample Information: Cryopreserved Human embryonic stem cell 3: line H9, H9-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: _____ **Reviewed:** _____

Testing conducted in accordance with current Good Manufacturing Practices.

Test Facility:

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: — 04-24-07 **Reviewed:** 04-24-07

Testing conducted in accordance with current Good Manufacturing Practices.

Test Facility:

AppTec

Report Number
744090
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 H9, H9-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: 04-20-07 **Reviewed:** 04-19-07

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:	H9-MCB.1
SPONSOR:	WiCell Research Institute 0
PERFORMING LABORATORY:	AppTec Laboratory Services
STUDY NUMBER:	57450
RESULT SUMMARY:	Considered **negative** for mycoplasma contamination and **non-inhibitory** for the detection of mycoplasma

Reference PO #

WCR01

57450

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
Reading	05/15/07	05/15/07	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

Madison, [illegible]

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; H9-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:	H9-MCB.1
Stability (Expiration):	Not Given
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

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

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

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: H9-MCB.1	*M. orale* spike	**39.7**	**1.1**	Non-Inhibitory
	M. pneumoniae spike	**43.7**	**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: H9-MCB.1	Negative	Negative	Negative	Negative
H9-MCB.1 Spiked with *M. orale*	Non-inhibitory Positive	Non-inhibitory Positive	Non-inhibitory Positive	Non-inhibitory Positive
H9-MCB.1 Spiked with *M. hyorhinis*	Non-inhibitory Positive			Non-inhibitory Positive
H9-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, H9-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 #6185

Report Date: July 26, 2007

Case Details:

Cell Line: *H9 (O)*

Passage #: *24*

Date Completed: *7/26/2007*

Cell Line Gender: *female*

Investigator: *National Stem Cell Bank*

Specimen: *hESC on MEF feeder*

Date of Sample: *7/20/2007*

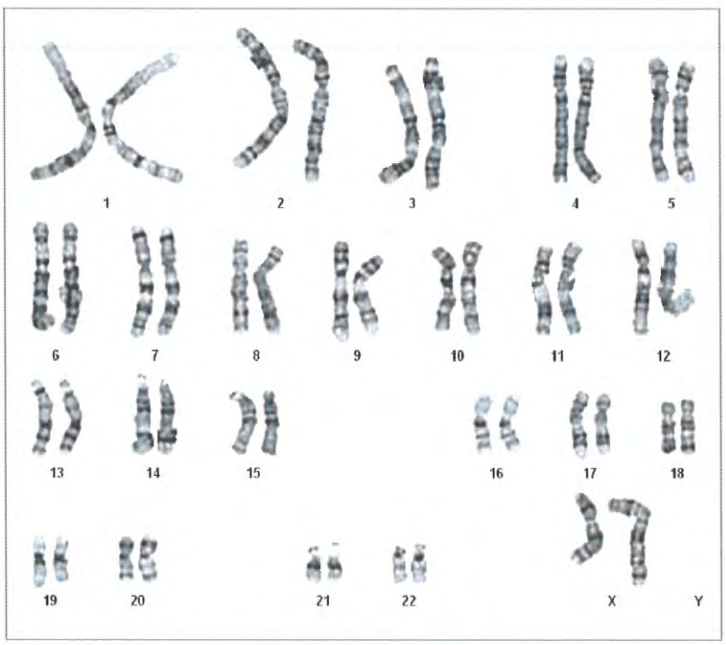
Tests,Reason for: *G-Band analysis, MCB release testing, NSCB #6185*

Results: *46,XX*

Completed by , CLSp(CG), on 7/25/2007

Reviewed and interpreted by PhD, FACMG, on 7/26/2007

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



Cell: *S01-04*

Slide: *B*

Slide Type: *Karyotyping*

Cell Results: *Karyotype: 46,XX*

# of Cells Counted: *40*

# of Cells Karyotyped: *4*

# of Cells Analyzed: *8*

Band Level: *450-550*

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: AC01UH.032901.BSV

Test Article ID: H9-MCB.1

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

Bovine viruses were not detected when the test article, H9-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: H9-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, H9-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.

Study Director

18 May 07
Date

TABLE 1

Observations for Cytopathic Effects in Cultures of BT and Vero Cells Inoculated with H9-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 H9-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 H9-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 H9-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

- Immunofluorescence not observed

+ Immunofluorescence observed

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

NA = Not Applicable

Quality Assurance Statement

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

Study Number: AC01UH.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: AC01UH.033901.BSV

Test Article ID: H9-MCB.1

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

Porcine viruses, BVDV, reovirus and Rabies were not detected when the test article H9-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: H9-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 H9-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 H9-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 H9-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 H9-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: AC01UH.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.

18May07
DATE

QUALITY ASSURANCE

Final Report

MOUSE ANTIBODY PRODUCTION (MAP) TEST

Study Number: AC01UH.004000.BSV

Test Article ID: H9-MCB.1

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

Based on the data obtained in the assays performed, the test article, H9-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: H9-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: 04/18/2007

Lab Completion: 05/22/2007

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

Study Director:

Technical Support Staff:

ry 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

[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 (≥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.

Study Director

8 Jun 07
Date

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 H9-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	I[4]	0	.01	0	0	.04	I[4]	.03	I[4]	.02	.01	0	.02	-	-	-
	.13	0	.03	0	0	.02	I[4]	.02	.14	.01	.01	0	.07	-	-	-
	.11	0	.03	0	0	0	I[4]	0	.14	.02	.03	0	.03	-	-	-
	I[4]	0	.02	.02	.01	0	I[4]	.09	I[4]	.03	.02	0	.03	-	-	-
Negative Control	.01	0	.05	.02	0	.16	.02	0	.01	0	.03	0	.01	-	-	-
	0	0	.01	0	.02	0	0	0	.01	.01	.03	0	.09	-	-	-
Serology Positive Control	1.10	1.05	1.12	1.10	1.15	1.00	1.09	1.14	1.12	1.04	1.11	.83	1.07	+	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 H9-MCB.1

Plasma from Animals Injected with	LDH Titer[a]
Test Article (1:10) (Group II)	142 200 272
Negative Control (Group III)	226 369
LDV Control	1050 1196

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

Quality Assurance Statement

Study Title: MOUSE ANTIBODY PRODUCTION (MAP) TEST

Study Number: AC01UH.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 Phase	07-Jun-07 - 07-Jun-07 To Study Dir 07-Jun-07 To Mgmt 08-Jun-07 Final Report and data audit
* Inspect On Phase	29-Mar-07 - 29-Mar-07 To Study Dir 29-Mar-07 To Mgmt 29-Mar-07 Systems Inspection - Administration of Test Substance to Test System
* Inspect On Phase	01-May-07 - 01-May-07 To Study Dir 01-May-07 To Mgmt 01-May-07 Systems Inspection - Observation of Test System/Data Collection and/or Analysis
* Inspect On Phase	22-May-07 - 22-May-07 To Study Dir 22-May-07 To Mgmt 22-May-07 Systems Inspection - Manipulation of Test System
* Inspect On Phase	06-Jun-07 - 06-Jun-07 To Study Dir 06-Jun-07 To Mgmt 06-Jun-07 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
QUALITY ASSURANCE DATE

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	Waisman Clinical BioManufacturing Facility
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

____ 29/May/07
Date

.....(Print Name)

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-MC6.1

Experimental Phase

Start Date: 6th June 2007

End Date: 10th August 2007

6/June/07.
Date

.....(Print Name)

Study Director

6th June 2007
Date

.....(Print Name)

Covance Biotechnology Management

SPONSOR ACCEPTANCE SHEET

Sponsor Name _____

Title Technical Director

Sponsors Company Waisman Clinical BioManufacturing Facility

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.

TEST FOR THE PRESENCE OF INAPPARENT VIRUSES

Study No.: AC01UH.005002.BSV

Test Article: H9-MCB.1

Final Report
For

WiCell Research Institute

By

BioReliance®

TABLE OF CONTENTS

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, H9-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:	AC01UH.005002.BSV
Test Article:	H9-MCB.1 was received by BioReliance on 04/05/2007 and 05/09/2007. 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: Sunrise Farms (BE Eggs) York Springs, Pennsylvania
Original assay	Embryonated Hens' Eggs (yolk sac route): forty, seven days old Source: Hy-Vac (BE Eggs) York Springs, Pennsylvania
Repeat Assay	Embryonated Hens' Eggs (yolk sac route): forty, seven days old Source: Hy-Vac (BE Eggs) York Springs, Pennsylvania

AC01UH.005002.BSV

Sponsor:	
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:	05/19/2007
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

The entire yolk sac portion of the assay was repeated utilizing the pertinent procedures and a back-up sample of the test article.

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°C ± 2°C and 25°C ± 2°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.

In the original yolk sac assay, 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 and 48 hours post injection. At examination on day 10 post injection, six of the test article injected eggs and all of the negative control article injected eggs contained viable embryos. Four of the test article injected eggs contained non-viable embryos. An investigation was performed. No growth was observed on blood agar plates streaked with fluid from the non-viable eggs. The cause of death of one of the non-viable embryos most likely resulted from injection related trauma as the egg appeared

non-viable at 24 and 48 hours post injection. The cause of death of the other three non-viable test article injected eggs could not be determined.

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.

Due to survival of the less than 80% of the test article injected eggs in the primary injection, the entire yolk sac portion of the assay was repeated utilizing the pertinent procedures and a back-up sample of the test article.

In repeat yolk sac assay, 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 and 48 hour post injection. At examination on day 10 post injection, eight of the ten test article injected eggs and nine of the ten negative control article injected eggs contained viable embryos. Two of the test article injected eggs and one of the negative control 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 one of the test article injected eggs most likely resulted from injection related trauma as the egg appeared non-viable at 24 and 48 hour post injection. The cause of death of the second non-viable test article injected egg and the non-viable negative control article injected egg could not be determined. (See Evaluation of Test Results/Criteria for a Valid Test.)

In the blind passage of the repeat yolk sac assay, 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 Criteria for a Valid Test.) See Table 4 for a summary of the data.

CONCLUSION

No evidence of viral contamination was observed due to the test article, H9-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.

_____ 8 Jan 07

Study Director Date

TABLE 1

Summary of Experimental Procedures for H9-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 H9-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 H9-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 H9-MCB.1

Guinea Pigs

Test Material	Animal Number	Clinical Signs	Day of Onset (Post-Injection)	Day of Death/Sacrifice (Post-Injection)
Test Article	7661	Normal		
	7662	Normal		
	7663	Normal		
	7664	Normal		
	7665	Normal		
	7666	Normal		
Negative Control	7657	Normal		
	7658	Normal		
	7659	Normal		
	7660	Normal		

TABLE 3 (Continued)

Summary of Daily Observations for H9-MCB.1

Adult Mice

Test Material	Animal Number	Clinical Signs	Day of Onset (Post-Injection)	Day of Death/Sacrifice (Post-Injection)
Test Article	7621	Normal		
	7622	Normal		
	7623	Normal		
	7624	Normal		
	7625	Normal		
	7626	Normal		
	7627	Normal		
	7628	Normal		
	7629	Normal		
	7630	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 H9-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 H9-MCB.1

Embryonated Hens' Eggs

	Allantoic Route		Yolk Sac Route			
			Original Assay		Repeat Assay	
	Primary Injection	Blind Passage	Primary Injection	Blind Passage	Primary Injection	Blind Passage
Test Article	10/10[a]	10/10	6/10	10/10	8/10	10/10
Negative Control	10/10	10/10	10/10	9/10	9/10	9/10

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

TABLE 5

Hemagglutination Observed by the Allantoic Route of Injection-Day 3 for H9-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 Phase	04-Jun-07 - 05-Jun-07 To Study Dir 05-Jun-07 To Mgmt 08-Jun-07 Final Report and data audit
* Inspect On Phase	29-Mar-07 - 29-Mar-07 To Study Dir 29-Mar-07 To Mgmt 29-Mar-07 Systems Inspection - Administration of Test Substance to Test System
* Inspect On Phase	01-May-07 - 01-May-07 To Study Dir 01-May-07 To Mgmt 01-May-07 Systems Inspection - Observation of Test System/Data Collection and/or Analysis
* Inspect On Phase	22-May-07 - 22-May-07 To Study Dir 22-May-07 To Mgmt 22-May-07 Systems Inspection - Manipulation of Test System
* Inspect On Phase	06-Jun-07 - 06-Jun-07 To Study Dir 06-Jun-07 To Mgmt 06-Jun-07 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-1-07-001914.30610

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
H9 MCB.1 1 passage in TeSR1	07-001914
H9 MCB.1 2 passages in TeSR1	07-001915
H9 MCB.1 3 passages in TeSR1	07-001916

SPONSOR:

PERFORMING LABORATORY: AppTec, Inc.

APPTEC ACCESSION NUMBER	RESULTS
07-001914	Transmission electron microscopic examination revealed no identifiable virus.
07-001915	Transmission electron microscopic examination revealed no identifiable virus.
07-001916	Transmission electron microscopic examination revealed no identifiable virus.

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 07-001914	September 20, 2007	November 14, 2007	November 14, 2007
Test Article 07-001915	September 19 and 20, 2007	November 14, 2007	November 14, 2007
Test Article 07-001916	September 19 and 20, 2007	November 14, 2007	November 14, 2007

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

Quality Assurance — 28Nov07 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 — 28NOV07 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: September 11, 2007
STUDY INITIATION DATE: September 12, 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:

APPTEC ACCESSION NUMBER	TEST ARTICLE IDENTIFICATION
07-001914	H9 MCB.1 1 passage in TeSR1
07-001915	H9 MCB.1 2 passages in TeSR1
07-001916	H9 MCB.1 3 passages 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 September 11, 2007 AppTec, Inc. received 3 vials each 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 articles were stored at 2-8°C until shipment to the subcontractor.

On September 12, 2007, 3 vials each 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.

Test Article 07-001914

13.1 Cellular Ultrastructure

Cells in the section were of moderate size and polygonal to irregular in shape (L22768). Cells had microvilli (MV: L22768)[1] unevenly distributed on the surface. Nuclei (N: L22768) tended to be polygonal to irregular, with chromatin relatively evenly dispersed or clumped along the periphery. Nuclei often had one or more nucleoli (NS: L22768) that were variably located, and nuclear pores (NP: L22761) were seen.

The cytoplasm of most cells contained varying numbers of mitochondria (MI: L22765). Profiles of rough endoplasmic reticulum (RER: L22762) were seen among the mitochondria. Ribosomes (RB: L22764) were abundant in the cytoplasm of most cells. Cells were observed to contain centrioles (CN: L22769), myelin figures (MF: L22763), glycogen (G: L22764, L22766) and autophagic vacuoles (AV L22767).

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: 07-001914
PAI EM Number: 07.469-1

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%

Twenty-nine percent of the cells were necrotic.

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

Test Article 07-001915

13.4 Cellular Ultrastructure

Cells in the section were of moderate size and polygonal to irregular in shape (L22775). Cells had microvilli (MV: L22778) unevenly distributed on the surface. Nuclei (N: L22775) tended to be polygonal to irregular, with chromatin relatively evenly dispersed or clumped along the periphery. Nuclei often had one or more nucleoli (NS: L22775) that were variably located. Some cells were observed to be under mitosis with chromosomes (CH: L22778) visible.

The cytoplasm of most cells contained varying numbers of mitochondria (MI: L22777). Profiles of rough endoplasmic reticulum (RER: L22777) were seen among the mitochondria. Ribosomes (RB: L22777) were abundant in the cytoplasm of most cells. Cells were observed to contain centrioles (CN: L22776), myelin figures (MF: L22774), glycogen (G: L22771). Golgi complexes (GO: L22770), lipid droplets (L: L22772) and autophagic vacuoles (AV: L22773) were also seen.

13.5 General Viral Particle Evaluation

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

13.6 Retrovirus-like Particle Evaluation and Tabulation

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

Test Article: 07-001915
PAI EM Number: 07.470-1

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%

Thirty-seven percent of the cells were necrotic.

Test Article 07-001916

13.7 Cellular Ultrastructure

Cells in the section were of moderate size and polygonal to irregular in shape (L22804). Cells had microvilli (MV: L22804) unevenly distributed on the surface. Nuclei (N: L22804) tended to be polygonal to irregular, with chromatin relatively evenly dispersed or clumped along the periphery. Nuclei often had one or more nucleoli (NS: L22804) that were variably located.

The cytoplasm of most cells contained varying numbers of dense mitochondria (MI: L22801). Profiles of rough endoplasmic reticulum (RER: L22799) were seen among the mitochondria. Ribosomes (RB: L22802) were abundant in the cytoplasm of most cells. Cells were observed to contain myelin figures (MF: L22798), glycogen (G: L22803), lipid droplets (L: L22802) and autophagic vacuoles (AV: L22800).

13.8 General Viral Particle Evaluation

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

13.9 Retrovirus-like Particle Evaluation and Tabulation

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

Test Article: 07-001916
PAI EM Number: 07.471-1

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%

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

14.0 CONCLUSION

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

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.

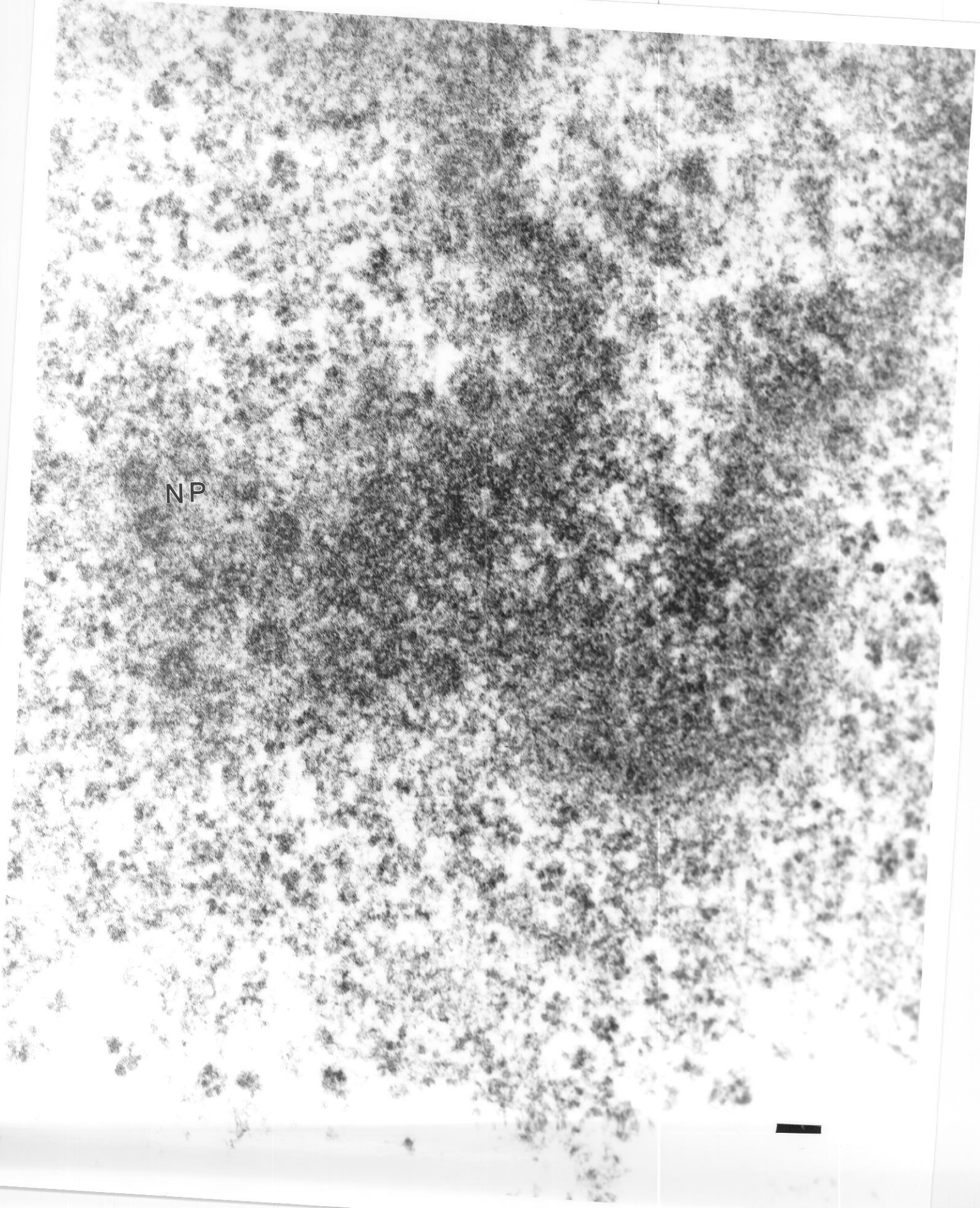
18.0 REFERENCES

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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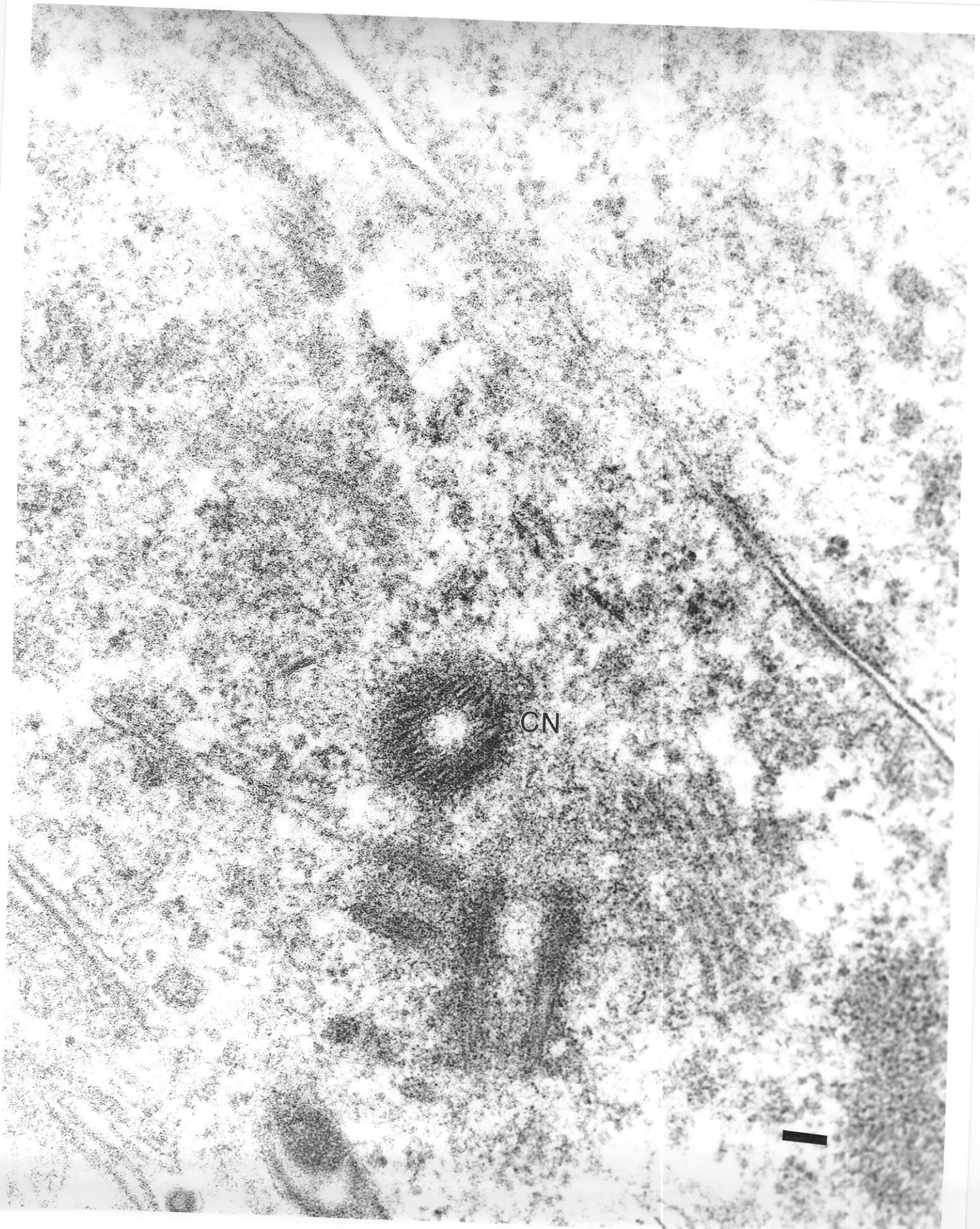
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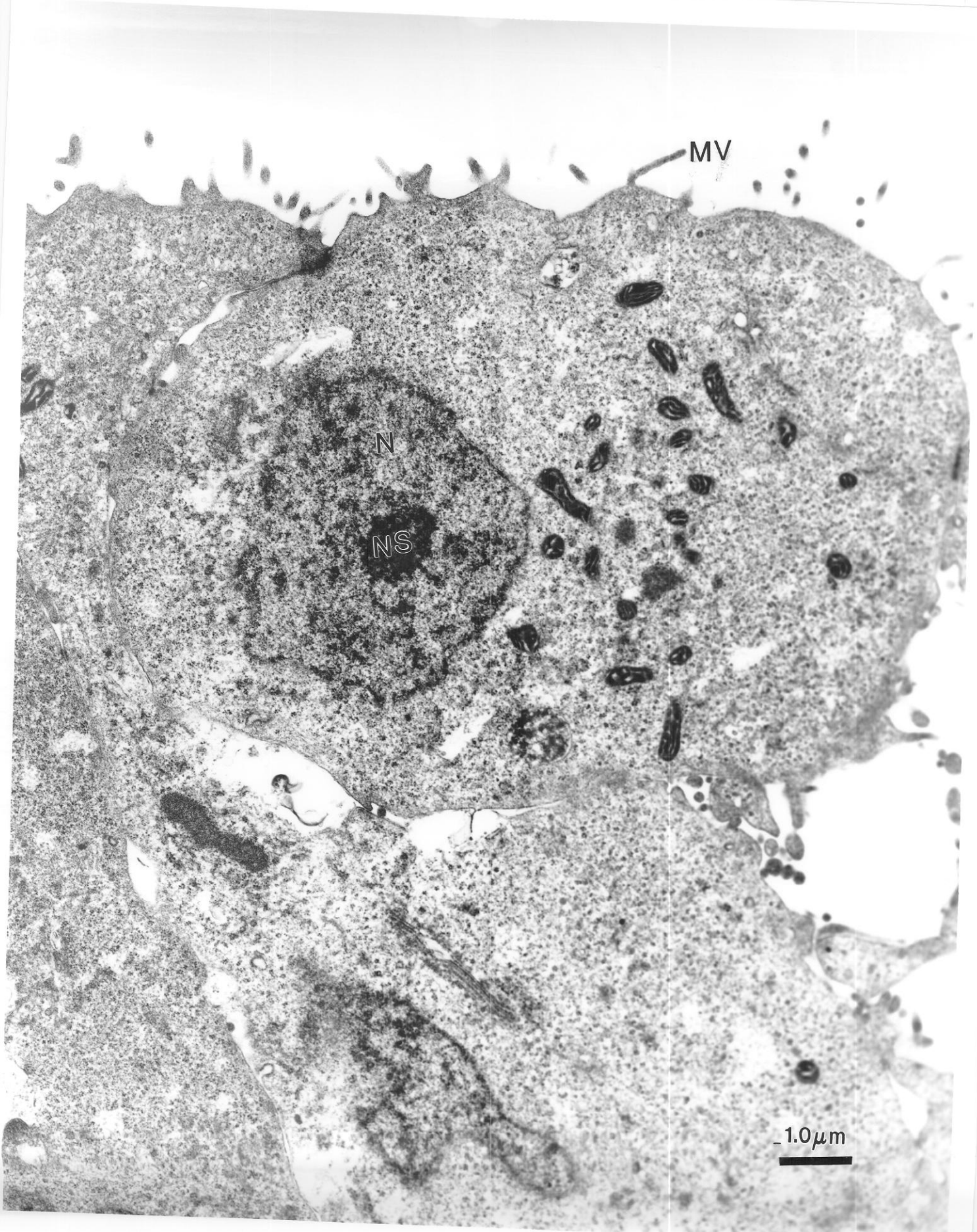
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N
NS
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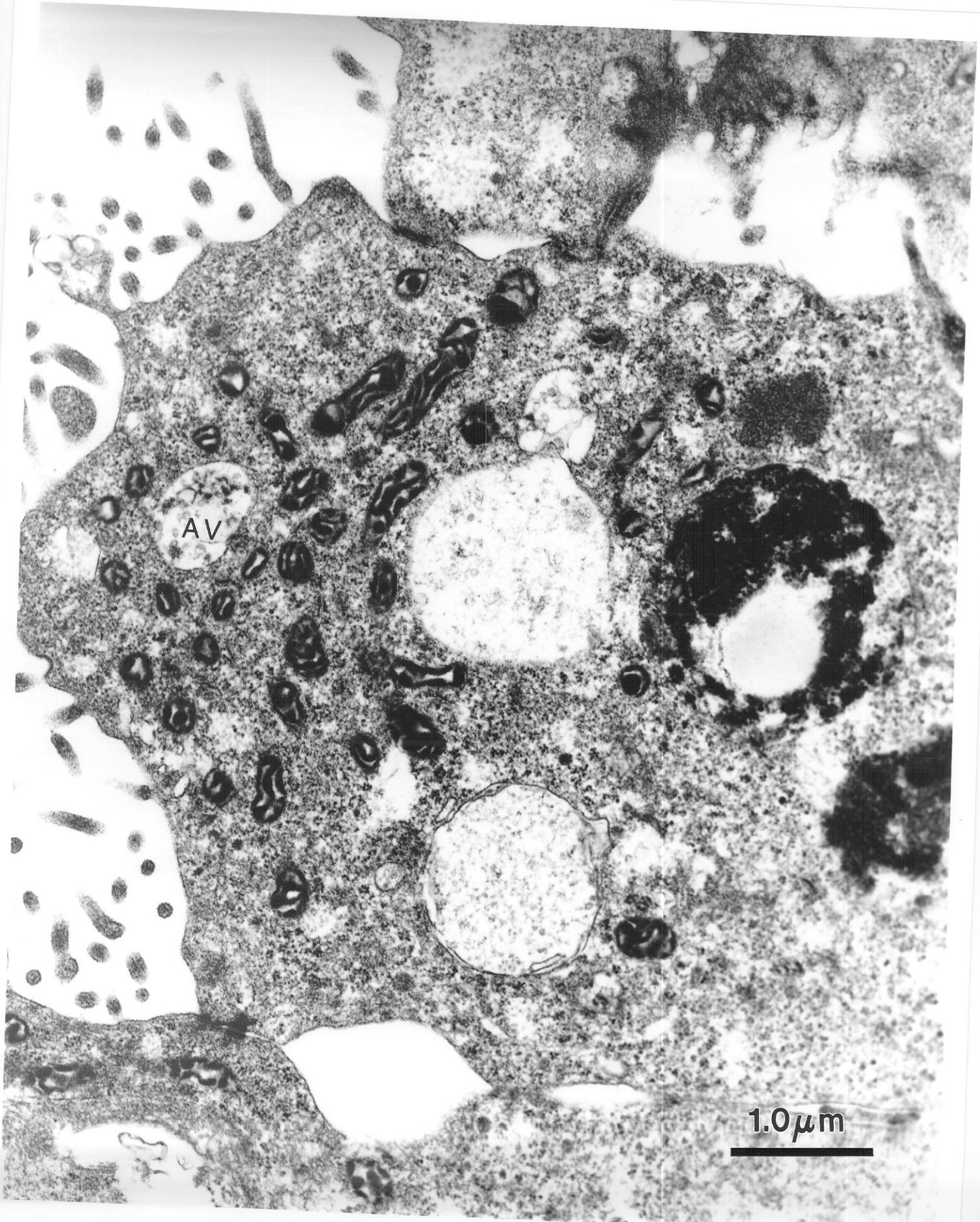
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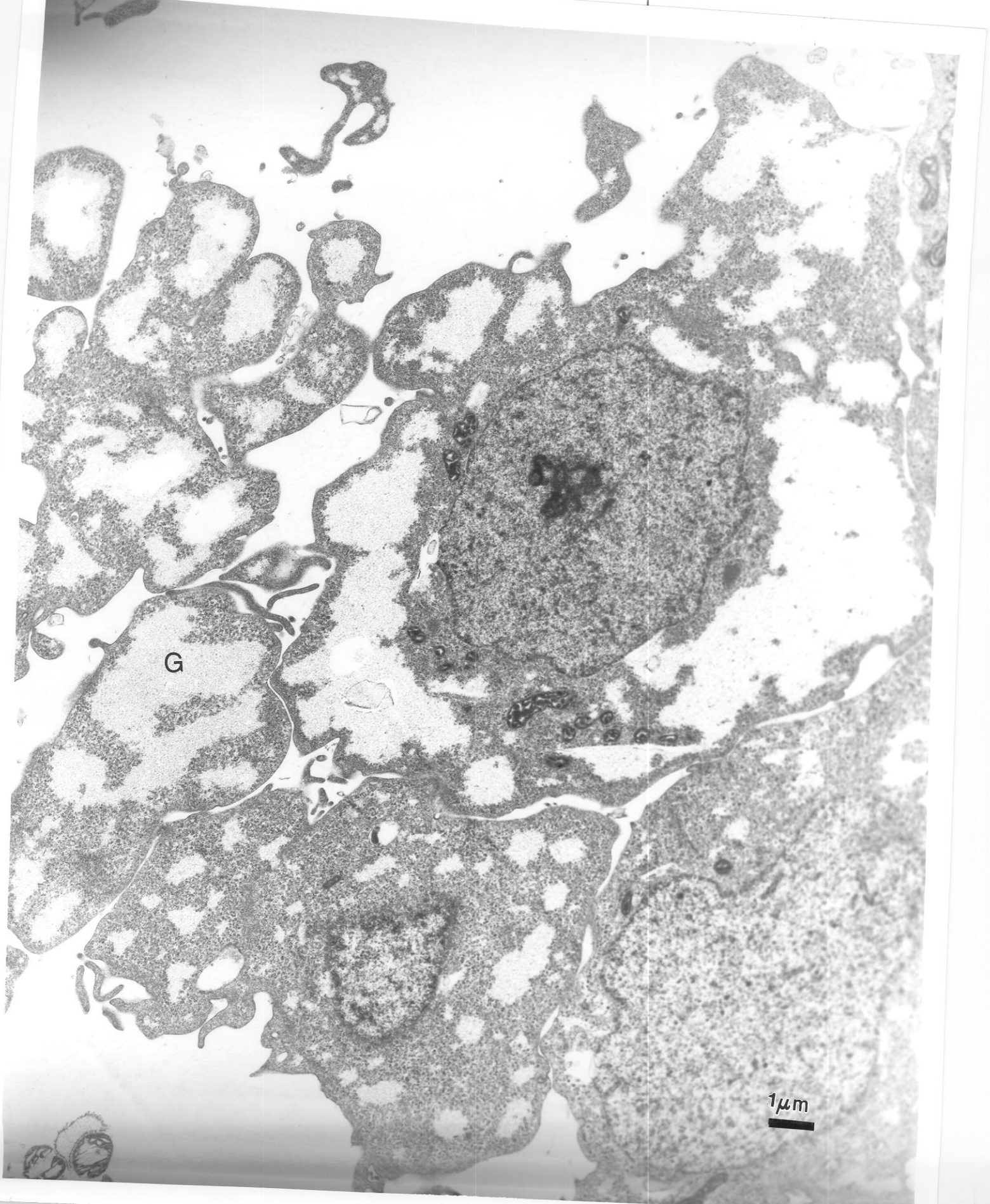
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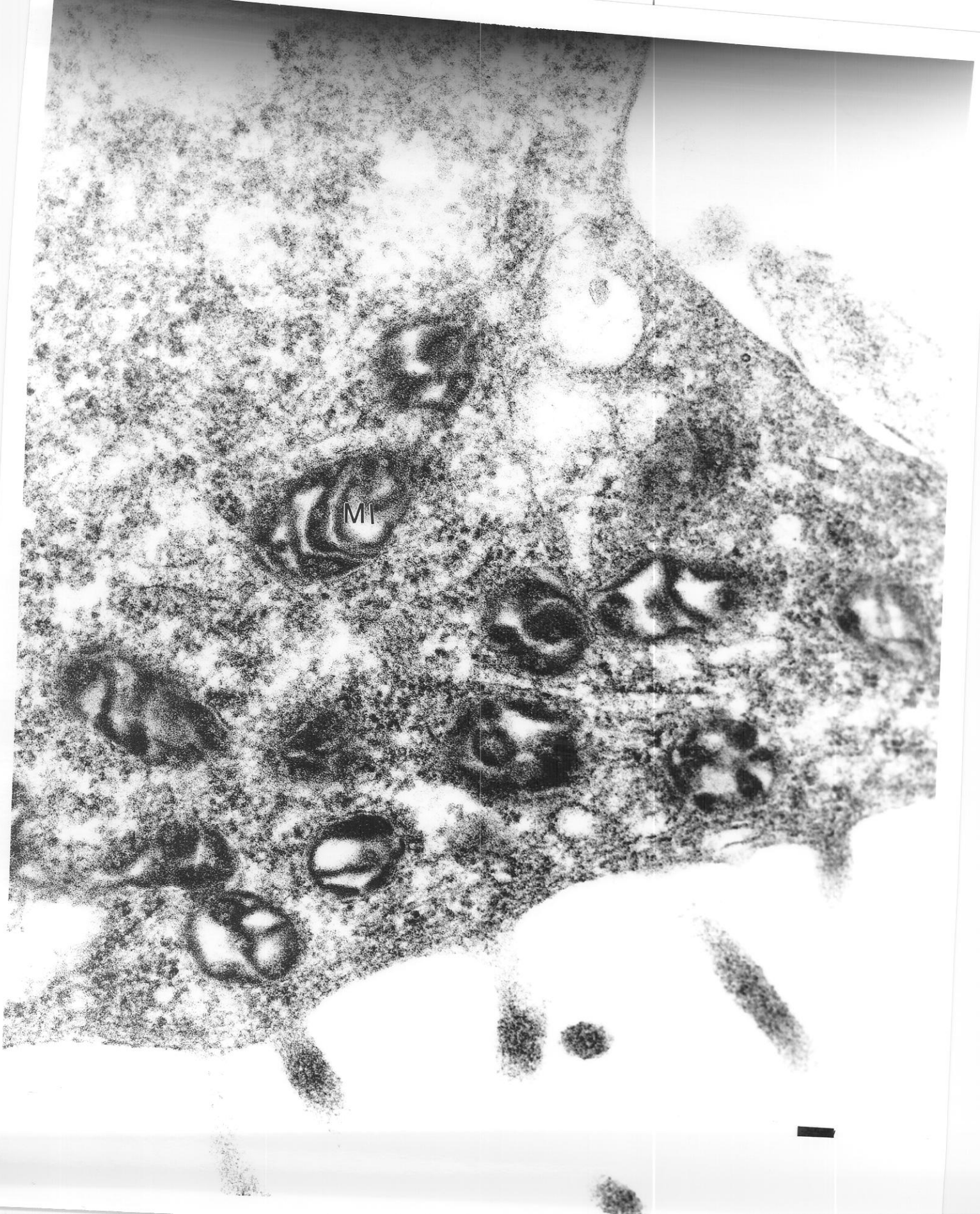
1μm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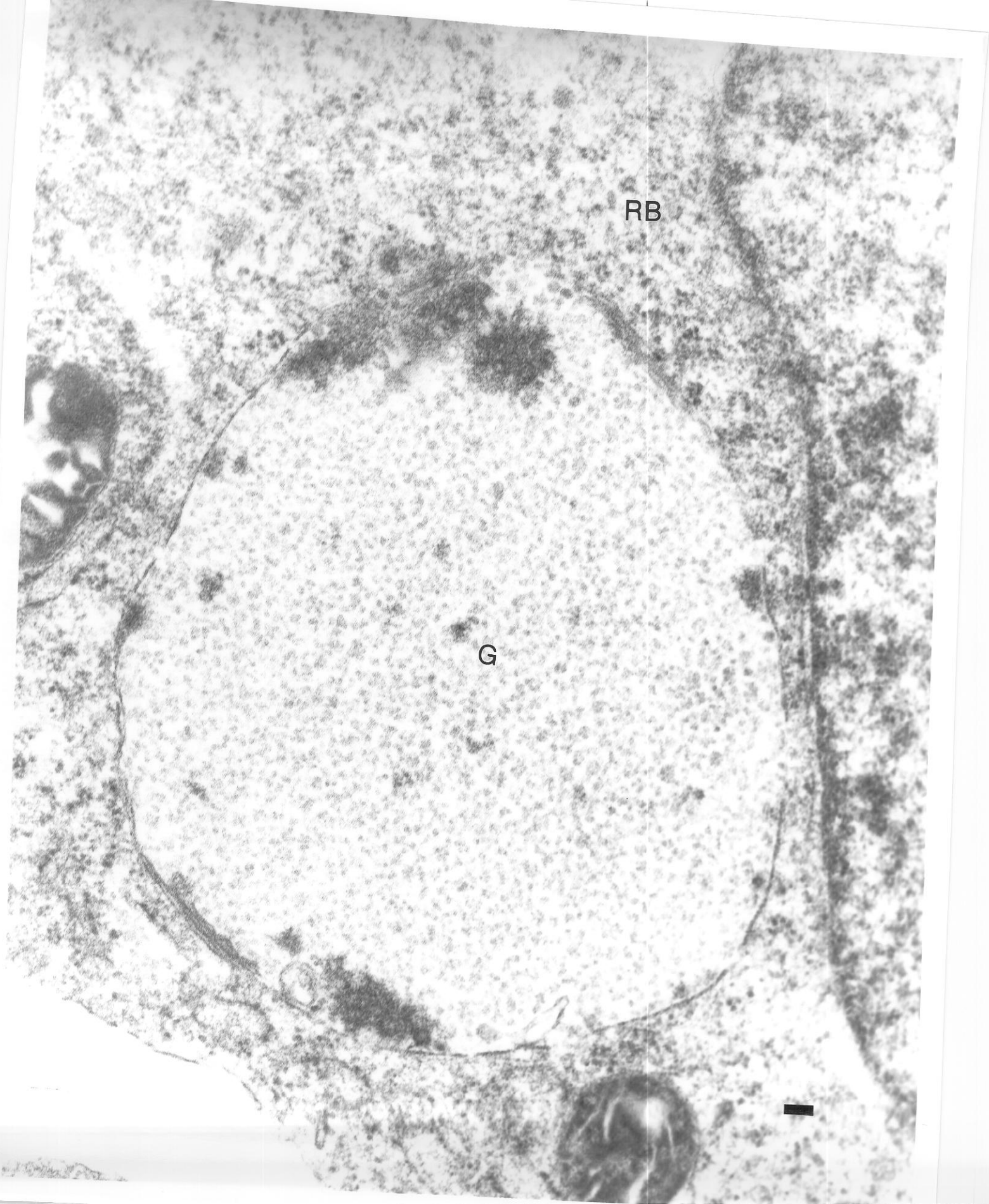
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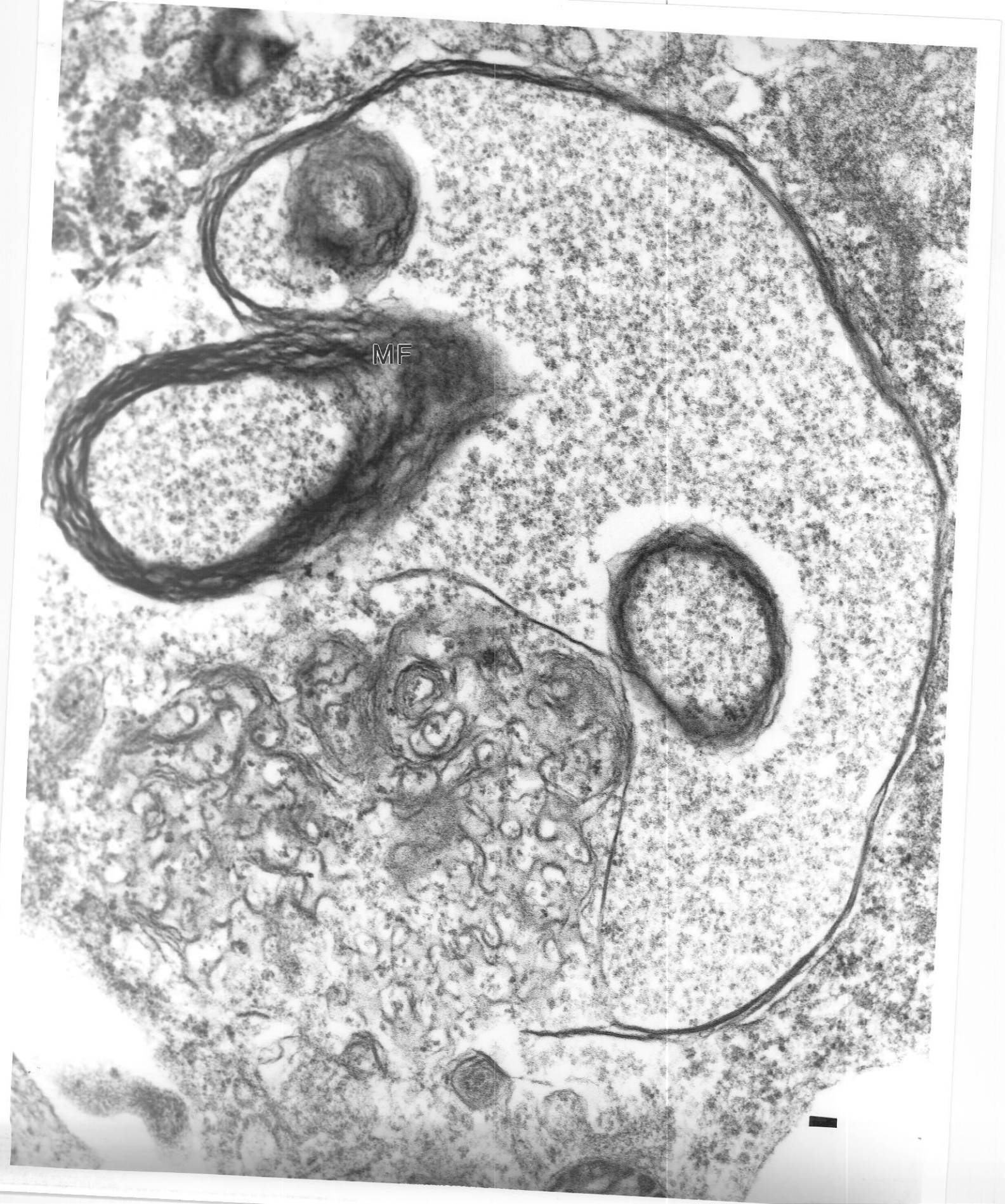
G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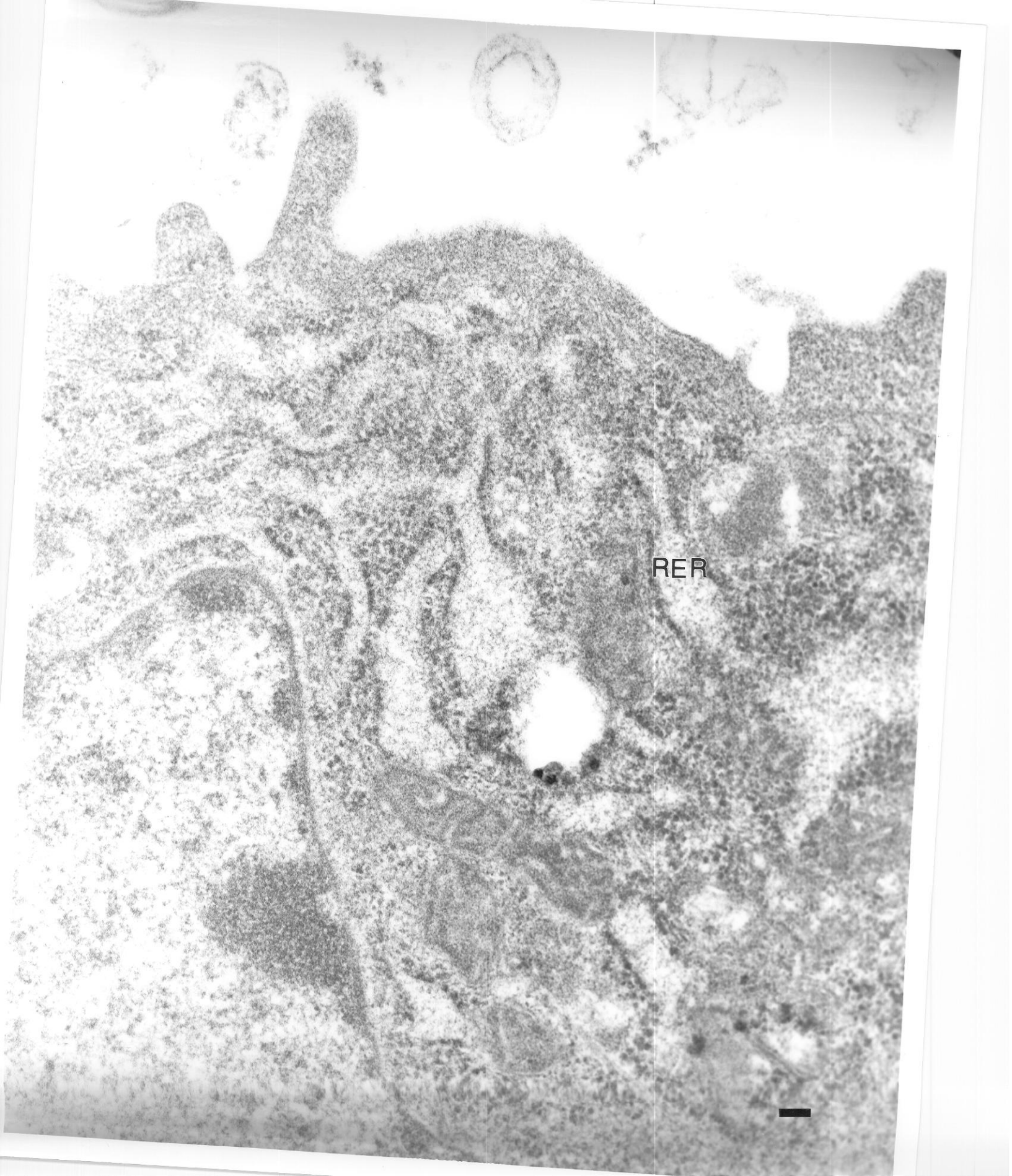
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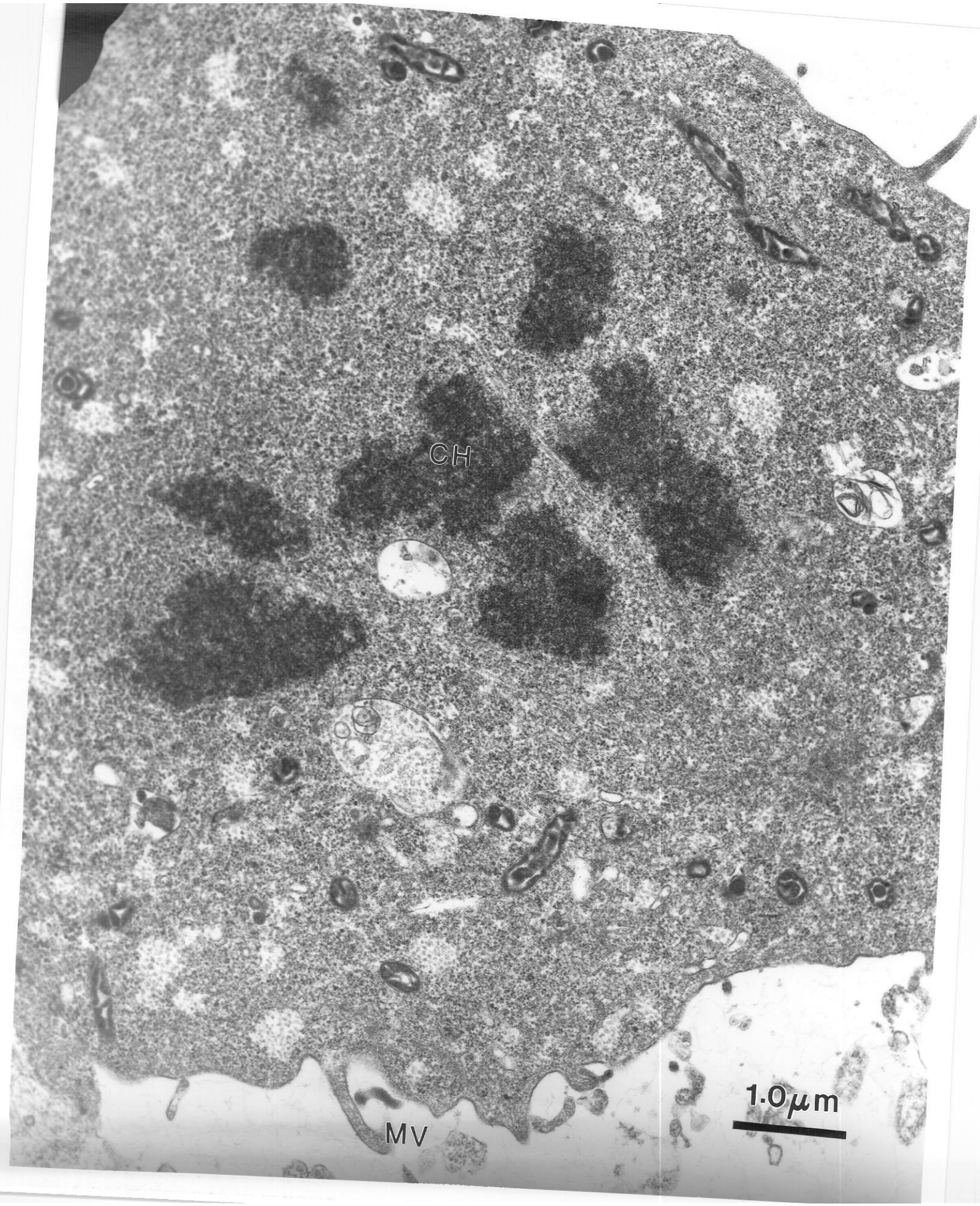
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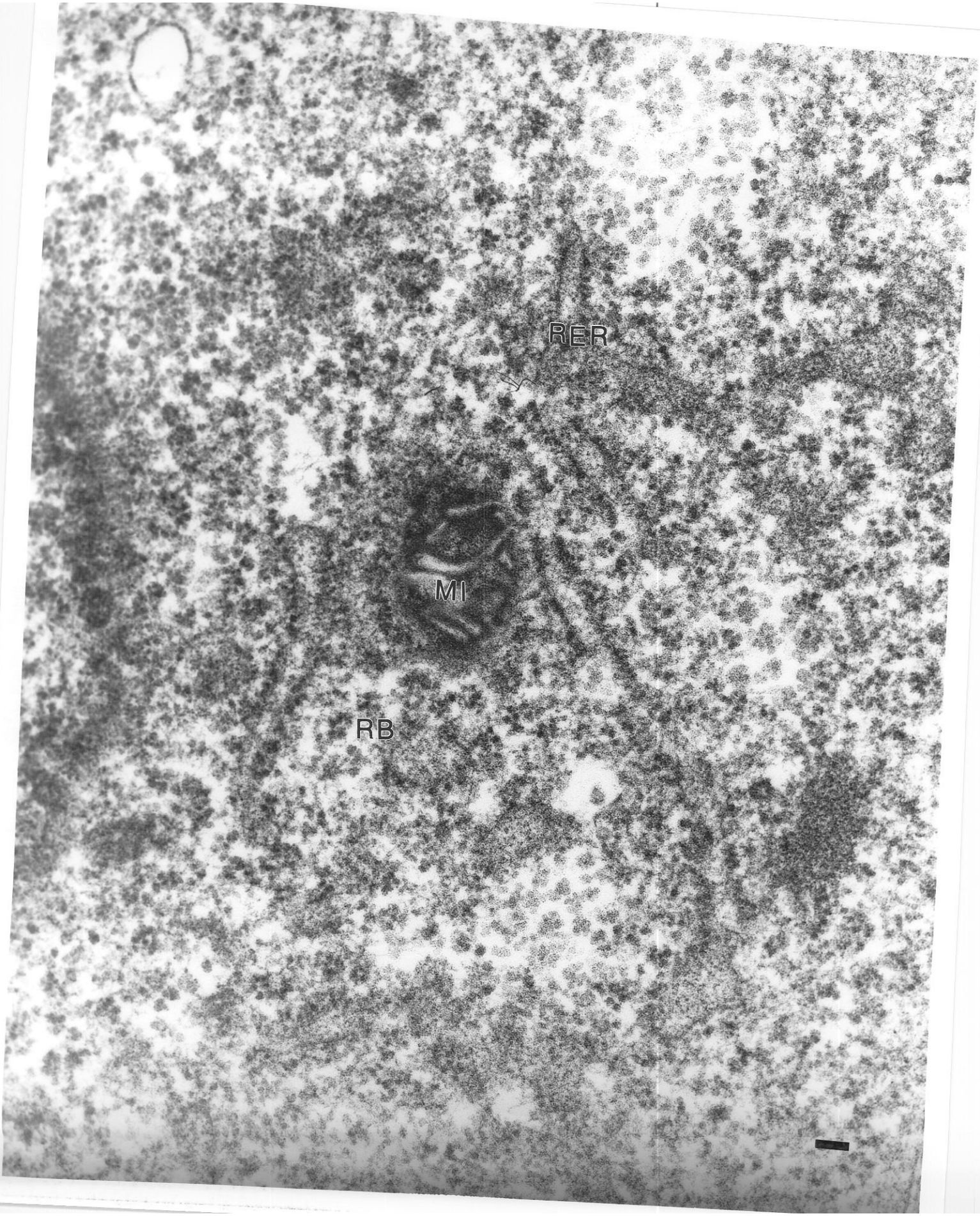
MV
1.0μm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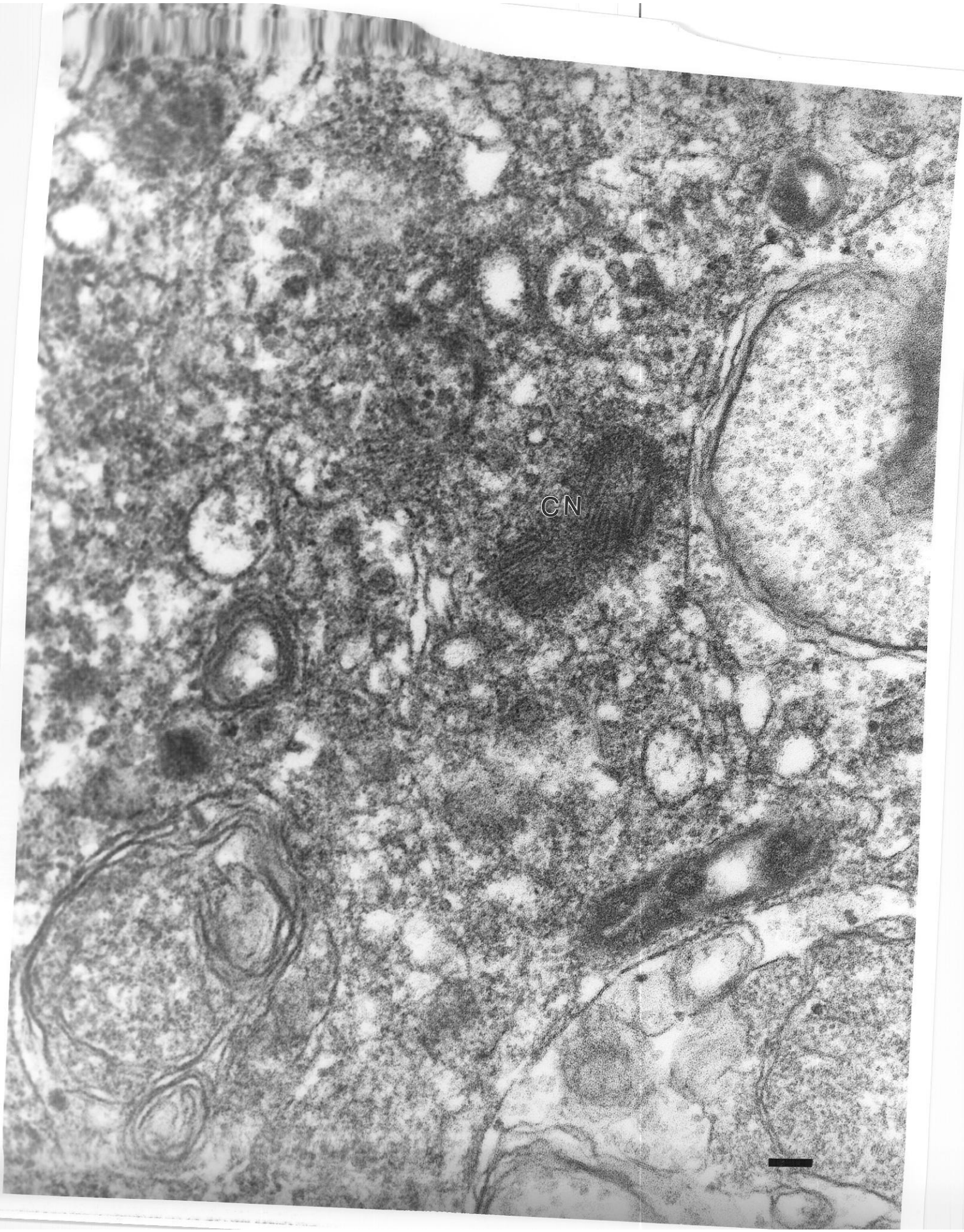
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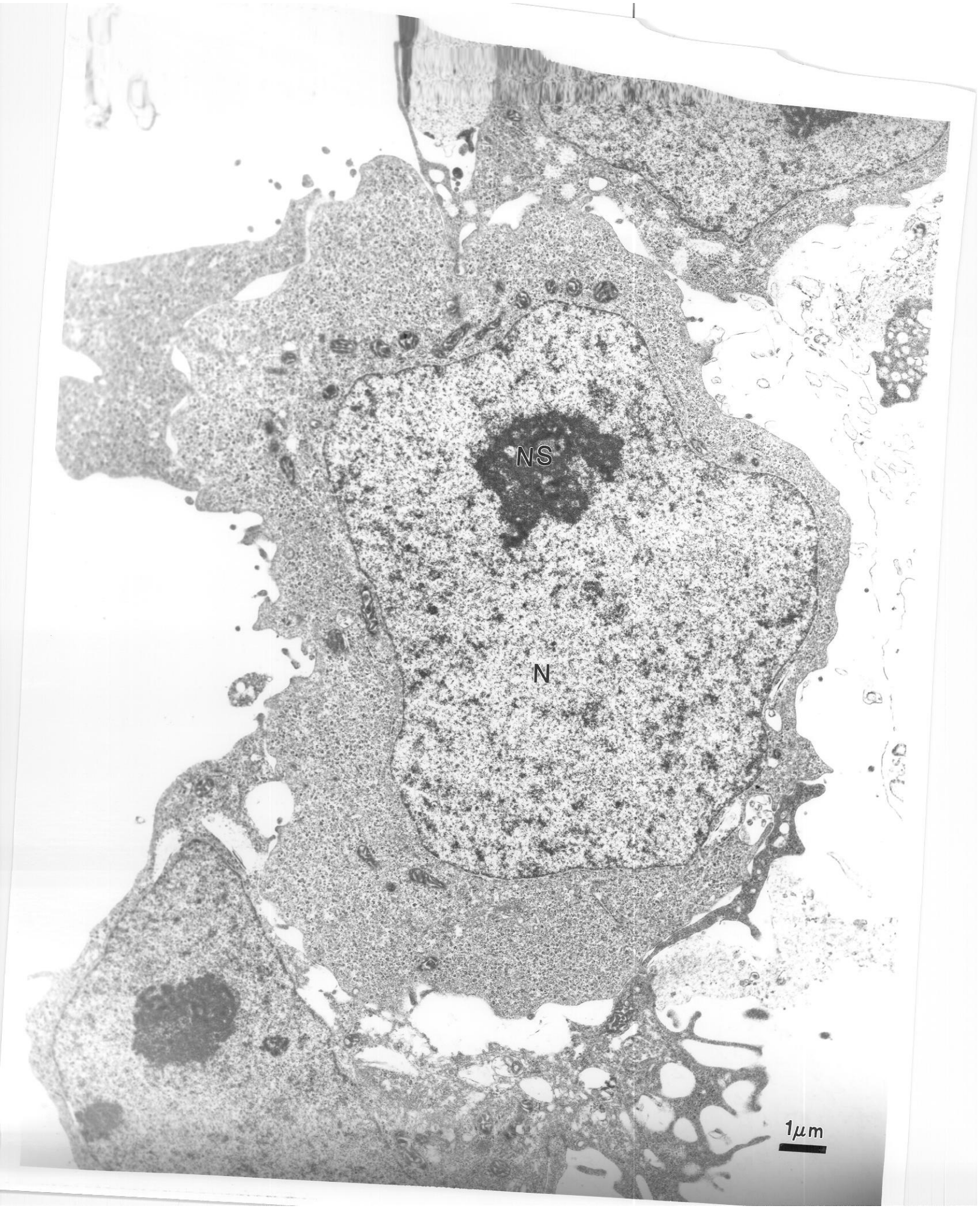
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N
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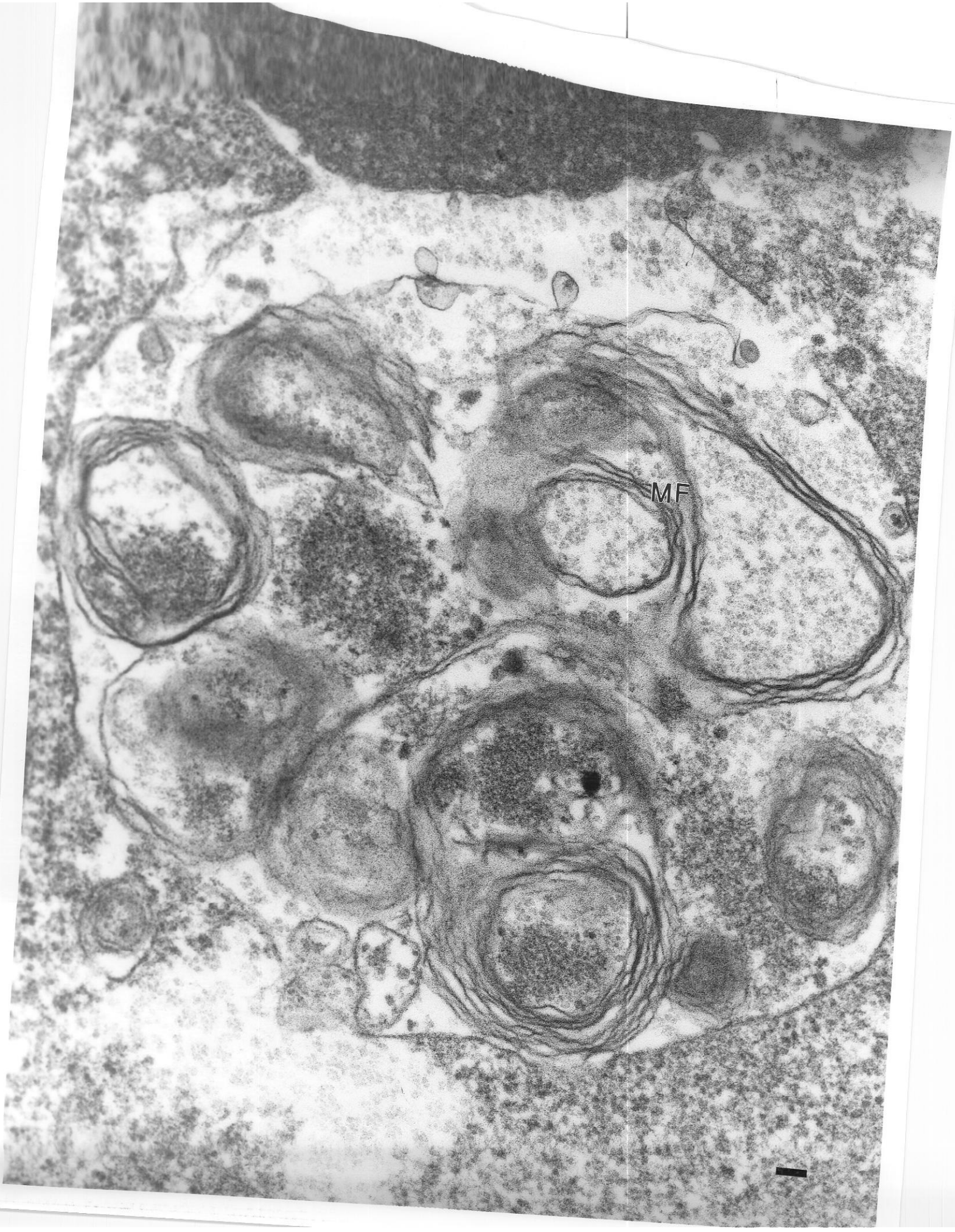
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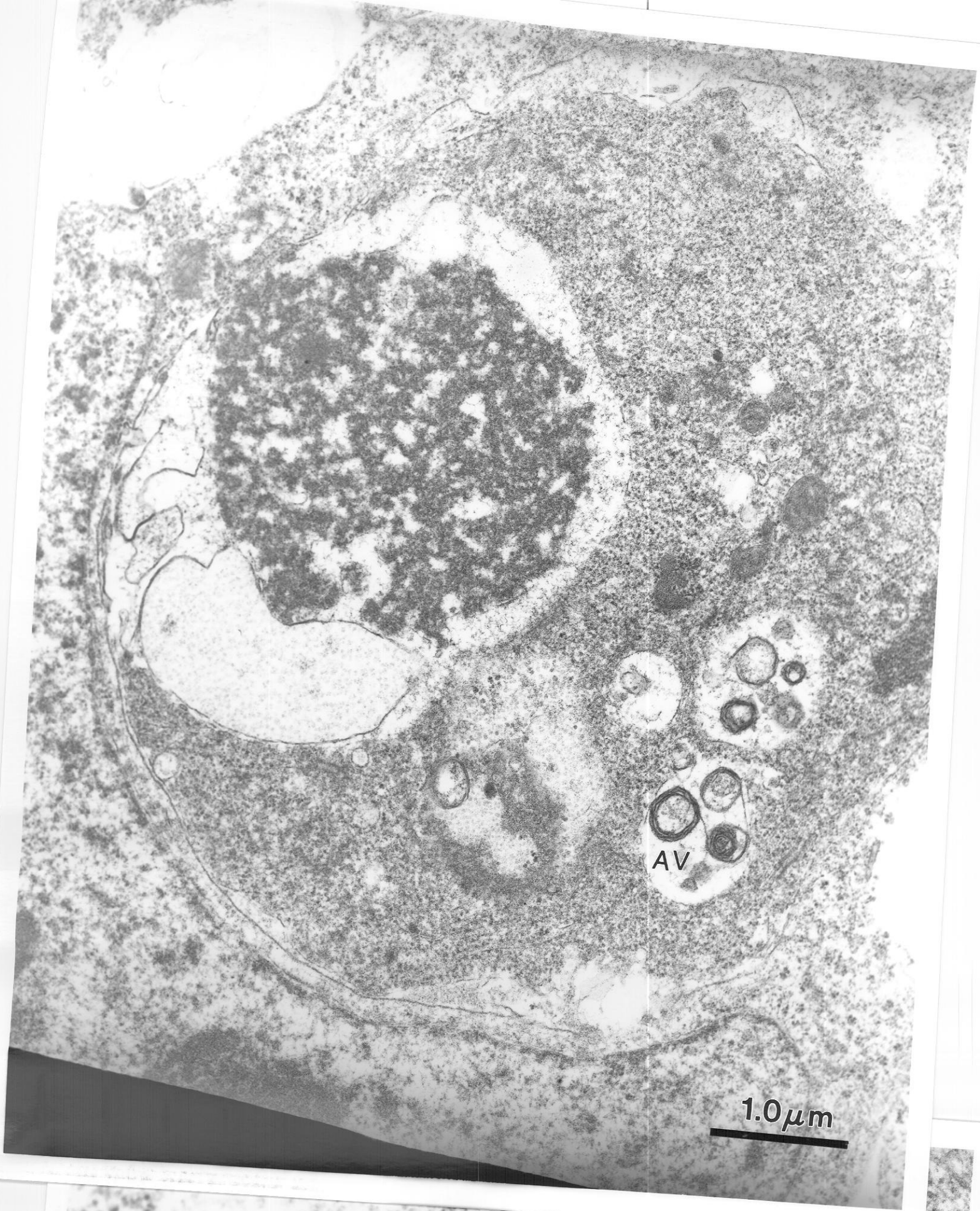
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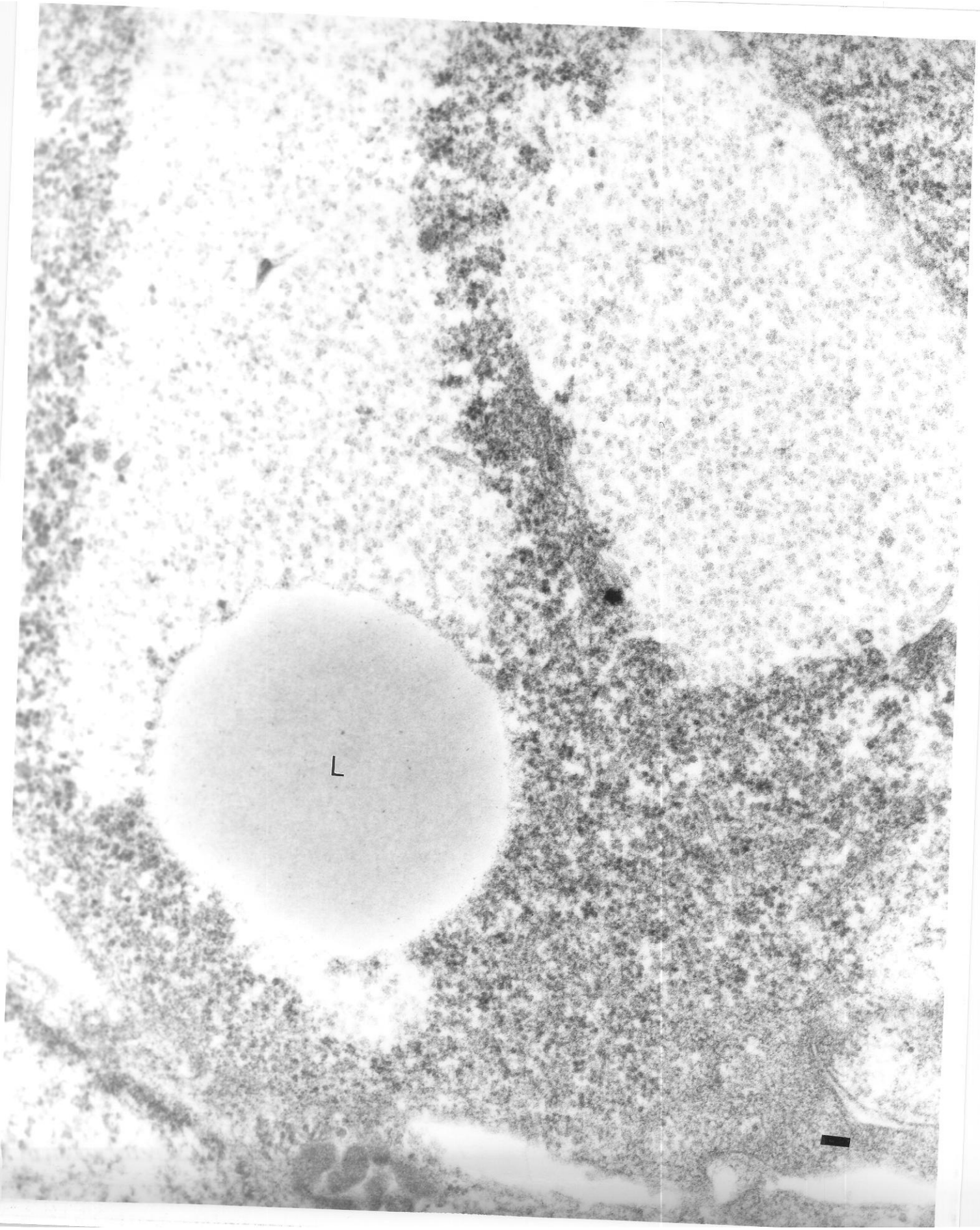
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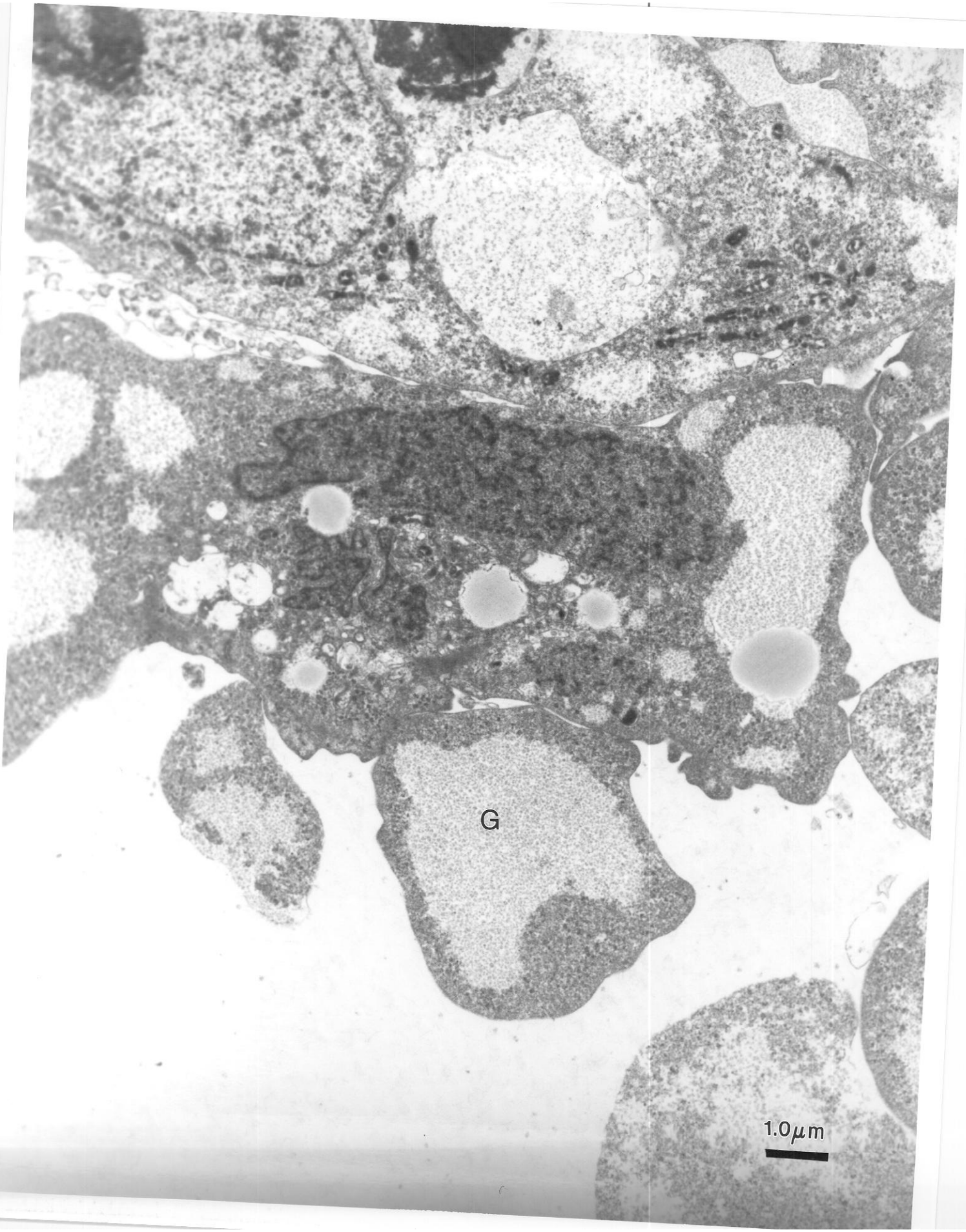
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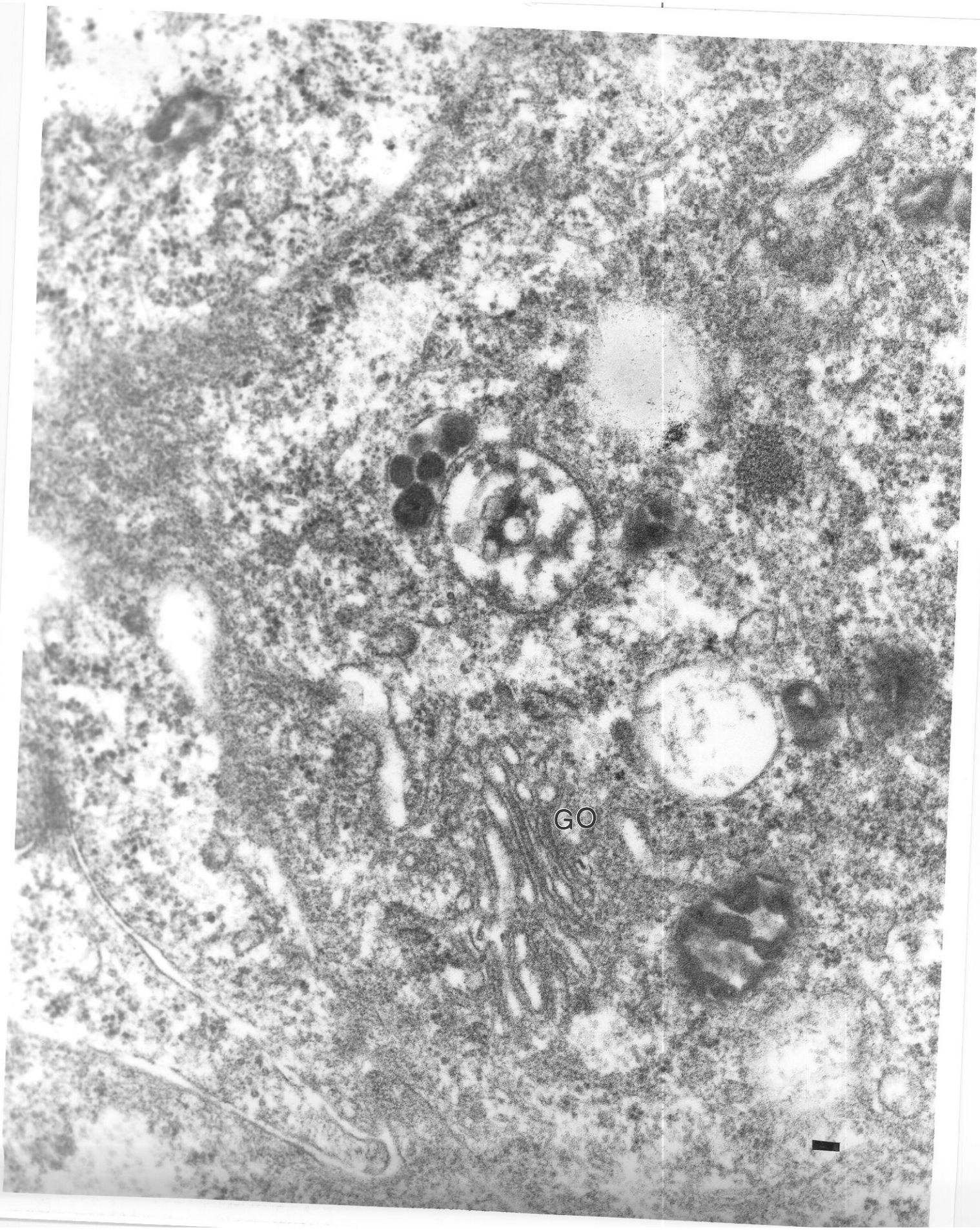
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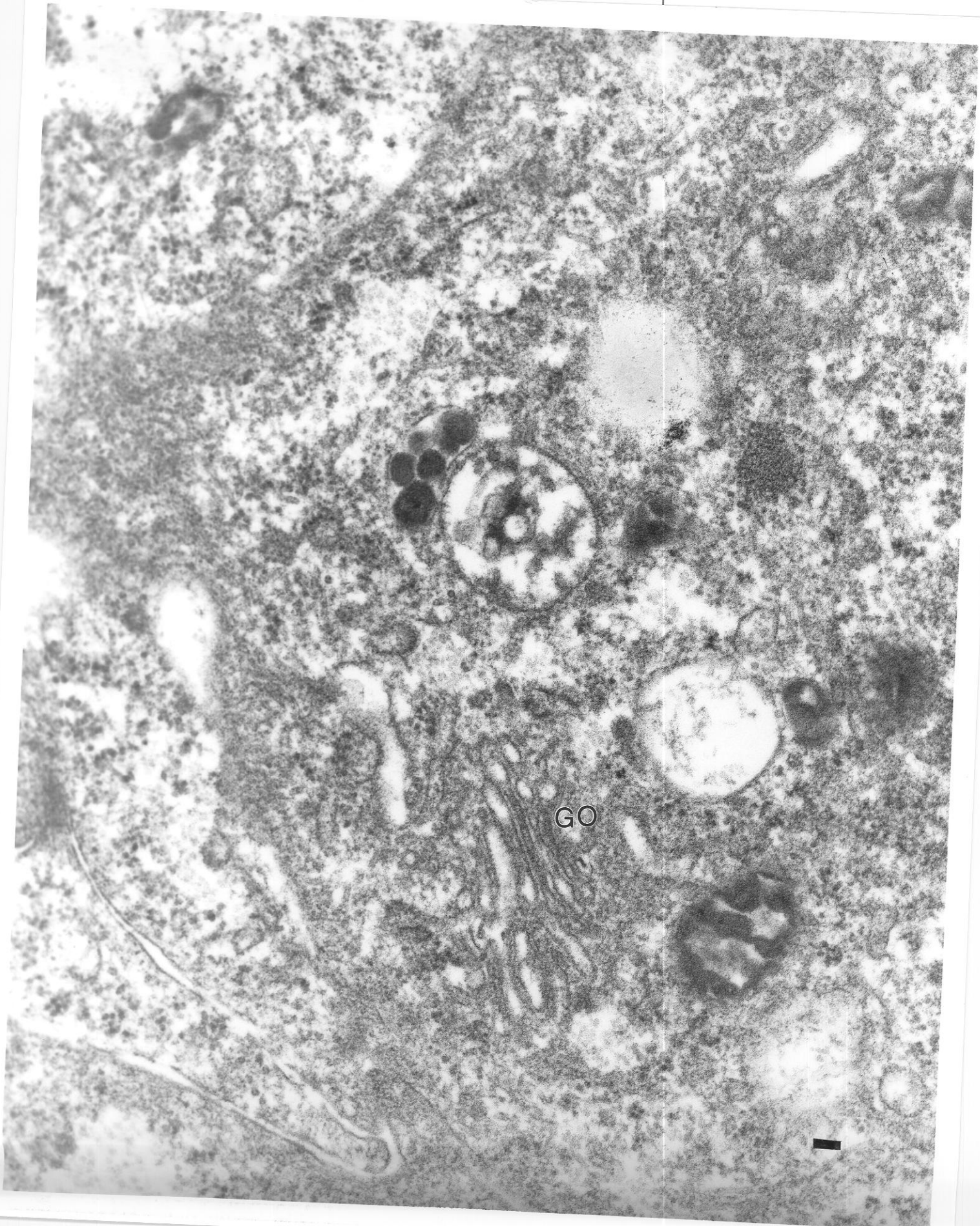
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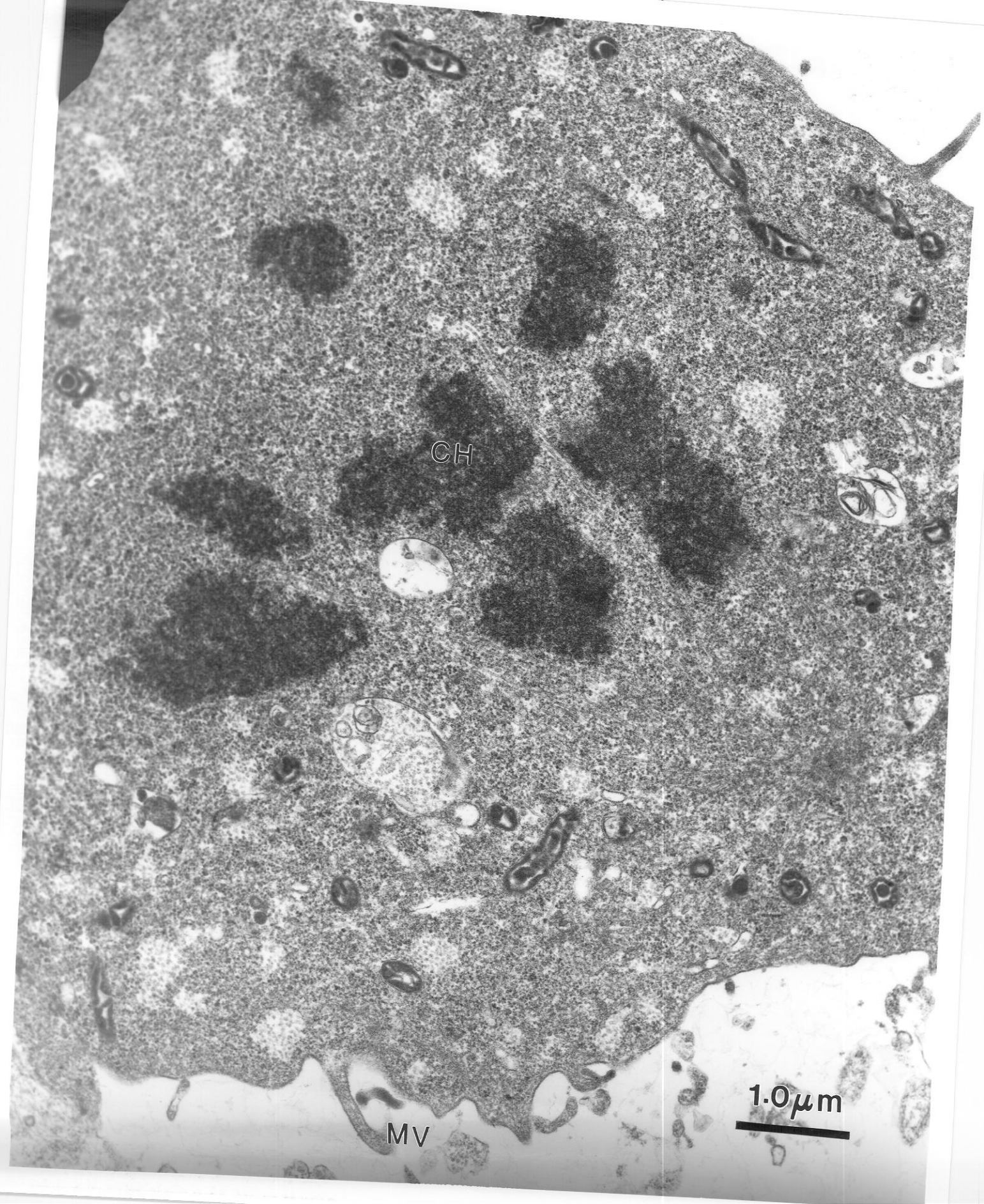
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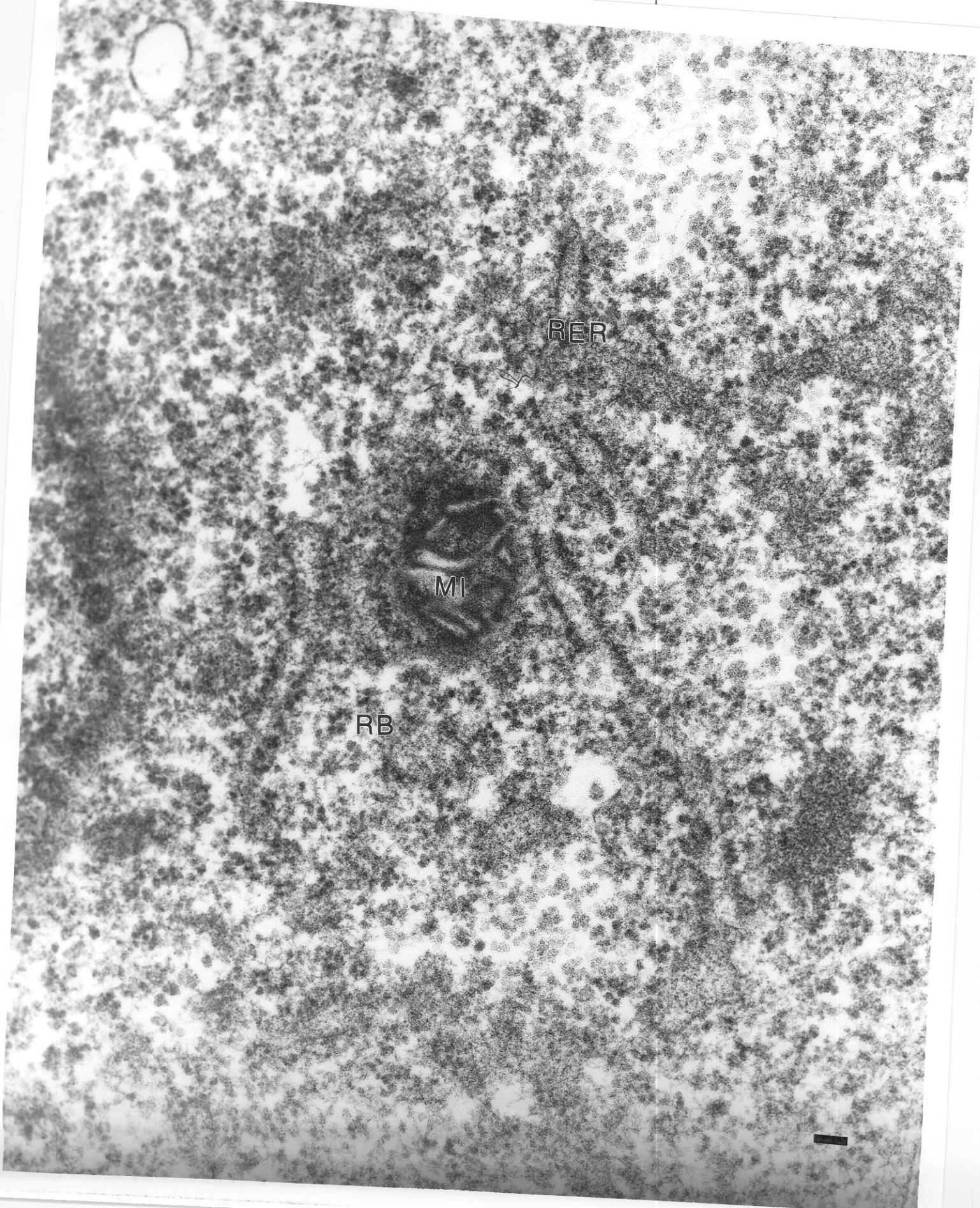
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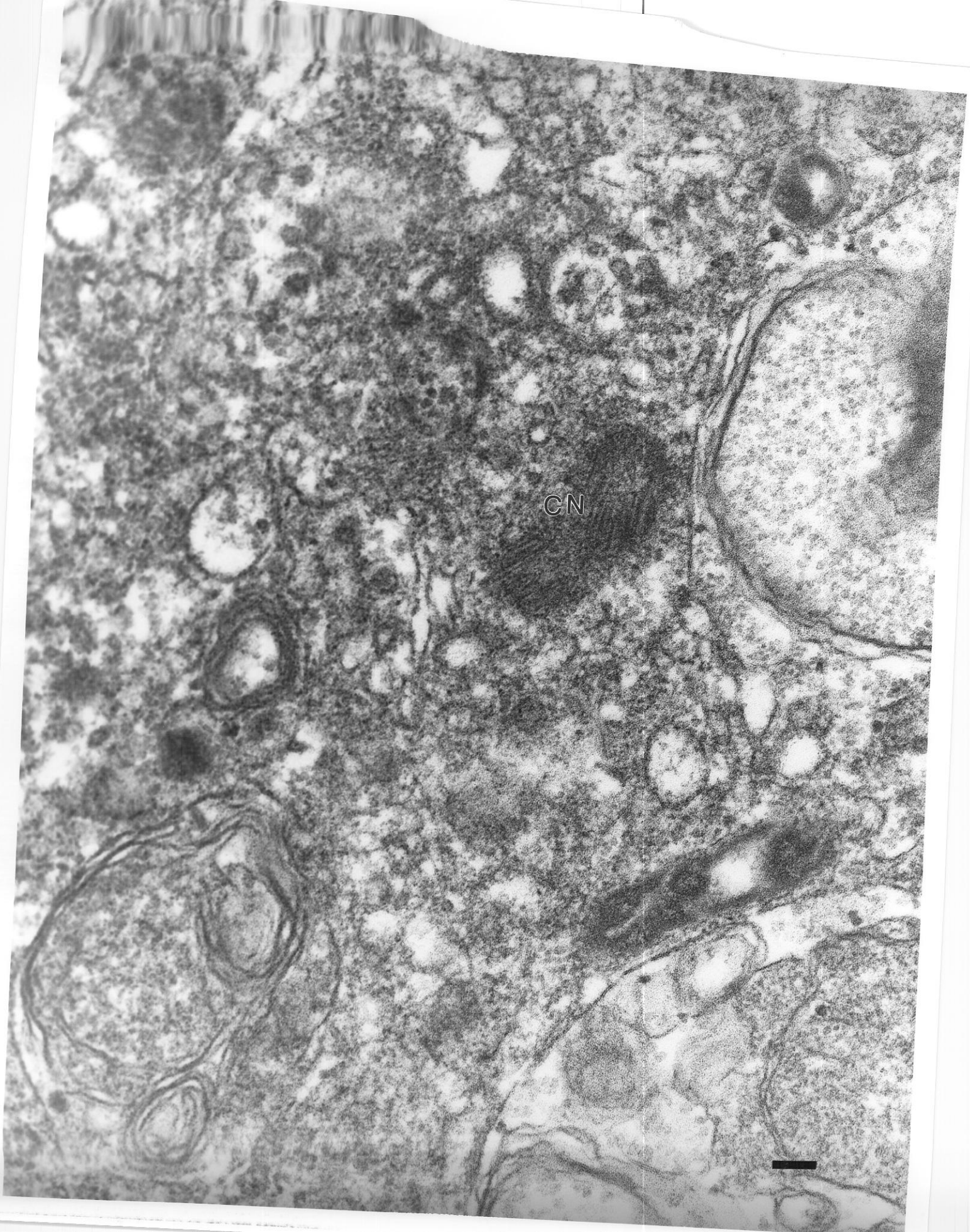
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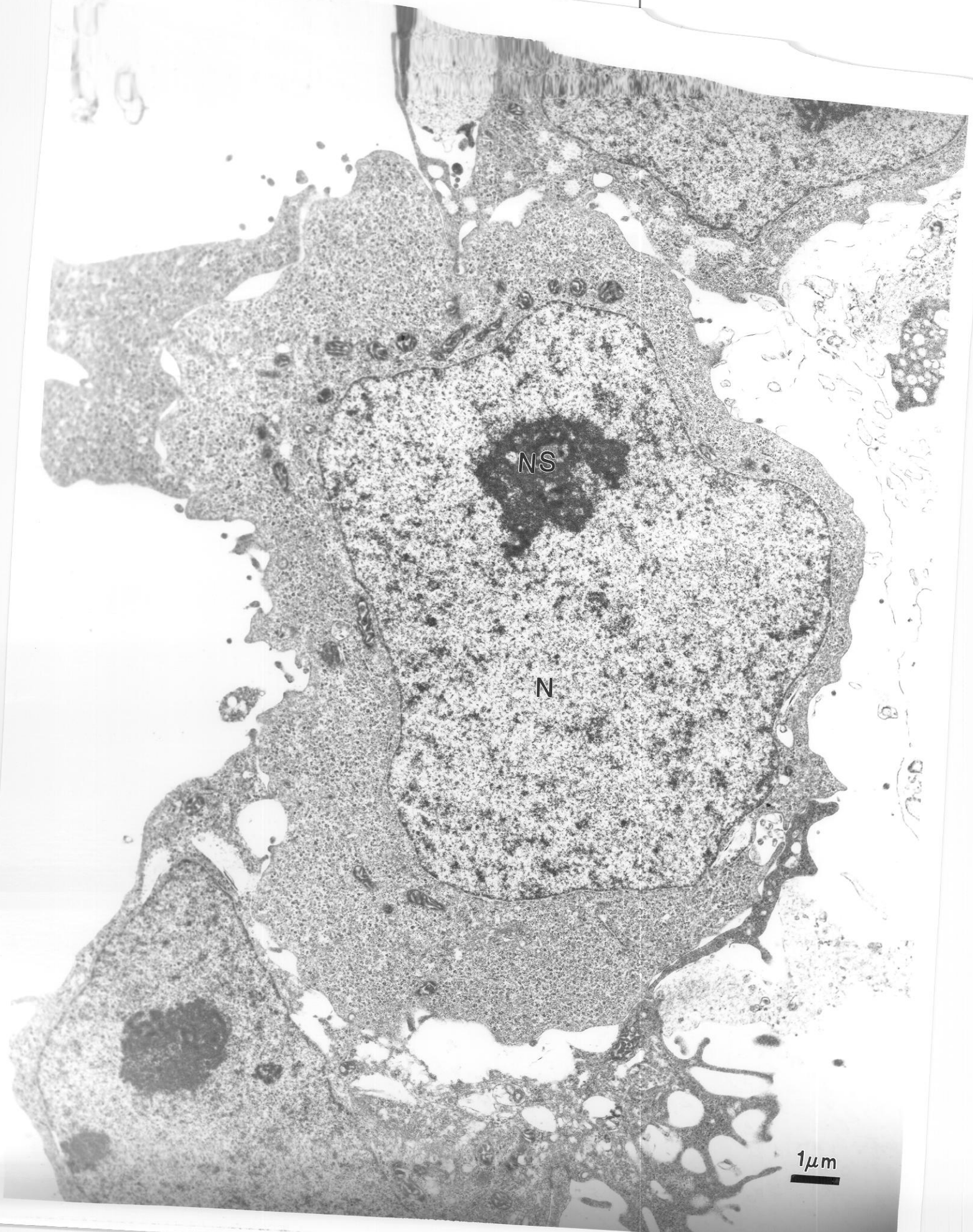
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N
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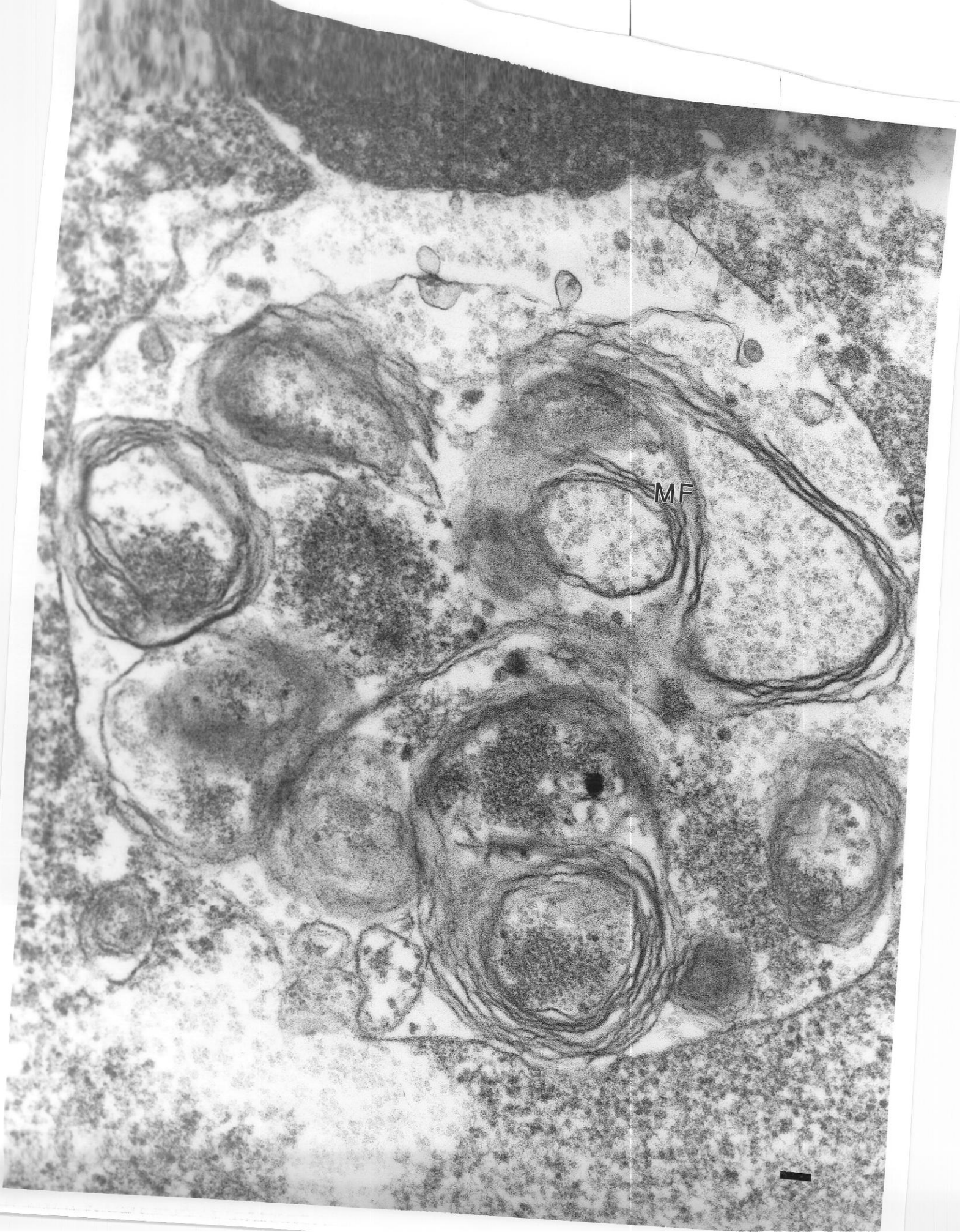
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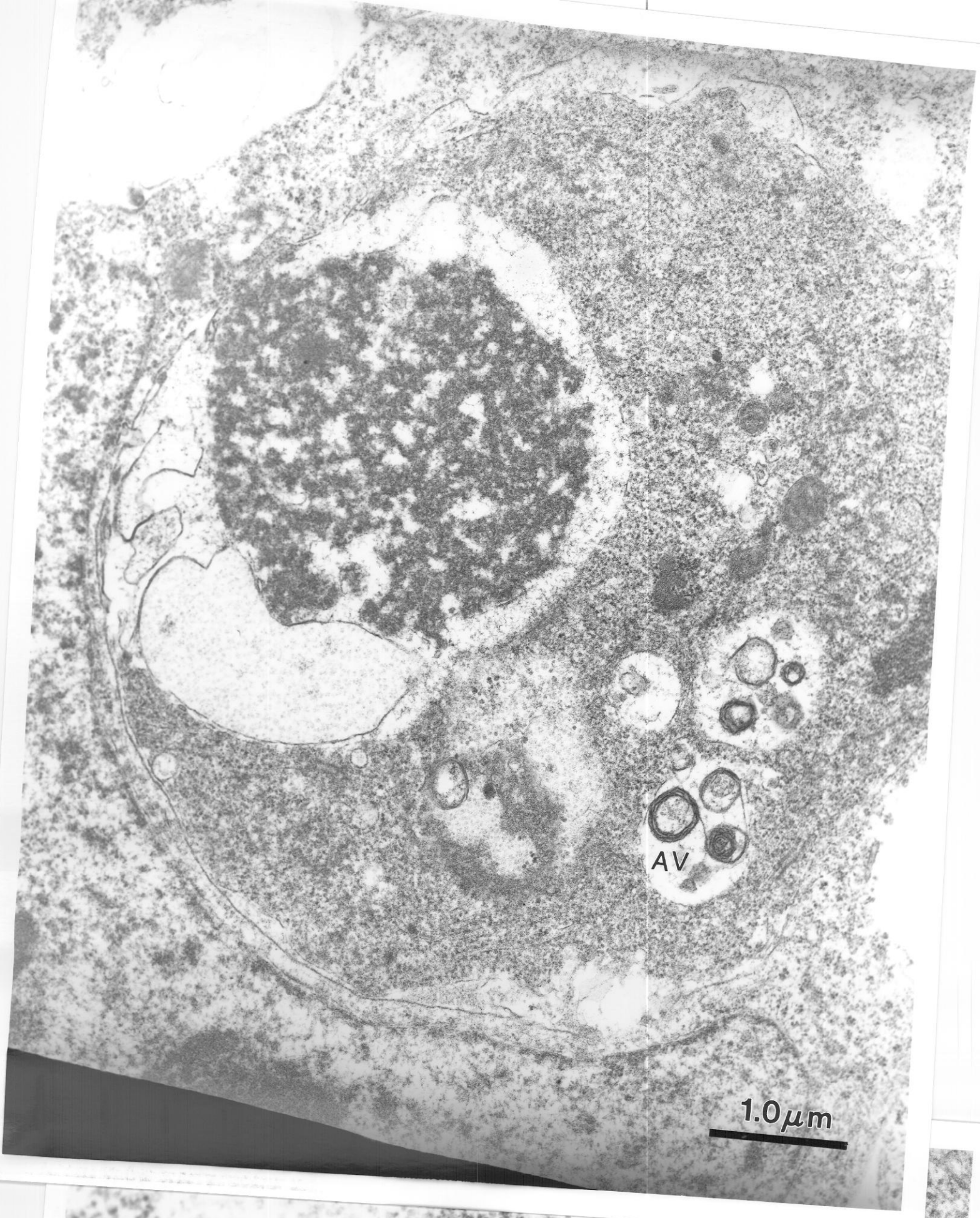
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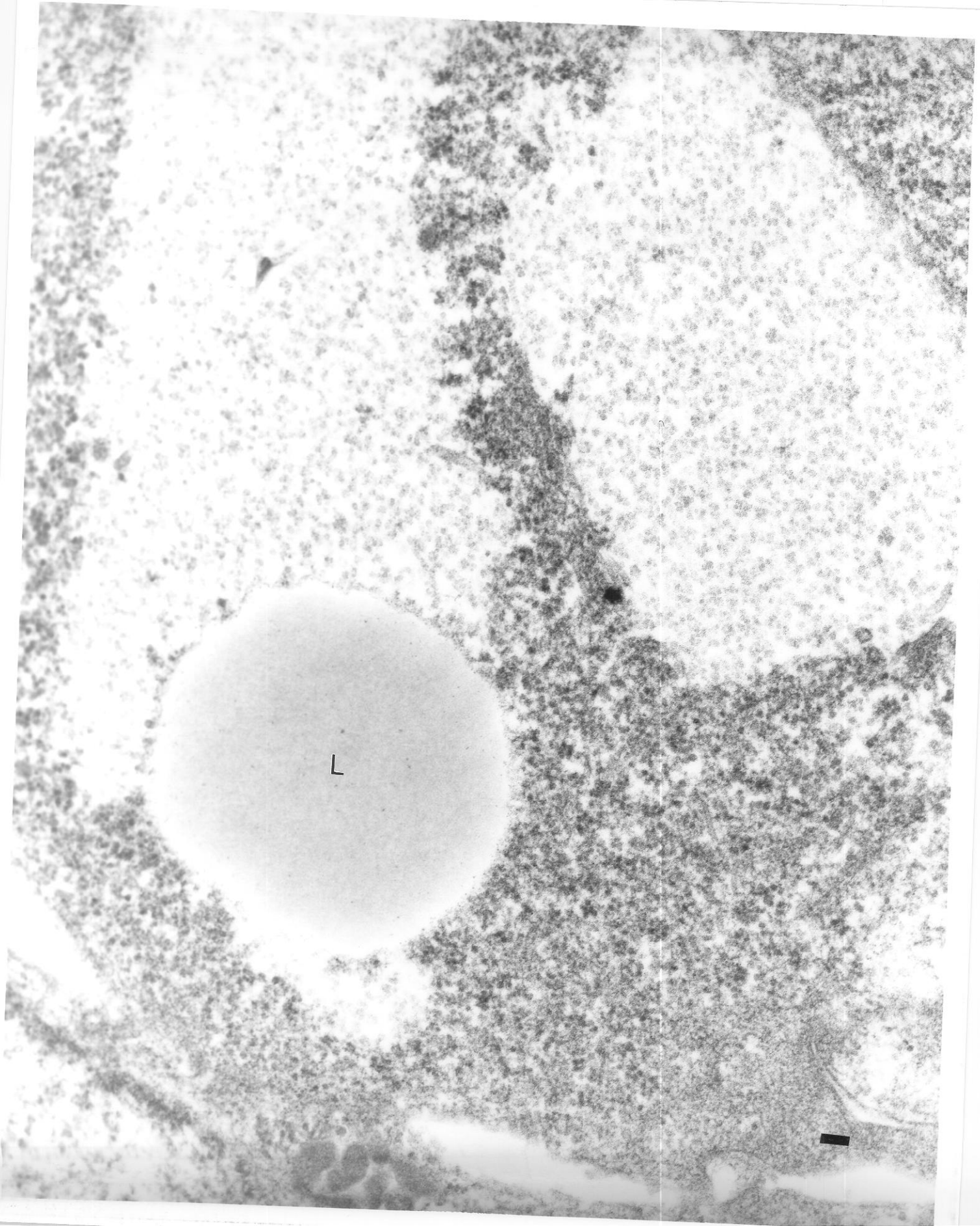
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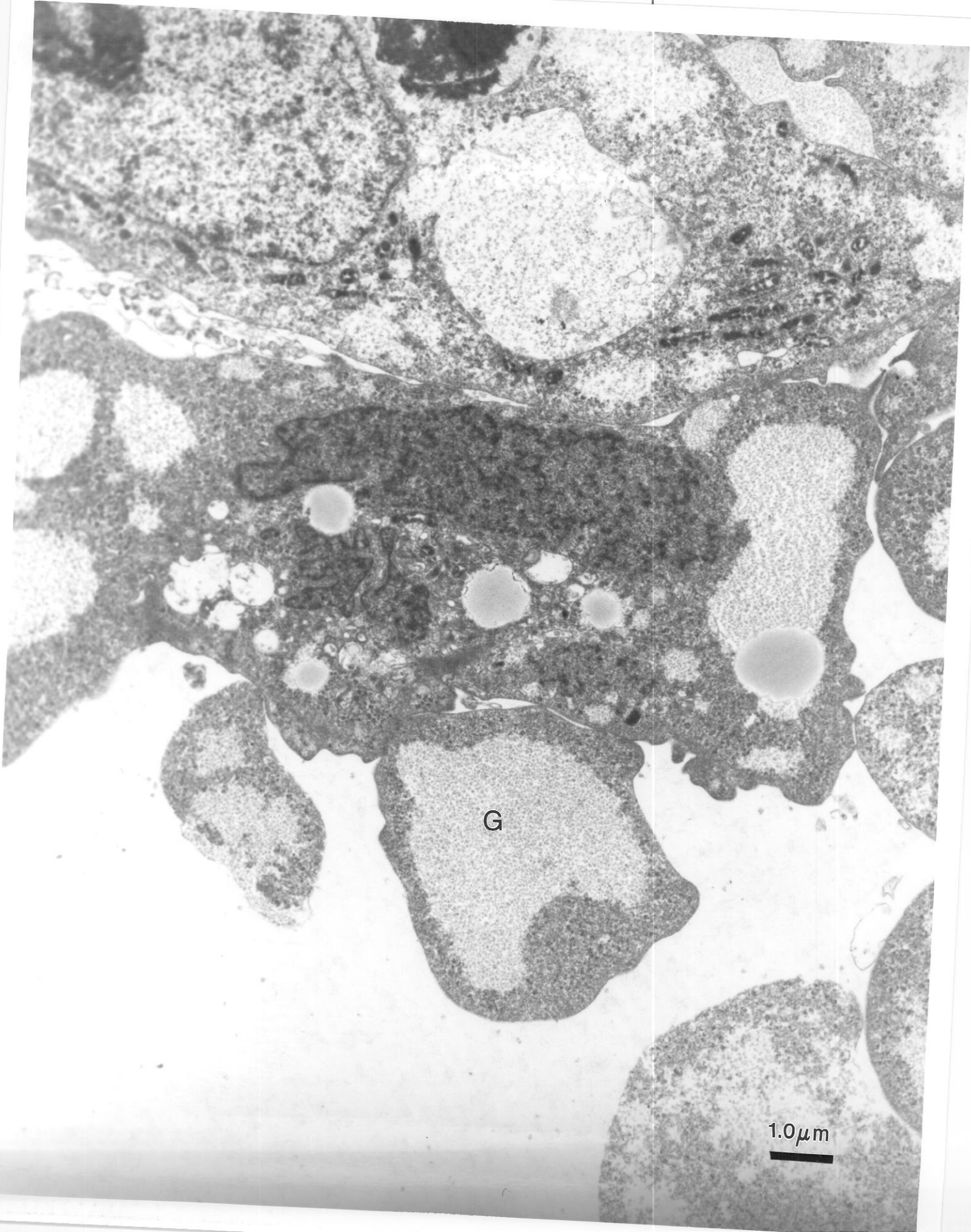
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1.0μm

E-FR-1-07-001214.30201

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
H9-MCB.1	07-001214

SPONSOR:

PERFORMING LABORATORY: AppTec, Inc.

APPTEC ACCESSION NUMBER	RESULTS
07-001214	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 13, 2007	June 13, 2007

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

Quality Assurance _____ 26 Jun 07
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 _____ 6-26-07
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: H9-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 T25 flask of "Human embryonic stem cell line H9 on mouse embryonic feeder layer" at room temperature, and 1 tube containing 25 mL of test sample material, cold on cold packs and designated for use in this assay. The flask of test article was stored at 37±2°C / 5±2% CO_2 atmosphere, while the tube was stored at 2-8°C 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) and 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 # 07-001214[1] (diluted 1:2)**	**T_0**	**ND**
	Accession # 07-001214 (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 reportable 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).

Final Report

Study Title	H9-MCB.1: Detection of HIV-1, HIV-2, HTLV-2, EBV, HHV-7, HHV-8 and HBV Sequences using the Polymerase Chain Reaction (PCR) Technique. Detection and Quantification of B19 Sequences using the Quantitative Polymerase Chain Reaction Technique (QPCR). Detection and Quantification of HCV Sequences using the Reverse Transcriptase Quantitative Polymerase Chain Reaction Technique (RT-QPCR)
Author	
Test Facility	Covance Laboratories Ltd., Otley Road, Harrogate, North Yorkshire HG3 1PY United Kingdom
Study Monitors	
Sponsor	Waisman Clinical BioManufacturing Facility
Covance Study Number	2823/004
Covance Report Number	2823/004-D5141
Report Issued	May 2008
Page Number	1 of 58

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STUDY DIRECTOR AUTHENTICATION AND GLP COMPLIANCE STATEMENT

H9-MCB.1: Detection of HIV-1, HIV-2, HTLV-2, EBV, HHV-7, HHV-8 and HBV Sequences using the Polymerase Chain Reaction (PCR) Technique. Detection and Quantification of B19 Sequences using the Quantitative Polymerase Chain Reaction Technique (QPCR). Detection and Quantification of HCV Sequences using the Reverse Transcriptase Quantitative Polymerase Chain Reaction Technique (RT-QPCR)

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 work and generated data are scientifically acceptable and valid and this Report provides a true and accurate record of the results obtained.

The study was conducted 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.

* With the exception of the plate reader Spectrafluor Plus and the XFLUOR (version: V 4.11) software and the Applied Biosystems 7900HT Real Time PCR System and software. The Spectrafluor Plus and the XFLUOR (version: V 4.11) software are not currently fully validated at Covance Harrogate. The Applied Biosystems 7900HT Real Time PCR System has completed and passed the validation process but is awaiting report finalisation. An internal risk assessment has been generated at Covance Harrogate detailing the acceptability of the system for use with this study.

_____ 30 May 2008
Date

Study Director

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QUALITY ASSURANCE STATEMENT

H9-MCB.1: Detection of HIV-1, HIV-2, HTLV-2, EBV, HHV-7, HHV-8 and HBV Sequences using the Polymerase Chain Reaction (PCR) Technique. Detection and Quantification of B19 Sequences using the Quantitative Polymerase Chain Reaction Technique (QPCR). Detection and Quantification of HCV Sequences using the Reverse Transcriptase Quantitative Polymerase Chain Reaction Technique (RT-QPCR)

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			Date Reported to SD and SD Management
From	To	Phase	
02 Jul 2007	02 Jul 2007	Protocol Review	02 Jul 2007
15 Jan 2008	15 Jan 2008	Protocol Amendment Review	15 Jan 2008
28 Jan 2008	28 Jan 2008	Draft Report and Data Review	30 Jan 2008
29 May 2008	29 May 2008	Final Report Review	29 May 2008

Process

Inspection Dates			Date Reported to SD and SD Management
From	To	Phase	
04 Jul 2007	04 Jul 2007	Test Article Receipt	04 Jul 2007
10 Jul 2007	10 Jul 2007	Polymerase Chain Reaction	10 Jul 2007
02 Aug 2007	02 Aug 2007	Extraction	02 Aug 2007
30 Aug 2007	31 Aug 2007	Data Review	31 Aug 2007
26 Sep 2007	26 Sep 2007	Polymerase Chain Reaction	26 Sep 2007
31 Oct 2007	31 Oct 2007	Test Article Preparation	31 Oct 2007
31 Oct 2007	31 Oct 2007	Test Article Preparation	31 Oct 2007
08 Nov 2007	08 Nov 2007	Extraction	08 Nov 2007
22 Nov 2007	22 Nov 2007	Test Article Preparation	23 Nov 2007

30 May 2008
Date

Quality Assurance Unit

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RESPONSIBLE PERSONNEL

H9-MCB.1: Detection of HIV-1, HIV-2, HTLV-2, EBV, HHV-7, HHV-8 and HBV Sequences using the Polymerase Chain Reaction (PCR) Technique. Detection and Quantification of B19 Sequences using the Quantitative Polymerase Chain Reaction Technique (QPCR). Detection and Quantification of HCV Sequences using the Reverse Transcriptase Quantitative Polymerase Chain Reaction Technique (RT-QPCR)

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:	28th June 2007 (Date Study Director signed Definitive Protocol).
Assay initiation date:	3rd July 2007 (Date of first study related data capture).
Assay completion date:	24th November 2007 (Date of last data capture).
Study completion date:	Date Study Director signed the Final Report.

ARCHIVE STATEMENT

H9-MCB.1: Detection of HIV-1, HIV-2, HTLV-2, EBV, HHV-7, HHV-8 and HBV Sequences using the Polymerase Chain Reaction (PCR) Technique. Detection and Quantification of B19 Sequences using the Quantitative Polymerase Chain Reaction Technique (QPCR). Detection and Quantification of HCV Sequences using the Reverse Transcriptase Quantitative Polymerase Chain Reaction Technique (RT-QPCR)

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. The Sponsor will be notified of the financial implications 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 in writing 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

At the Sponsor's request, the testing of assays for HTLV-1, HCMV and HHV-6 sequences was terminated; hence results are not documented in this Report. This Report now includes analysis of assays for HIV-1, HIV-2, HTLV-2, HBV, EBV, HHV-7, HHV-8, B19 and HCV sequences.

DNA and RNA were extracted from the test article (H9-MCB.1) and analysed for the presence of viral sequences in individual, specific PCR, QPCR and RT-QPCR assays. In the PCR assays, quadruplicate aliquots of the test article DNA (0.1µg per replicate) were assayed to increase sampling. In the QPCR and RT-QPCR assays, triplicate aliquots of the test article DNA or RNA (<1.0µg per replicate) were assayed. Spiked controls were also included in the PCR, QPCR and RT-QPCR assays to monitor for sample specific inhibition.

Following PCR, QPCR and RT-QPCR analysis, target-specific products were not amplified from the test article DNA or RNA, in assays for any of the viral specific sequences tested.

Target specific amplicons were detected in all test article samples spiked with the relevant positive control on each occasion, thus confirming that no factors inhibitory to PCR were present in the DNA or RNA, or were at levels high enough to be significant to the assays.

Following PCR, the additional PCR amplicons that were observed from the test article DNA replicates in the HHV-8, HTLV-2, HIV-1 and EBV assays were deemed to be non-specific.

To summarise, within the limits of assay sensitivity, the test article was determined to be negative for the presence of HIV-1, HIV-2, HTLV-2, EBV, HHV-7, HHV-8, HBV, B19 and HCV by PCR, QPCR or RT-QPCR.

INTRODUCTION

It is important to demonstrate that Master Cell Bank stock is free from the presence of viruses. A number of viruses are of particular concern because they are latent or non-cultivable, and therefore broad-spectrum tissue culture based assays would not detect such viruses. The polymerase chain reaction (PCR) technique is an *in vitro* method for the amplification of DNA molecules from low copy number target molecules. PCR is a highly specific technique as it relies upon the hybridisation of oligonucleotide primers to the target nucleic acid sequence of interest. The quantitative PCR assay allows highly sensitive and specific detection of DNA sequences, as well as offering the ability to quantify the target sequence. The ABI PRISM 7900™ is able to detect fluorescence during PCR. This allows the 'real-time' detection of PCR product accumulation via the hydrolysis of probes labelled with both a fluorescent reporter and a quencher. Quantitative data are derived from a determination of the cycle at which the amplification product signal crosses a pre-set detection threshold (threshold cycle, C_T value). This cycle number is proportional to the amount of starting material. Therefore, PCR and QPCR can be used to detect viral sequences in infected cell lines. Either PCR or QPCR assays were performed for each virus of concern.

Following binding, internalisation and uncoating of the virion of retroviruses HIV-1 (*Human Immunodeficiency Virus-1*), HIV-2 (*Human Immunodeficiency Virus-2*) and HTLV-2 (*Human T Cell Lymphotrophic Virus-2*), the genomic RNA is reverse transcribed to yield double-stranded DNA copies which can integrate into the host cell genome. Therefore, the presence, or absence, of HIV-1, HIV-2 and HTLV-2 can be determined by extracting DNA from the test article, followed by amplification of specific retroviral sequences using the PCR (polymerase chain reaction) technique (Refs. 1, 2 and 3) with primers specific to the particular retroviral genome. The primers for the detection of HIV-2 are also capable of detecting SIV sequence.

EBV (*Epstein Barr Virus*), HHV-7 (*Human Herpes Virus-7*) and HHV-8 (*Human Herpes Virus-8*) are double-stranded DNA viruses, B19 (*Human Parvovirus*) is a single-stranded DNA virus and HBV (*Hepatitis B Virus*) is partially double stranded. Therefore, the presence or absence of these viruses can be determined by extracting DNA from the test article, followed by amplification of specific DNA viral sequences, using the PCR technique with primers specific to the relevant viral genome.

HCV (*Hepatitis C Virus*) is a single-stranded RNA virus. Therefore, its presence or absence in a test article can be determined by extracting RNA from the sample, followed by RT-QPCR (reverse transcriptase quantitative polymerase chain reaction). This involves the synthesis of first strand cDNA and subsequent amplification by QPCR and quantification using primers and probe specific to the HCV sequences.

OBJECTIVE

The objective of this study was to determine whether HIV-1, HIV-2, HTLV-2, EBV, HHV-7, HHV-8, HBV, B19 and HCV were present in the test article (H9-MCB.1) using the polymerase chain reaction (PCR) technique, the quantitative polymerase chain reaction (QPCR) technique and the reverse transcriptase quantitative polymerase chain reaction (RT-QPCR) technique.

MATERIALS

Protocol Adherence

The study described in this Report was carried out according to the agreed Definitive Protocol and one Protocol Amendment, see Annex for details. Minor deviations deemed not to have affected the study are presented in the Appendix.

Test Article

Identification: H9-MCB.1.

Source: WiCell Research Institute.

The test article cell pellets were received at Covance Laboratories on the following dates, 4 x 15ml vials on 29th March 2007, 1 x 1.5ml vial on 12th July 2007 and 2 x 1.5ml vials on 6th September 2007. The cell pellets were stored at -80°C until being tested.

The exact details as presented on the test article vessel:
COVANCE 1 pellet @ $1x10^6$ cells Human Virus Panel MCB.A.H9p27. 22 JAN 07. DF.

Additional vials of test article were received and these were labelled as follows: H9MCB-1 RNA extract 7.08.07, and: MCB.01 H9 (0) 7.31.07 and all were stored at -80°C until being tested.

The Sponsor provided the following details on the test article by completing a Test Article Safety & Pre-Study Questionnaire:

Appearance:	Cell Pellet.
Concentration:	$1x10^6$cells.
Cell Line Information:	Human Embryonic Stem Cells.
Storage Temperature:	-70°C.
Expiry Date:	N/A.

Unused test article to be disposed of by incineration.
The test article does not contain chemicals which may be hazardous.

This study, for the presence of extraneous agents was conducted to define the purity of the test article. 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 this study was to test for extraneous agents that may be present in the test materials.

PCR and QPCR Test Systems

The Polymerase Chain Reaction (PCR), Quantitative Polymerase Chain Reaction (QPCR), and Reverse Transcriptase Quantitative Polymerase Chain Reaction (RT-QPCR).

Controls

Positive Controls:	DNA extracted from HIV-1. DNA extracted from HIV-2. DNA extracted from HTLV-2. DNA extracted from EBV. DNA extracted from HHV-7. DNA extracted from HHV-8.
Source:	Advanced Biotechnologies Inc.
Positive Control:	B19 DNA synthetic oligonucleotide.
Source:	Eurogentec.
Positive Control:	HBV, full length genome in pEco63, extracted DNA.
Source:	American Type Tissue Culture Collection.
Positive Control:	Armored RNA® HCV (Genotype 1a) in TSM III buffer.

Source:	Ambion Diagnostics.
Negative Control:	DNA extracted from uninfected (i.e. HIV-1, HIV-2, HTLV-2, EBV, HHV-7, HHV-8, HBV and B19 negative) cells.
	RNA extracted from uninfected (i.e. HCV negative) cells.
Source:	Prepared in-house from MRC-5 or MDBK cells, as documented in laboratory records.
Blank, Sentinel and RNA Extraction Control:	Purified water (DNase, RNase none detected, 0.1µm filtered).
Source:	Sigma-Aldrich Company Ltd.
DNA Extraction Control:	Dulbecco's Phosphate Buffered Saline (DPBS).
Source:	Invitrogen Ltd.

PROCEDURES

The procedures were performed as documented in the Definitive Protocol and one Protocol Amendment, see Annex for details. Minor deviations deemed not to have affected the study are presented in the Appendix.

RESULTS

Human Immunodeficiency Virus-1 (HIV-1) Results

Following PCR analysis, no PCR amplicon of the expected size (125 bp) was detected in any of the four replicates of test article DNA (0.1µg per replicate) tested (Table 1). Therefore, within the sensitivity of the assays, the test article was negative for the presence of HIV-1 sequences by PCR. However, PCR products were observed over a range of higher, and lower, molecular weights that differed to those of the expected HIV-1 amplicon, in two of the four unspiked test article DNA replicates. These additional products were deemed non-specific.

A HIV-1 specific PCR product was amplified from both of the test article DNA samples spiked with 100pg of HIV-1 positive control DNA (Table 1). These data demonstrated that the negative results generated with the test article DNA were valid. Furthermore, the data confirmed that no factors inhibitory to PCR were present in the DNA, or were at levels high enough to be significant.

The PCR assay was performed using HIV-1 positive control DNA at approximately 10, 100 and 1000pg. A PCR amplicon of the correct size was amplified from 100, and 1000pg of positive control; therefore, the PCR assay sensitivity was at least 100pg HIV-1 positive control DNA (Table 1).

Human Immunodeficiency Virus-2 (HIV-2) Results

No PCR product of the expected size (159 bp) was detected in any of the four replicates of test article DNA (0.1µg per replicate) tested (Table 1), following PCR analysis. Therefore, within the sensitivity of the assays, the test article was negative for the presence of HIV-2 sequences by PCR analysis.

An HIV-2-specific PCR product was amplified from both of the test article DNA samples spiked with 1pg of HIV-2 positive control DNA (Table 1). These data demonstrated that the negative results generated with the test article DNA were valid. Furthermore, the data confirmed that no PCR inhibitory factors were present in the DNA, or were at levels high enough to be significant.

The PCR assay was performed using HIV-2 positive control DNA at approximately 1, 10 and 100pg HIV-2 DNA. A PCR amplicon of the correct size (159 bp) was amplified from all positive controls therefore, the PCR assay was sensitive to at least 1pg of HIV-2 DNA (Table 1).

Human T Cell Lymphotrophic Virus-2 (HTLV-2) Results

Following PCR analysis, no PCR product of the expected size (185 bp) was detected in any of the four replicates of test article DNA (0.1µg per replicate) tested. Additional amplicons, of molecular weights that differed to those of the expected HTLV-2 amplicon, were observed in all four test article replicates, again these bands were deemed non-specific. Therefore, within the sensitivity of the assay, the test article was negative for the presence of HTLV-2 sequences by PCR.

PCR amplicons were detected in both of the replicates spiked with 10pg of HTLV-2 positive control. The data demonstrates that the negative results generated with the test article DNA were valid, and that no PCR inhibitory factors were apparent.

The PCR assay was performed using HTLV-2 positive control DNA at approximately 10, 100 and 1000pg. A PCR amplicon of the correct size (185 bp) was amplified from replicates containing 100 and 1000pg positive control therefore, the PCR assay sensitivity was at least 100pg of HTLV-2 positive control DNA.

Epstein Barr Virus (EBV) Results

No PCR product of the expected size (171 bp) was detected in any of the four replicates of test article DNA (0.1µg per replicate) tested (Table 1), following PCR analysis.

Additional amplicons, of molecular weights that differed to those of the expected EBV-specific amplicon, were observed in all four test article replicates, again these bands were deemed non-specific. Therefore, within the sensitivity of the assay, the test article was negative for the presence of EBV sequences by PCR analysis.

An EBV-specific PCR product was amplified from both of the test article DNA samples spiked with EBV positive control DNA (1 in 10 dilution) (Table 1). These data demonstrated that the negative results generated with the test article DNA were valid. Furthermore, the data confirmed that no PCR inhibitory factors were present in the DNA, or were at levels high enough to be significant.

The PCR assay was performed using EBV positive control DNA at approximate dilutions of 1 in 100, 1 in 10 and neat EBV samples. A PCR amplicon of the correct size (171 bp) was amplified from all positive controls therefore, the PCR assay was sensitive to at least 1 in 100 dilutions of EBV DNA (Table 1).

Human Herpes Virus 7 (HHV-7) Results

No PCR product of the expected size (186 bp) was amplified from any of the four replicates of test article DNA (0.1µg per replicate), following PCR analysis (Table 1). Therefore, within the sensitivity of the assays, the test article was negative for the presence of HHV-7 sequences by PCR.

A HHV-7-specific PCR product was amplified from both of the test article DNA samples spiked with 10vp of HHV-7 positive control DNA (Table 1).

The data demonstrates that the negative results generated with the test article DNA were valid. Furthermore, the data confirmed that no factors inhibitory to PCR were present in the DNA, or were at levels high enough to be significant.

The PCR assay was performed using HHV-7 positive control DNA at approximately 1, 10 and 100vp. A PCR amplicon of the correct size (186 bp) was amplified in positive controls containing 10 and 100vp. Therefore, the PCR assay was sensitive to at least 10vp HHV-7 positive control DNA (Table 1).

Human Herpes Virus 8 (HHV-8) Results

Following PCR analysis, no PCR product of the correct size (233 bp) was detected in the four replicates of test article DNA (0.1µg per replicate) assayed (Table 1). However, PCR products were observed over a range of higher and lower molecular weights that differed to those of the expected HHV-8 amplicon, in all four of the unspiked test article DNA replicates and in the sentinel control.

These additional products were deemed non-specific. Therefore, within the sensitivity of the assay, the test article was negative for the presence of HHV-8 sequences by PCR.

HHV-8-specific PCR products were amplified from both of the test article DNA samples spiked with 100pg of HHV-8 positive control (Table 1). These data demonstrated that the negative results generated with the test article DNA were valid. Furthermore, the data confirmed that no factors inhibitory to PCR were present in the DNA, or were at levels high enough to be significant.

The PCR assay was performed using HHV-8 positive control DNA at approximately 50, 100 and 1000pg/reaction. A PCR amplicon of the correct size (233 bp) was amplified in all three positive controls therefore, the PCR assay was sensitive to at least 50pg HHV-8 positive control DNA (Table 1).

Hepatitis B Virus (HBV) Results

The PCR analysis of HBV was repeated due to there being no amplification in one of the replicates spiked with 100 copies of HBV positive control DNA. Following repeat PCR analysis, no PCR product of the expected size (269 bp) was detected in any of the four replicates of test article DNA (0.1µg per replicate) tested (Table 1). Therefore, within the sensitivity of the assay, the test article was negative for the presence of HBV sequences by PCR.

An HBV-specific PCR product was amplified from both of the test article DNA samples spiked with 100 copies of HBV positive control DNA (Table 1). These data demonstrated that the negative results generated with the test article DNA were valid. Furthermore, the data confirmed that no factors inhibitory to PCR were present in the DNA, or were at levels high enough to be significant.

The PCR assay was performed using HBV positive control DNA at approximately 100, 1000 and 10000 copies. A PCR amplicon of the correct size was amplified from all three positive controls therefore, the PCR assay sensitivity was at least 100 copies HBV positive control DNA (Table 1).

Human Parvovirus (B19) Results

No amplification was detected in any of the QPCR negative controls (the sentinel, water blank, extraction and negative controls (MRC-5 cell DNA), in the QPCR assay (Table 2).

Following QPCR analysis of the extracted test article DNA, amplification was detected in all of the positive controls, containing 1×10^7, 1×10^6, 1×10^5, 1×10^4, 1×10^3 and 1×10^2 copies DNA, which were subsequently used to generate a standard curve (Table 2). Linear regression of this data produced a slope of –3.51 and a correlation coefficient (R^2) of 0.98 (data not shown).

Three replicates of test article DNA (0.8µg per replicate) extracted from test article were assayed directly, with no further treatment. Three replicates of test article DNA (0.8µg per replicate) were spiked with 1000 copies of B19 positive control to assess for any test article related inhibitory factors to B19 QPCR assay.

Within the sensitivity of the assay, the test article was negative for the presence of B19 DNA sequences. The three spiked test article replicates had a mean C_T value of 33.13 (Table 2). A spiked sample with a C_T value 3.3 cycles or greater than the mean C_T value of the mean of the 1000 copy standards would be considered partially inhibitory. Therefore if the spiked sample had a C_T value greater than or equal to 35.87 (32.57 + 3.3 = 35.87) it would have been considered partially inhibitory. A C_T value of undetermined in the spiked test article replicates would have been considered totally inhibitory. Therefore, no total or partial inhibition was detected in the QPCR assay, demonstrating that the negative results generated with the test article DNA were valid.

In addition, the TaqMan® exogenous internal positive control (IPC) reagents included in all QPCR replicates established that all negative PCR results were truly negative and not due to failed amplification of the QPCR assay. Furthermore, the data confirmed that no factors inhibitory to QPCR were present in the DNA, or were at levels high enough to be significant.

Hepatitis C Virus (HCV) Results

No amplification was detected in any of the RT-QPCR negative controls (MRC-5, sentinel control, water blank and extraction control) in the QPCR assay (Table 3).

Amplification was detected in the positive controls after RT-QPCR analysis of the RNA extracted from test article. HCV positive controls containing 2.34×10^4, 1×10^4, 1×10^3 and 1×10^2copies. Linear regression of this data produced a slope of –4.24 and a correlation coefficient (R^2) of 0.96 (data not shown).

Three replicates of test article RNA (0.6µg in total) extracted from test article were assayed directly, with no further treatment. Three replicates of test article RNA (0.6µg in total) were spiked with 1000 copies of HCV positive control to assess for any test article related inhibitory factors to HCV RT-QPCR assay.

Within the sensitivity of the assay, the test article was negative for the presence of HCV RNA sequences. The three spiked test article replicates had a mean C_T value of 32.74 (Table 3). A spiked sample with a C_T value 3.3 cycles or greater than the mean C_T value of the mean of the 1000 copy standards would be considered partially inhibitory. Therefore if the spiked sample had a C_T value greater than or equal to 35.98 (32.68 + 3.3 = 35.98) it would have been considered partially inhibitory. A C_T value of undetermined in the spiked test article replicates would have been considered totally inhibitory. Therefore, no total or partial inhibition was detected in the RT-QPCR assay, demonstrating that the negative results generated with the test article RNA were valid.

In addition, the TaqMan® exogenous internal positive control (IPC) reagents included in all RT-QPCR replicates established that all negative PCR results were truly negative and not due to failed amplification of the RT-QPCR assay. Furthermore, the data confirmed that no factors inhibitory to RT-QPCR were either present in the RNA, or were at levels high enough to be significant.

CONCLUSIONS

Within the limits of assay sensitivity, the test article DNA and RNA analysed at 0.1µg per reaction (PCR assays) and <1.0µg per reaction (QPCR & RT-QPCR assays) was determined to be negative for the presence of HIV-1, HIV-2, HTLV-2, EBV, HHV-7, HHV-8, HBV, B19 and HCV sequences, following PCR, QPCR and RT-QPCR analysis. Additional PCR products amplified from the test article DNA in the HHV-8, HTLV-2, HIV-1 and EBV PCR assays were deemed to be non-specific.

The inclusion of positive control spiked reactions in each PCR, QPCR and RT-QPCR validated the negative results generated.

In summary, within the sensitivity of the assays performed the test article (H9-MCB.1) was determined to be negative for the presence of HIV-1, HIV-2, HTLV-2, EBV, HHV-7, HHV-8, HBV, B19 and HCV viral specific sequences.

TABLES

Table 1: Results Summary for Test Article: H9-MCB.1

Virus Target (0.1µg DNA Tested)	PCR Result			Southern Blot Result			Final Result
	Assay Sensitivity (per 5µl)	Unspiked	Spiked	Assay Sensitivity (per 5µl)	Unspiked	Spiked	
HIV-1	100 pg	4N*, 0P	2P*	NA	NA	NA	Negative[3]
HIV-2	1 pg	4N, 0P	2P	NA	NA	NA	Negative
HTLV-2	100 pg	4N*, 0P	2P*	NA	NA	NA	Negative[3]
EBV	Pos b (1 in 100)	4N*, 0P	2P	NA	NA	NA	Negative
HHV-7	10 vp	4N, 0P	2P	NA	NA	NA	Negative[3]
HHV-8	50 pg	4N*, 0P	2P	NA	NA	NA	Negative
HBV	100 copies	4N, 0P	2P	NA	NA	NA	Negative
HCV (0.2µg/reaction)[1]	100 copies	3N, 0P	3P	NA	NA	NA	Negative
B19 (0.80µg/reaction)[2]	100 copies	3N, 0P	3P	NA	NA	NA	Negative

N = Negative.
P = Positive.
vp = Virus particles.
* = Non-specific PCR product(s) present.
pg = Picograms.
NA = Not applicable.

[1] = RT-QPCR assay.
[2] = QPCR assay.
[3] = Two positive control dilutions containing the highest levels of positive control were detected. This meets the PCR acceptance criteria.

Table 2: Results of B19 QPCR

Sample	C_T Value	Mean C_T Value	Copies/ Reaction	Mean Copies/Reaction
Sentinel Control	U	U	0	0
	U		0	
	U		0	
Blank Water Control	U	U	0	0
	U		0	
	U		0	
Negative Control (MRC-5 cell DNA)	U	U	0	0
	U		0	
	U		0	
Extraction Control 070703	U	U	0	0
	U		0	
	U		0	
Test Article: H9-MCB.1	U	U	0	0
	U		0	
	U		0	
Spiked Test Article: H9-MCB.1. – Spiked with Positive Control (1000 copies)	33.372948	33.130542	849.60090	1003.11334
	33.092247		1021.23840	
	32.926430		1138.50070	
Positive Control: 1 x 10^7 copies	19.734076	18.868398	1 x 10^7	1 x 10^7
	19.366602		1 x 10^7	
	17.504517		1 x 10^7	
Positive Control: 1 x 10^6 copies	23.66314	23.240631	1 x 10^6	1 x 10^6
	23.64256		1 x 10^6	
	22.416193		1 x 10^6	
Positive Control: 1 x 10^5 copies	27.062052	26.144311	1 x 10^5	1 x 10^5
	26.289484		1 x 10^5	
	25.081396		1 x 10^5	
Positive Control: 1 x 10^4 copies	29.486250	29.025994	1 x 10^4	1 x 10^4
	29.293701		1 x 10^4	
	28.298030		1 x 10^4	
Positive Control: 1 x 10^3 copies	32.995323	32.570467	1 x 10^3	1 x 10^3
	32.424877		1 x 10^3	
	32.291200		1 x 10^3	
Positive Control: 1 x 10^2 copies	38.182602	37.282490	1 x 10^2	1 x 10^2
	37.630493		1 x 10^2	
	36.034374		1 x 10^2	

U = Undetermined.

Partial inhibition: Spiked sample with a C_T value equal to or greater than the mean C_T value of 1000 copies + 3.3 = 32.57 + 3.3 = 35.87. Therefore, the test article is not partially or totally inhibitory.

Table 3: Results of HCV RT-QPCR

Sample	C_T Value	Mean C_T Value	Copies/ Reaction	Mean Copies/Reaction
Sentinel Control	U	U	0	0
	U		0	
	U		0	
Blank Water Control	U	U	0	0
	U		0	
	U		0	
Negative Control (MRC-5 Cell RNA)	U	U	0	0
	U		0	
	U		0	
Extraction Control 070716	U	U	0	0
	U		0	
	U		0	
Test Article: H9-MCB.1	U	U	0	0
	U		0	
	U		0	
Spiked Test Article: H9-MCB.1. – Spiked with Positive Control (1000 copies)	33.276516	32.736638	1214.8655	1680.6198
	32.777540		1593.2953	
	32.155857		2233.6985	
Standard: 2.34×10^4	28.571940	28.450141	2.34×10^4	2.34×10^4
	28.468647		2.34×10^4	
	28.309837		2.34×10^4	
Standard: 1×10^4	29.482330	29.145277	1×10^4	1×10^4
	28.994808		1×10^4	
	28.958694		1×10^4	
Standard: 1×10^3	32.717216	32.678206	1×10^3	1×10^3
	32.680336		1×10^3	
	32.637066		1×10^3	
Standard: 1×10^2	U*	38.759730	1×10^2	1×10^2
	38.795590		1×10^2	
	38.723870		1×10^2	

U = Undetermined.
* = Omitted from calculations as undetermined.

Partial inhibition: Spiked sample with a C_T value equal to or greater than the mean C_T value of 1000 copies + 3.3 = 32.68 + 3.3 = 35.98. Therefore, the test article is not inhibitory.

APPENDIX

Minor Deviations from the Definitive Protocol

1. On page 7 of the Definitive Protocol for the RNA extraction it states that the test article will be resuspended in 20µl of DNase, RNase none detected 0.1µm filtered water. However, the test article was resuspended in 30µl of DNase, RNase none detected 0.1µm filtered water in order to ensure that there was sufficient volume for spectrophotometry and RT-PCR analysis. This minor deviation is deemed not to have affected the integrity or outcome of the study.

2. On page 7 of the Definitive Protocol for the RNA extraction it states that the pellets will be washed with 1ml of 75% (v/v) ethanol. However, the pellets were washed with 1ml of 76% (v/v) ethanol in error. This minor deviation is deemed not to have affected the integrity or outcome of the study.

3. On page 14 of the Definitive Protocol for the EBV PCR it states that the spike will be 0.1pg and the positive control dilutions (approximate range) will be 0.01, 0.1 and 1pg. However, this should have read 1 in 10 for the spike and 1 in 100, 1 in 10 and neat EBV for the positive control dilutions (approximate range). This deviation is deemed not to have affected the integrity or outcome of the study.

4. On page 11 of the Definitive Protocol for the B19 QPCR it states 'Three replicates of 1µg of DNA will be assayed directly, with no further treatment and three replicates of 1µg of DNA will be spiked (1000 copies) to assess for inhibition. Where 1µg of DNA is unavailable, neat aliquots of the test article sample will be assayed. The extraction control will also be included in triplicate in QPCR.' The DNA was diluted to a concentration of 0.2µg/µl instead of 0.25µg/µl and therefore 0.8µg of DNA was tested spiked and un-spiked in triplicate. This minor deviation is deemed not to have affected the integrity or outcome of the study.

5. On page 11 of the Definitive Protocol for the B19 QPCR the reaction conditions are incorrect and should have included a cycle of 50°C for 2 minutes. The B19 QPCR run included this cycle and therefore this minor deviation is deemed not to have affected the integrity or outcome of the study.

6. On page 13 of the Definitive Protocol for the HCV probe concentration it states 0.15µM this should read 0.1µM as in the table on page 9 for the HCV master mix. This minor deviation is deemed not to have affected the integrity or outcome of the study.

7. On page 9 of the Definitive Protocol for the HCV RT-QPCR it states 'Three replicates of 1µg of RNA will be assayed directly, with no further treatment and three replicates of 1µg of RNA will be spiked (e.g. 100pg) to assess for inhibition. If 1µg of RNA is unattainable, neat aliquots of the test article will be tested.' However, the DNA was diluted to a concentration of 0.2µg/µl instead of being used neat, therefore, 0.6µg of DNA was tested spiked and un-spiked in triplicate. This minor deviation is deemed not to have affected the integrity or outcome of the study.

8. MRC-5 cell RNA batch 050722 expired on 22/07/07, however, was used on 06/08/07. The RT-QPCR passed all the assay acceptance criteria and the assay was valid. Therefore, this minor deviation is deemed not to have affected the integrity or outcome of the study.

9. On page 7 of the Definitive Protocol the second paragraph of the RNA extraction section states that, 'Each positive control sample will be resuspended in 10.5µl DNase, RNase none detected 0.1µm filtered water'. This is a typographical error and should not have been included.

10. On page 10 of the Definitive Protocol it states: 'In addition, a second "master mix" will be prepared containing all the reagents used with the exception of Reverse Transcriptase (EuroScript RT), which will be replaced with water. The RT negative control will be analysed in triplicate and will control for the presence of contaminating DNA'. This second master mix was not prepared. This second master mix controls for the presence of contaminating DNA, however as the test article samples were shown to be negative using only the master mix containing Reverse Transcriptase (EuroScript RT), viral RNA and contaminating DNA was therefore not present in the test article. This minor deviation is deemed not to have affected the integrity or outcome of the study.

11. On page 5 of the Definitive Protocol, for the Preparation of Test Article for DNA Extraction, it states: 'The appropriate number of cells will be centrifuged at 300 x 'g' for approximately 5 minutes. The supernatant will be removed and discarded and cell pellet used directly'.

As the Sponsor provided the sample as a cell pellet it was not necessary to prepare cell pellets of the test article as detailed here. The cell pellets provided by the Sponsor were extracted directly as detailed in the DNA extraction section. This minor deviation is deemed not to have affected the integrity or outcome of the study.

12. On page 7 of the Definitive Protocol, for the Preparation of Test Article for RNA Extraction, it states: 'The appropriate number of cells will be centrifuged at 160 x 'g' for approximately 10 minutes at approximately 20°C. The supernatant will be removed and the cell pellet resuspended in 0.25ml of supernatant. The concentrated cell suspension will be used directly'. As the Sponsor provided the sample as a cell pellet it was not necessary to prepare cell pellets of the test article as detailed here. The cell pellets provided by the Sponsor were extracted directly as detailed in the RNA Extraction section. This minor deviation is deemed not to have affected the integrity or outcome of the study.

ANNEX

The Annex consists of 32 pages, including this one, and includes:

- Definitive Protocol (28 pages)
- Protocol Amendment 1 (3 pages)

Definitive Protocol

Title	H9-MCB.1: Detection of HIV-1, HIV-2, HTLV-1, HTLV-2, HCMV, EBV, HHV-7, HHV-8 and HBV Sequences using the Polymerase Chain Reaction (PCR) Technique. Detection and Quantification of HHV-6 and B19 Sequences using the Quantitative Polymerase Chain Reaction Technique (QPCR). Detection and Quantification of HCV Sequences using the Reverse Transcriptase Quantitative Polymerase Chain Reaction Technique (RT-QPCR)
Study Director	
Testing Facility	Covance Laboratories Ltd.,
Study Monitors	
Sponsor	Waisman Clinical BioManufacturing Facility
Covance Study Number	2823/004
Page Number	1 of 28

COVANCE
THE DEVELOPMENT SERVICES COMPANY

INTRODUCTION

It is important to demonstrate that a Master Cell Bank stock is free from the presence of viruses. A number of viruses are of particular concern because they are latent or non-cultivable, and therefore broad-spectrum tissue culture based assays would not detect such viruses. The Polymerase Chain Reaction (PCR) technique is an *in vitro* method for the amplification of DNA molecules from low copy number target molecules. PCR is a highly specific technique as it relies upon the hybridisation of oligonucleotide primers to the target nucleic acid sequence of interest. The quantitative PCR assay allows highly sensitive and specific detection of DNA sequences, as well as offering the ability to quantify the target sequence. The ABI PRISM 7900™ is able to detect fluorescence during PCR. This allows the 'real-time' detection of PCR product accumulation via the hydrolysis of probes labelled with both a fluorescent reporter and a quencher. Quantitative data are derived from a determination of the cycle at which the amplification product signal crosses a pre-set detection threshold (threshold cycle, C_T value). This cycle number is proportional to the amount of starting material. Therefore, PCR and QPCR can be used to detect viral sequences in infected cell lines. Either PCR or QPCR assays will be performed for each virus of concern.

Following binding, internalisation and uncoating of the virion of retroviruses HIV-1 (*Human Immunodeficiency Virus-1*), HIV-2 (*Human Immunodeficiency Virus-2*), HTLV-1 (*Human T Cell Lymphotrophic Virus-1*) and HTLV-2 (*Human T Cell Lymphotrophic Virus-2*), the genomic RNA is reverse transcribed to yield double-stranded DNA copies which can integrate into the host cell genome. Therefore, the presence or absence of HIV-1, HIV-2, HTLV-1 and HTLV-2 can be determined by extracting DNA from the test article, followed by amplification of specific retroviral sequences using the PCR (Polymerase Chain Reaction) technique (Refs. 1, 2 and 3) with primers specific to the particular retroviral genome. The primers for the detection of HIV-2 are also capable of detecting SIV sequence.

EBV (*Epstein Barr Virus*), HCMV (*Human Cytomegalovirus*), HHV-6 (*Human Herpes Virus-6*), HHV-7 (*Human Herpes Virus-7*) and HHV-8 (*Human Herpes Virus-8*) are double-stranded DNA viruses, B19 (*Human Parvovirus*) is a single-stranded DNA virus and HBV (*Hepatitis B Virus*) is partially double stranded. Therefore, the presence or absence of these viruses can be determined by extracting DNA from the test article, followed by amplification of specific DNA viral sequences, using the PCR technique with primers specific to the relevant viral genome.

HCV (*Hepatitis C Virus*) is a single-stranded RNA virus. Therefore, its presence or absence in a test article can be determined by extracting RNA from the sample, followed by RT-QPCR (reverse transcriptase quantitative polymerase chain reaction). This involves the synthesis of first strand cDNA and subsequent amplification by QPCR and quantification using primers and probe specific to the HCV sequences.

OBJECTIVE

The objective of this study is to determine whether HIV-1, HIV-2, HTLV-1, HTLV-2, HCMV, EBV, HHV-6, HHV-7, HHV-8, HBV, HCV and B19 are present in the test article using the Polymerase Chain Reaction (PCR) technique, the Quantitative Polymerase Chain Reaction (QPCR) technique and the Reverse Transcriptase Quantitative Polymerase Chain Reaction (RT-QPCR) technique.

TEST ARTICLE

Identification: H9-MCB.1.

Source: WiCell Research Institute.

The exact details as presented on the test article vessel are: COVANCE 1 pellet @ $1x10^6$ cells Human Virus Panel MCB.A.H9p27. 22JAN07 DF.

The Sponsor provided the following details on the test article by completing a Test Article Safety & Pre-Study Questionnaire:

Appearance: Cell pellet
Concentration: $1x10^6$ cells
Vial size: 1.5mL
Cell line information: Human Embryonic Stem Cells
Storage temperature: -70°C
Expiry date: N/A
Unused test article to be disposed of by incineration.
The test article does not contain chemicals which may be hazardous.

This study, for the presence of extraneous agents will be conducted to define the purity of the test article. 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 that may be present in the test materials.

PCR AND QPCR TEST SYSTEMS

Controls

Positive controls:	DNA extracted from HIV-1, DNA extracted from HIV-2, DNA extracted from HTLV-1, DNA extracted from HTLV-2, DNA extracted from EBV, DNA extracted from HCMV, DNA extracted from HHV-7, DNA extracted from HHV-8.
Source:	Advanced Biotechnologies Inc.
Positive control:	B19 DNA synthetic oligonucleotide, HHV-6 DNA synthetic oligonucleotide.
Source:	Eurogentec.
Positive control:	HBV, full length genome in pEco63, extracted DNA.
Source	American Type Tissue Culture Collection.
Positive control:	Armored RNA® HCV (Genotype 1a) in TSM III buffer.
Source	Ambion Diagnostics.
Negative control:	DNA extracted from uninfected (i.e. HIV-1, HIV-2, HTLV-1, HTLV-2, HCMV, EBV, HHV-6, HHV-7, HHV-8, HBV and B19) cells. RNA extracted from uninfected (i.e., HCV negative) cells.
Source:	Prepared in-house from MRC-5 or MDBK cells, as documented in laboratory records.

Blank, sentinel and RNA extraction control:

Purified water (DNase, RNase none detected, 0.1µm filtered).

Source: Sigma-Aldrich Company Ltd.

DNA extraction control: Dulbecco's Phosphate Buffered Saline (DPBS).

Source: Invitrogen Ltd.

PROCEDURES

All items (e.g. tubes, plates, containers) holding test article material will be appropriately and uniquely identifiable.

Due to the extreme sensitivity of PCR and therefore, the technical problems associated with this technique, each key step will be carried out in a separate air space. Furthermore, where appropriate, the recommended precautions (Refs. 4 and 5) will be adhered to.

Preparation of Test Article for DNA Extraction

A maximum of $5x10^6$ cells can be extracted per column using the DNeasy™ Tissue and Blood Kit. Total cell count (not viable cell count) is required, therefore no trypan blue should be added. The appropriate number of cells will be centrifuged at 300 x 'g' for approximately 5 minutes. The supernatant will be removed and discarded and cell pellet used directly.

DNA Extraction

If necessary, multiple aliquots of each test article will be extracted. The test article cell pellets (maximum of $5x10^6$ cells per column) will be resuspended in DPBS. DNA will be extracted from test article cells using Qiagen DNeasy™ Tissue and Blood kit. Proteinase K will be added to each sample (20µl) followed by 200µl of Buffer AL and each sample then mixed by vortexing for 15 seconds. The samples will be incubated in a waterbath at 70 ± 1°C for 10 minutes. Following incubation, the samples will be briefly centrifuged to remove drops from the inside of the lid and 200µl of 100% (v/v) ethanol will be added to each sample and vortexed for 15 seconds. The samples will be briefly centrifuged to remove drops from the inside of

the lid. Each sample (approximately 620µl) will then be pipetted onto a Qiagen DNeasy™ column and centrifuged at 6 000 x 'g' for 1 minute at room temperature. On any occasion where the supernatant does not completely pass through the column, the column will be respun at 12 000 x 'g' for 3 minutes at room temperature. This step will be repeated if the filtrate does not completely pass through the column. The filtrate will be discarded and 500µl of buffer AW1 then applied to each Qiagen DNeasy™ column. The columns will then be centrifuged at 6 000 x 'g' for 1 minute at room temperature. The filtrate will be discarded and 500µl of buffer AW2 then applied to each Qiagen DNeasy™ column. The columns will be centrifuged at 20 000 x 'g' for 3 minutes at room temperature. The filtrate will be discarded and the columns centrifuged at 20 000 x 'g' for 1 minute at room temperature to eliminate possible buffer AW2 carryover. The filtrate will again be discarded and 100µl of buffer AE applied to each Qiagen DNeasy™ column. The columns are centrifuged at 6 000 x 'g' for 1 minute at room temperature to elute the DNA. If necessary, the DNA from multiple aliquots of the test article extracted may be pooled.

An extraction control (200µl of DPBS) will be included in the extraction. The extraction control will be treated in the same manner as the test article and functions as a control for cross-contamination during the extraction process.

The eluted DNA will be stored at -20°C or below until required for analysis. Following isolation the DNA concentration of the test article samples will be determined spectrophotometrically and diluted with sterile, purified water to a working concentration (e.g. 0.02µg/µl for PCR and 0.2µg/µl for QPCR).

Determination of the DNA Concentration of Samples

The concentration of the test article stock DNA will be determined spectrophotometrically by taking optical density (OD) readings in triplicate at 260nm (1 OD_{260} = 50µg/ml). OD readings at 280nm will be also recorded and the purity of each DNA sample estimated by calculation of the OD260:280nm ratio. Finally, an aliquot of each DNA sample will be diluted with purified water to a working concentration, e.g. 0.02µg/µl for PCR and 0.2µg/µl for QPCR (if concentration is below these values, samples will be analysed neat and this will be recorded into the data report). Samples will be stored frozen at approximately -20°C until required for further use.

Ideally, the DNA should have an OD260:280nm value of 1.6 to 1.9 for PCR analysis. However, samples in the range of 1.0 to 2.5 will be accepted for PCR analysis. If a sample has a ratio outside the range of 1.6 to 1.9 and is shown to be inhibitory, the Sponsor will be contacted with the results prior to proceeding with further extraction.

Preparation of Test Article for RNA Extraction

Between $5x10^6$ and $1x10^7$ cells can be extracted per 0.75ml of TRIzol reagent. Total cell count (not viable cell count) is required, therefore no trypan blue should be added. The appropriate number of cells will be centrifuged at 160 x 'g' for approximately 10 minutes at approximately 20°C. The supernatant will be removed and the cell pellet resuspended in 0.25ml of supernatant. The concentrated cell suspension will be used directly.

RNA Extraction

RNA will be extracted using TRIzol LS Reagent. Aliquots (250µl) of the test article samples and an extraction control (250µl of DNase, RNase none detected 0.1µm filtered water) will be included in each extraction. The extraction control will be treated in the same manner as the test samples and functions as a control for cross-contamination during the extraction process.

TRIzol LS Reagent will then be added to each sample in a 3:1 ratio (i.e. 750µl for every 250µl of sample), vortexed for 15 seconds and incubated at room temperature for 10 minutes. Following incubation samples will be centrifuged, for 15 seconds on pulse at approximately 4°C, to collect the contents and 200µl of chloroform: isoamyl alcohol (24:1) will then be added to each sample and vortexed for 15 seconds. The phases will be separated by centrifugation at 21 000 x 'g' for 15 minutes at approximately 4°C (a slow deceleration will be used to avoid the mixing of the phases). The upper aqueous phase (400µl) of each sample will then be transferred to an appropriately labelled sterile tube and 500µl of isopropanol containing 40µg of glycogen added. The samples will be mixed by vortexing for 5 seconds and then placed in the freezer at approximately -20°C overnight. The samples will be removed from the freezer the following day and the RNA pelleted by centrifugation at 21 000 x 'g' for 15 minutes at approximately 4°C. The supernatant will then be removed and the pellets washed with 1ml of 75% (v/v) ethanol. Each sample will be mixed by vortexing and the RNA collected by centrifugation at 21 000 x 'g' for 5 minutes at approximately 4°C. The supernatant will then be removed and each sample re-spun for 10 seconds at 21 000 x 'g' at approximately 4°C and any remaining ethanol wash removed. Each sample will be resuspended in DNase, RNase, none detected 0.1µm filtered water as follows. Each positive control sample will be resuspended in 10.5µl DNase, RNase, none detected 0.1µm filtered water; the test article will be resuspended in approximately 20µl DNase, RNase, none detected 0.1µm filtered water (to ensure sufficient volume for spectrophotometric and RT-PCR analysis); the extraction control will be resuspended in 40µl DNase, RNase, none detected 0.1µm filtered water (to ensure sufficient volume for RT-PCR analysis). If

necessary, the RNA from the multiple aliquots of the test article extracted, may be pooled.

Following extraction, the RNA will be stored frozen at approximately -70°C until required for analysis. The RNA concentration of the test article will be determined spectrophotometrically and diluted with sterile, purified water to a working concentration (e.g. 0.2µg/µl).

Determination of the RNA Concentration of Samples

The concentration of the test article stock RNA will be determined spectrophotometrically by taking optical density (OD) readings in triplicate at 260nm (1 OD_{260} = 40µg/ml). OD readings at 280nm will be also recorded and the purity of each RNA sample estimated by calculation of the OD260:280nm ratio. Finally, an aliquot of each RNA sample will be diluted with purified water to a working concentration, e.g. 0.2µg/µl for RT-QPCR (if concentration is below these values, samples will be analysed neat and this will be recorded into the data report). Samples will be stored frozen at approximately -20°C until required for further use.

Ideally, the RNA should have an OD260:280nm value of 1.65 to 2.1 for PCR analysis. However, samples in the range of 1.4 to 2.1 will be accepted for PCR analysis. If a sample has a ratio outside the range of 1.65 to 2.1 and is shown to be inhibitory, the Sponsor will be contacted with the results prior to proceeding with further extraction.

PCR Amplification

A "master mix" of all the reagents used in each PCR will be prepared and aliquoted in to the required number of appropriately labelled tubes. The concentration of each reagent in the final reaction mixture will be as follows:

1 x PCR buffer	50 mM KCl, 10 mM Tris pH 8.3
dNTPs	200 µM each
$MgCl_2$	as optimised for each primer pair, 1.5 - 3.5 mM
Primers	0.03 - 0.3 µM each
Amplitaq Gold DNA polymerase	1.25 - 2.5 units

Refer to primers section for precise reaction component concentrations.

Aliquots (5µl) of the extraction controls and test article DNA sample will then be added to each appropriately labelled reaction tube and the tubes closed. The test article DNA sample will be analysed once in quadruplicate (i.e. 4 replicates of 0.1 µg DNA) in each PCR assay.

Reverse Transcriptase (RT)-QPCR Amplification

HCV RT-QPCR

A "1.25 x master mix" containing sufficient reagents for the number of reactions will be prepared, each reaction to contain a total volume of 20µl as detailed in the following table.

For each well, 17.6µl (16.0µl + 10%) of 1.25 x master-mix will be prepared (10% of this volume is added on to compensate for any pipette variation). Volumes of 52.8µl (3 x 17.6µl) of the master mix will be aliquotted into appropriately labelled tubes for each triplicate sample to be tested.

A total volume of 13.2µl ((4.0µl + 10%) x 3 replicates) of water, sample, spiked sample, and positive control will be added to each tube containing the master mix and vortexed. Total volumes of 20µl of master mix and sample/control will be aliquotted into three wells in the 384-well plate.

	Volume per reaction (µl)	Concentration in master mix	Final Concentration [3]
Water	[1]	N/A	N/A
2 x reaction buffer [2]	11.0	1.25 x reaction	1 x reaction
200 x EuroScript RT (50 Units/µl EuroScript RT and 20 Units/µl)	0.11	1.25 x EuroScript RT	1 x EuroScript RT (0.25 Units/ml EuroScript RT and 0.1 Units/ml)
10 x IPC Mix [4]	2.2	1.25 x IPC Mix	1 x IPC Mix
50 x IPC DNA [4]	0.44	1.25 x IPC DNA	1 x IPC DNA
20 µM Primers	0.33	375 nM of each primer	300 nM of each primer
5 µM Probe	0.44	125 nM	100 nM

[1] The amount of water added will bring the total volume per reaction to 20µl after the addition of sample.
[2] Reaction buffer contains HotGoldStar, dNTP's, $MgCl_2$, ROX™ passive reference and stabilisers (proprietary formulation).
[3] The final concentration is based on a total volume of 66 µl of reaction buffer plus water/sample prepared and then 20µl of this used for each of the three replicates to be tested.
[4] TaqMan® exogenous internal positive control (IPC) reagents.
N/A = Not applicable.

Three replicates of 1µg of RNA will be assayed directly, with no further treatment and three replicates of 1µg of RNA will be spiked (e.g. 100pg) to assess for inhibition. If 1µg of RNA is unattainable, neat aliquots of the test article will be tested.

The extraction control will also be included in triplicate. In addition, a second "master mix" will be prepared containing all the reagents used with the exception of Reverse Transcriptase (EuroScript RT), which will be replaced with water. The RT negative control will be analysed in triplicate and will control for the presence of contaminating DNA.

Once the reactions are prepared and capped, and placed in the ABI PRISM 7900™ sequence detection system. Amplification will then be performed using the following reaction conditions:

One cycle of:

48°C for 30 minutes
95°C for 10 minutes

Then 40 cycles of:

95°C for 15 seconds
60°C for 1 minute

A computer, attached to the sequence detection system, will collect the fluorescence data generated during amplification. Data analysis will also be performed using this computer including generation of quantification data for the test article samples. The data produced will be held both electronically and as a printed hard copy. Electronic data will be backed up onto the Covance Network.

QPCR Amplification

B19 QPCR

A "1.25 x master mix" containing sufficient reagents for the number of reactions will be prepared, each reaction to contain a total volume of 20µl as detailed in the following table.

For each well, 17.6µl (16.0µl + 10%) of 1.25 x master-mix will be prepared (10% of this volume is added on to compensate for any pipette variation). Volumes of 52.8µl (3 x 17.6µl) of the master mix will be aliquotted into appropriately labelled tubes for each triplicate sample to be tested.

A total volume of 13.2µl ((4.0µl + 10%) x 3 replicates) of water, sample, spiked sample, and positive control will be added to each tube containing the master mix and vortexed. Total volumes of 20µl of master mix and sample/control will be aliquotted into three wells in the 384-well plate.

	Volume per reaction (µl)	Concentration 1.25x Master Mix	Final Concentration [3]
2x Reaction buffer [2]	11.00	1.25x	1x
Forward primer 20µM	0.33	375 nM	300nM
Reverse primer 20µM	0.33	375 nM	300nM
TaqMan Probe 5 µM	0.44	125 nM	100nM
10 x IPC Mix [4]	2.20	1.25x	1x
50 x IPC DNA [4]	0.44	1.25x	1x
RNase free water [3]	[1]	N/A	[3]

[1] The amount of water added will bring the total volume per reaction to 20µl after the addition of sample.
[2] Reaction buffer contains HotGoldStar, dNTP's, $MgCl_2$, ROX™ passive reference and stabilisers (proprietary formulation).
[3] The final concentration is based on a total volume of 66 µl of reaction buffer plus water/sample prepared and then 20µl of this used for each of the three replicates to be tested.
[4] TaqMan® exogenous internal positive control (IPC) reagents.
N/A = Not applicable.

Three replicates of 1µg of DNA will be assayed directly, with no further treatment and three replicates of 1µg of DNA will be spiked (1000 copies) to assess for inhibition. Where 1µg of DNA is unavailable, neat aliquots of the test article sample will be assayed. The extraction control will also be included in triplicate in QPCR.

Once the reactions are prepared and capped, and placed in the ABI PRISM 7900™ sequence detection system. Amplification will then be performed using the following reaction conditions:

One cycle of:

95°C for 10 minutes

Then 40 cycles of:

95°C for 15 seconds
60°C for 1 minute

A computer, attached to the sequence detection system, will collect the fluorescence data generated during amplification. Data analysis will also be performed using this computer including generation of quantification data for the test article samples. The data produced will be held both electronically and as a printed hard copy. Electronic data will be backed up onto the Covance Network.

HHV-6 QPCR

A master mix containing sufficient reagents for the number of reactions will be prepared as detailed in the following table.

	Volume per reaction (25µl) *	Final concentration
Water [2]		
2x Universal Master Mix [1]	12.5	1x
20µM Primers	0.375	1x (300nM of each)
5µM Probe	0.75	1x (150nM)
10 x IPC Mix [2]	2.5	1x
50 x IPC DNA [2]	0.5	1x

* The total volume per reaction will be modified when spike is added to the sample, however this would not affect the outcome of the results.

[1] Universal master mix contains AmpliTaq Gold® DNA polymerase, AmpErase UNG, dNTP's (with dUTP), passive reference 1 (ROX™) and pre-optimised buffer components (proprietary formulation).

[2] The amount of water added will bring the total volume per reaction to 25µl after the addition of sample.

Three replicates of 1µg of DNA will be assayed directly, with no further treatment and three replicates of 1µg of DNA will be spiked (1000 copies) to assess for inhibition. Where 1µg of DNA is unavailable, neat aliquots of the test article sample will be assayed. The extraction control will also be included in triplicate in QPCR.

Once the reactions are prepared and capped, and placed in the ABI PRISM 7900™ sequence detection system. Amplification will then be performed using the following reaction conditions:

One cycle of:

95°C for 10 minutes

Then 40 cycles of:

95°C for 15 seconds
60°C for 1 minute

A computer, attached to the sequence detection system, will collect the fluorescence data generated during amplification. Data analysis will also be performed using this computer including generation of quantification data for the test article samples. The data produced will be held both electronically and as a printed hard copy. Electronic data will be backed up onto the Covance Network.

PCR Primers

The following primers targeted to the specified viral genomes will be used:

Virus target	Gene/ sequence	Primer 1 (5')	Primer 2 (3')	$MgCl_2$ conc. (mM)	Primer conc. (µM)	AmpliTaq Gold (units)
HIV-1/O	LTR	HIV-1 LTR OS	HIV-1 LTR AO	1.5	0.075	1.25
HIV-1/O (nested)	LTR	HIV-1 LTR IS	HIV-1 LTR AI	1.5	0.3	1.25
HIV-2/SIV	LTR	HIV-2 LTR OS	HIV-2 LTR AO	2	0.03	1.25
HIV-2/SIV (nested)	LTR	HIV-2 LTR IS	HIV-2 LTR AI	2	0.3	1.25
HTLV-1	POL	HTLV-1 POL 1	HTLV-1 POL 2	2	0.3	1.25
HTLV-2	POL	HTLV-1 POL 1	HTLV-1 POL 2	2	0.3	1.25
HCMV	glycoprotein	HCMV gB 1	HCMV gB 2	1.5	0.3	1.25
EBV	EBNA-1	EBV BKRF 1	EBV BKRF 2	2	0.3	1.25
HHV-7	43L3a	HHV-7 1	HHV-7 2	1.5	0.3	1.25
HHV-8	OFR 26	KS330 233A	KS330 233E	2.5	0.3	1.25
HBV	Pre-core/core	HBV CC 1	HBV CC 2	1.5	0.3	1.25

RT-QPCR and QPCR Primers and Probe

The following primers targeted to the specified viral genomes will be used:

Virus target	Gene/ sequence	Primer 1 (5')	Primer 2 (3')	Probe	Primer conc. (µM)	Probe conc. (µM)
B19	Accession number DQ357065	B19F	B19R	B19T	0.3	0.1
HHV-6	Accession number AF157706.1	HHV-6B U67.txt-257F	HHV-6B U67.txt-322R	HHV-6B U67.txt-277T	0.3	0.1
HCV	5' NCR	HCV 146F	HCV 232R	HCV 166T	0.3	0.15

The probe T is labelled with the fluorescent dye FAM at the 5'end and with the fluorescence quencher TAMRA at the 3' end. The fluorescent dye FAM is released from its quencher by *Taq* polymerase provided the probe is bound to the PCR template during primer extension.

PCR Controls

A pre-determined range of specific positive control will be included in each PCR assay to monitor for assay sensitivity and specificity. Furthermore, a pre-determined amount of each positive control will be used to spike duplicate aliquots of the test article DNA or RNA sample prior to each PCR assay. These sample specific spiked controls are used to control against the presence of inhibitors to PCR in the individual test article DNA or RNA sample, thus validating any negative test result and preventing false negative results.

Virus target	Spike	Positive control dilutions (approximate range)
HIV-1/O	100 pg	10, 100, 1000 pg
HIV-2/SIV	1 pg	1, 10, 100 pg
HTLV-1	10 pg	10, 100, 1000 pg
HTLV-2	10 pg	10, 100, 1000 pg
HCMV	0.1 vp	0.1, 1, 10 vp
EBV	0.1 pg	0.01, 0.1, 1 pg
HHV-7	10 vp	1, 10, 100 vp
HHV-8	100 pg	50, 100, 1000 pg
HBV	100 copies	100, 1000, 10 000 copies

Duplicate blank reactions, i.e. using water as the template will be used as assay blank controls in each PCR run. One blank reaction tube remains open throughout the assay preparation steps until initiation of PCR, functioning as a sentinel control to monitor for air-borne contamination. A negative control i.e. using DNA which does not contain the viral sequences of interest, will be included in each PCR run to monitor for specificity of each set of virus-specific primers.

B19 QPCR Controls

The positive control will be serially diluted in TE Buffer and aliquots containing a range of DNA included in triplicate (e.g. 10, 100, 1000, 10 000, $1x10^5$, $1x10^6$, and $1x10^7$ copies per reaction), ensuring comparability between QPCR runs. The data generated from this dilution series will be used to construct a standard curve and generate quantitative data. Six blank reactions using water as the template will also be used as negative controls. Three wells will remain open throughout the assay preparation steps until initiation of QPCR. These wells function as sentinel controls by monitoring for airborne contamination. The second set of three wells function as assay blank controls to monitor for reagent contamination. A negative control DNA sample will be included in triplicate in the assay to ensure specificity of the QPCR primers.

HHV-6 QPCR Controls

The positive control will be included and will containing a range of DNA (e.g. $1x10^7$ - $1x10^2$ copies/µl), ensuring comparability between QPCR runs. The data generated from this dilution series will be used to construct a standard curve and generate quantitative data. Four blank reactions using water as the template will also be used as negative controls. Two wells will remain open throughout the assay preparation steps until initiation of QPCR. These wells function as sentinel controls by monitoring for airborne contamination. The second set of wells function as assay blank controls to monitor for reagent contamination. A negative control DNA sample will be included in triplicate in the assay to ensure specificity.

HCV RT-QPCR Controls

The positive control will be serially diluted in purified water and aliquots containing a range of DNA included (e.g. 100, 1000, 10 000 and $2.34x10^4$ pg per reaction), ensuring comparability between RT-QPCR runs. The data generated from this dilution series will be used to construct a standard curve and generate quantitative data. Six blank reactions using water as the template will also be used as negative controls. Three wells will remain open throughout the assay preparation steps until initiation of RT-QPCR. These wells function as sentinel controls by monitoring for airborne contamination. The second set of three wells function as assay blank controls to monitor for reagent contamination. A negative control RNA sample will be included in triplicate in the assay to ensure specificity of the RT-QPCR primers.

PCR Thermal Profiles

Each set of prepared reactions will be incubated in a thermal cycler using the relevant profile as follows:

HIV-1 LTR AO/ HIV-1 LTR OS primers

cycle 1	10* min, 95°C	0.5 min, 50°C	0.5 min, 72°C
cycles 2 - 29	0.5 min, 95°C	0.5 min, 50°C	0.5 min, 72°C
cycle 30	0.5 min, 95°C	0.5 min, 50°C	10 min, 72°C

HIV-1 LTR AI/ HIV-1 LTR IS primers

cycle 1	10* min, 95°C	0.5 min, 50°C	0.5 min, 72°C
cycles 2 - 34	0.5 min, 95°C	0.5 min, 50°C	0.5 min, 72°C
cycle 35	0.5 min, 95°C	0.5 min, 50°C	10 min, 72°C

HIV-2 LTR AO/ HIV-2 LTR OS primers

cycle 1	10* min, 95°C	0.5 min, 55°C	0.5 min, 72°C
cycles 2 - 14	0.5 min, 95°C	0.5 min, 55°C	0.5 min, 72°C
cycle 15	0.5 min, 95°C	0.5 min, 55°C	10 min, 72°C

HIV-2 LTR AI/ HIV-2 LTR IS primers

cycle 1	10* min, 95°C	0.5 min, 60°C	15 sec, 72°C
cycles 2 - 34	0.5 min, 92°C	0.5 min, 60°C	15 sec, 72°C
cycle 35	0.5 min, 92°C	0.5 min, 60°C	10 min, 72°C

HTLV-1 Pol 1/HTLV-1 Pol 2 primers (for detection of HTLV-1 and HTLV-2)

cycle 1	10* min, 95°C	0.5 min, 60°C	0.5 min, 72°C
cycles 2 - 39	0.5 min, 95°C	0.5 min, 60°C	0.5 min, 72°C
cycle 40	0.5 min, 95°C	0.5 min, 60°C	10 min, 72°C

HCMV gB1/HCMV gB 2 primers

cycle 1	10* min, 95°C	0.5 min, 65°C	0.5 min, 72°C
cycles 2 - 39	0.5 min, 95°C	0.5 min, 65°C	0.5 min, 72°C
cycle 40	0.5 min, 95°C	0.5 min, 65°C	10 min, 72°C

EBV BKRF1/EBV BKRF2, HHV-7 1/HHV-7 2 and HBV CC 1/ HBV CC 2, primers

cycle 1	10* min, 95°C	0.5 min, 55°C	0.5 min, 72°C
cycles 2 - 39	0.5 min, 95°C	0.5 min, 55°C	0.5 min, 72°C
cycle 40	0.5 min, 95°C	0.5 min, 55°C	10 min, 72°C

KS330 233A/KS330 233E primers

cycle 1	10* min, 95°C	0.5 min, 58°C	0.5 min, 72°C
cycles 2 - 39	0.5 min, 95°C	0.5 min, 58°C	0.5 min, 72°C
cycle 40	0.5 min, 95°C	0.5 min, 58°C	10 min, 72°C

* A 10 minute incubation will be used in the first PCR cycle to activate the endogenous chemical hot start activity of the DNA polymerase enzyme used.

Gel Electrophoresis

After amplification, an aliquot from each reaction tube will be removed and added to a portion of gel loading buffer. The samples, including an appropriate DNA size marker, are then electrophoresed on an agarose gel (e.g. 1.8% w/v), containing ethidium bromide, and photographed under UV illumination.

Southern Blot Hybridisation

If bands are observed that are similar in size to the expected positive bands, then southern blot hybridisation will be performed using a labelled virus specific probe to confirm the presence of potential contaminants. A digoxigenin labelled probe will be prepared using a DIG labelling mix in place of dNTPs in the PCR / RT-PCR reaction for amplification of the sequence from the positive control template and virus specific primers. PCR / RT-PCR reactions will be electrophoresed on an agarose gel along with DIG-labelled molecular weight size markers. The agarose gel will then be Southern blotted, i.e. denatured and nucleic acid fragments from the gel transferred to hybridisation membrane. The membrane will be baked to immobilise the DNA and then hybridised with the DIG labelled virus specific probe. The position of the hybridised probe is located on the membrane by chemiluminescent detection and will be visualised by autoradiography.

DATA ANALYSIS

PCR Assay Acceptance Criteria

The assay for the detection of each viral sequence will be considered acceptable if:

1) No viral specific amplicon is detected in the negative control, blank reaction or sentinel control following each PCR assay.

2) No viral specific amplicon is detected in the extraction control(s) following each PCR assay.

3) Viral specific amplicon is detected in at least the two positive control dilutions containing the highest levels of positive control, following each PCR.

4) Viral specific amplicon is detected in each spiked test article DNA or RNA sample following each PCR assay.

Preparation of DNA will be repeated if amplicon is detected in the extraction control(s) following PCR, suggesting a contamination during the extraction process.

A PCR assay will be repeated if viral specific amplicon is not detected in at least two of the positive control dilutions containing the highest levels of positive control; or if viral specific amplicon is detected in the blank, sentinel or negative control reactions, following each PCR.

A PCR assay will be repeated once only for any sample where one or both of the test article spiked samples are not amplified following PCR. If one or both of the test article spiked samples are not amplified following the repeat PCR assay then the sample will be deemed to be inhibitory to the PCR assay. Samples that are deemed inhibitory will be re-analysed by PCR at e.g. 1:10 dilution in an attempt to remove the inhibition (unless otherwise requested by the Sponsor). If, following the repeat PCR analysis, the samples remain inhibitory, but are within the specified range for OD 260:280nm the samples will be described as inhibitory. If, following the repeat QPCR analysis, the samples remain inhibitory, but are outside of the specified range for OD 260:280nm then the Sponsor will be contacted with the results prior to proceeding with further extraction of these samples. Refer to the section entitled 'Determination of the DNA Concentration of Samples' for the specified range for OD 260:280nm.

PCR Evaluation Criteria

The assay for the detection of viral DNA is evaluated as follows:

1) By comparing the electrophoretic migration distances of the PCR product obtained using each positive control DNA with the migration distance of any PCR products obtained using the test article DNA sample, for each primer pair used.

   If, following PCR, none of the replicates of the test article DNA sample generate a PCR product which co-migrates with the positive control PCR product, then the test article will be considered negative for viral DNA.

   If, following PCR, one or more of the replicates of the test article DNA sample generate a PCR product which co-migrates with the positive control PCR product, then the test article will be considered positive for viral DNA.

2) By comparing the hybridisation signals obtained using the positive control DNA with any hybridisation signals obtained using the test article DNA.

   If following Southern blot hybridisation, no signal is observed in a sample which generated a weak positive product in PCR analysis, then the DNA sample will be confirmed as negative for the presence of viral DNA.

   If following Southern blot hybridisation, a signal is observed in any of the sample replicates, then the sample will be confirmed as positive for the presence of viral DNA.

QPCR Assay Acceptance Criteria

The assays for the detection of viral DNA by QPCR will be considered acceptable in each assay run if:

1. Amplification is detected in the appropriate positive control dilutions by QPCR.

2. No amplification is detected in the negative, extraction, water or sentinel controls by QPCR.

3. Linear regression analysis of the standard curve dilutions produces a slope of less than -2.2 and greater than -4.4 and a correlation coefficient (R^2) of greater than 0.9.

The QPCR analysis will be repeated if the assay acceptance criteria are not achieved.

QPCR Evaluation Criteria

The assay for the quantification of test article by QPCR will be evaluated as follows:

1) The samples will be judged positive for the test article if the level of fluorescence (e.g. from FAM) rises >10 standard deviations above background fluorescence, once, and the accumulation of fluorescence is indicative of exponential amplification.

2) By comparing the C_T values generated from the spiked test article samples with those generated from the spiked control material that forms the standard curve. Test article samples will be judged totally inhibitory if the spiked sample produced a C_T value of 40. Test article samples will be judged partially inhibitory if the spiked sample produced a C_T value, 3.3 cycles or more, greater than the C_T predicted from the standard curve.

   Following QPCR analysis, samples that are deemed inhibitory in the first QPCR analysis will be re-analysed by QPCR at 1:10 and 1:100 dilutions (e.g. where 1µg was used, 0.1 and 0.01 µg will be use in the repeat) in attempt to remove the inhibition (unless otherwise requested by the Sponsor). If, following the repeat QPCR analysis, the samples remain inhibitory, but are within the specified range for OD 260:280nm the samples will be described as inhibitory. If, following the repeat QPCR analysis, the samples remain inhibitory, but are outside of the specified range for OD 260:280nm then the Sponsor will be contacted with the results prior to proceeding with further extraction of these samples. Refer to the section entitled 'Determination of the DNA Concentration of Samples' for the specified range for OD 260:280nm. The details of this further analysis will be recorded in the raw data and reported.

   Any decision to carry out further testing will only be made by the Study Director in consultation with the Sponsor and will be fully documented. Such work may have cost implications.

RT-QPCR Assay Acceptance Criteria

The assays for the detection of test article/positive control by RT-QPCR will be considered acceptable in each assay run if:

4. Amplification is detected in the appropriate positive control dilutions by RT-QPCR.

5. No amplification is detected in the negative, RT negative, extraction, water or sentinel controls by RT-QPCR.

6. Linear regression analysis of the standard curve dilutions produces a slope of less than -2.2 and greater than -4.4 and a correlation coefficient (R^2) of greater than 0.9.

The RT-QPCR analysis will be repeated if the assay acceptance criteria are not achieved.

RT-QPCR Evaluation Criteria

The assay for the quantification of test article by RT-QPCR will be evaluated as follows:

1. The samples will be judged positive for the test article if the level of fluorescence (e.g. from FAM) rises >10 standard deviations above background fluorescence, once, and the accumulation of fluorescence is indicative of exponential amplification.

2. By comparing the C_T values generated from the spiked test article samples with those generated from the spiked control material that forms the standard curve. Test article samples will be judged totally inhibitory if the spiked sample produced a C_T value of 40. Test article samples will be judged partially inhibitory if the spiked sample produced a C_T value, 3.3 cycles or more, greater than the C_T predicted from the standard curve.

Following RT-QPCR analysis, samples that are deemed inhibitory in the first RT-QPCR analysis will be re-analysed by RT-QPCR at 1:10 and 1:100 dilutions (e.g. where 1µg was used, 0.1 and 0.01 µg will be use in the repeat) in attempt to remove the inhibition (unless otherwise requested by the Sponsor). If, following the repeat QPCR analysis, the samples remain inhibitory, but are within the specified range for OD 260:280nm the samples will be described as inhibitory. If, following the repeat QPCR analysis, the samples remain inhibitory, but are outside of the specified range for OD 260:280nm then the Sponsor will be contacted with the results prior to proceeding with further extraction of these samples. Refer to the section entitled 'Determination of the RNA Concentration of Samples' for the specified range for OD

260:280nm. The details of this further analysis will be recorded in the raw data and reported.

Any decision to carry out further testing will only be made by the Study Director in consultation with the Sponsor and will be fully documented. Such work may have cost implications.

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.

* with the exception of the plate reader Spectrafluor Plus and the XFLUOR (version: V 3.21) software which are not currently fully validated at Covance Harrogate.

* with the exception of the Applied Biosystems 7900HT Real Time PCR System and software. The machine has completed and passed the validation process, but is awaiting report finalisation. An internal risk assessment has been generated at Covance Harrogate detailing the acceptability of the system for use with this study.

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.

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.

REFERENCES

1. Saiki R K, Scharf S, Faloona F, Mullis K B, Horn G T, Erlich H A and Arnheim N (1985) Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anaemia. Science *230* 1350 - 1354.

2. Mullis K B and Faloona F (1987) Specific synthesis of DNA in vitro via a polymerase-catalysed chain reaction. Methods in Enzymol. *155* 335 - 350.

3. Saiki R K, Gelfand D H, Stoffel S, Scharf S J, Higuchi R, Horn G T, Mullis K G and Erlich H A (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science *239* 487 - 491.

4. Bootman J S and Kitchin P A (1992) An international collaborative study to assess a set of reference reagents for HIV-1 PCR. J. Virological Methods *37* 23 - 42.

5. Kwok S and Higuchi R (1989) Avoiding false positives with PCR. Nature *339* 237 - 238.

APPENDIX 1

Study Records

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

Definitive Protocol

Amendment(s)*

File note(s)*

Study related correspondence*

Test article description

Test article receipt and utilisation

Metrology#

Records for reagents and stock solutions#

Test article cell culture records*

Work sheets

* where appropriate
# some records held centrally

APPENDIX 2

Draft Report

The Draft Report will be prepared to contain the following information:

- The objectives stated in the approved Protocol.
- 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.
- Location and storage of all raw data.

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. Any minor deviation(s) from the Protocol (if applicable) will be presented in the report.

Final Report

The Final Report will be issued following QAD evaluation of the complete Draft Report. The report will include all details described above with the following additions:

- The signature of the Study Director as study completion date and authentication of the report.
- Quality assurance statement.

APPENDIX 3

RESPONSIBLE PERSONNEL AND STUDY SCHEDULE

Study Director
Any change in Study Director will be documented by Protocol Amendment

Distribution
In addition to the above, the following personnel should receive copies or have access to the Protocol, Protocol Amendments and e Notes

Hard Copy
Head of Quality Systems[1]
Resource Management
Resource Management

Electronic Access
Study Supervisor[1]
Analytical Staff
Study Co-ordinator
Project Manager
VP of Biotechnology

[1] = Any change documented in study records

PROPOSED DATES
Analytical programme

Analysis Start:	July 2007
Anticipated Finish:	August 2007
Draft Report:	September 2007

PROTOCOL APPROVAL

_____ 7/11/07

Study Monitor
Waisman Clinical Biomanufacturing Facility

7/13/07

Quality Assurance
WiCell Research Institute

_____ 28 June 2007
Date

Study Director
Covance Laboratories Ltd

_____ 28 Jun 2007
Date

Head of Biosafety
Covance Laboratories Ltd

Protocol Amendment

Title	H9-MCB.1: Detection of HIV-1, HIV-2, HTLV-2, EBV, HHV-7, HHV-8 and HBV Sequences using the Polymerase Chain Reaction (PCR) Technique. Detection and Quantification of B19 Sequences using the Quantitative Polymerase Chain Reaction Technique (QPCR). Detection and Quantification of HCV Sequences using the Reverse Transcriptase Quantitative Polymerase Chain Reaction Technique (RT-QPCR)
Study Director	
Testing Facility	Covance Laboratories Ltd.,
Study Monitors	
Sponsor	Waisman Clinical BioManufacturing Facility
Covance Study Number	2823/004
Amendment number	1
Page Number	1 of 3

COVANCE
THE DEVELOPMENT SERVICES COMPANY

This Amendment documents the following:

At the Sponsor's request the testing of assays for HTLV-1, HCMV and HHV-6 sequences will be terminated. A Draft and Final Report will be issued including only PCR, RT-PCR, QPCR, RT-QPCR or Southern blot hybridisation analysis of assays for HIV-1, HIV-2, HTLV-2, HBV, EBV, HHV-7, HHV-8, HCV and B19 sequences.

Due to the termination of assays for HTLV-1, HCMV and HHV-6 sequences the title for study 2823/004 has been updated and is on the title page of this Amendment. Also the objective has been changed to the following:

OBJECTIVE

The objective of this study is to determine whether HIV-1, HIV-2, HTLV-2, EBV, HHV-7, HHV-8, HBV, HCV and B19 are present in the test article using the Polymerase Chain Reaction (PCR) technique, the Quantitative Polymerase Chain Reaction (QPCR) technique and the Reverse Transcriptase Quantitative Polymerase Chain Reaction (RT-QPCR) technique.

PROTOCOL APPROVAL

11/2/07

Study Monitor
Waisman Clinical Biomanufacturing Facility

11/2/07

Quality Assurance
WiCell Research Institute

01 NOV 2007
Date

Study Director
Covance Laboratories Ltd

01 November 2007
Date

Project Manager
Covance Laboratories Ltd

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: AC08DS.105013.BSV

Test Article ID: H9 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 H9 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: H9 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 H9 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 H9 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: AC08DS.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: AC08DS.105012.BSV

Test Article ID: H9 MCB.1

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

One-half (0.5) µg of DNA isolated from test article H9 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: H9 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 (0.5 µg), 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 H9 MCB.1 tested negative for the presence of CMV DNA.

APPROVAL

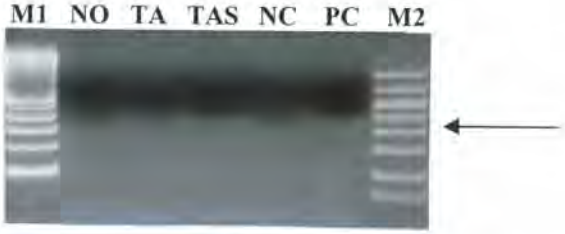
I accept responsibility for the conduct of this study which was performed in compliance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the UK GLP Regulations, the Japanese GLP Standard, and the Organization for Economic Cooperation and Development (OECD) Principles of Good Laboratory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

23 Oct 07

Date

Study Director

FIGURE 1



Detection of CMV specific sequences in the test article H9 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: AC08DS.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: AC08DS.105020.BSV

Test Article ID: H9 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 H9 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: H9 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 OPBT0924. 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 BPBT0920.

DNA Amplification

PCR amplification was performed on 0.5µg 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 (0.5µg), 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 H9 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 H9 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-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

Arrows indicate specific amplification products.

Quality Assurance Statement

Study Title: POLYMERASE CHAIN REACTION ASSAY FOR THE DETECTION OF HUMAN HERPESVIRUS 6 (HHV-6) IN BIOLOGICAL SAMPLES

Study Number: AC08DS.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.

_____ 23 Oct 07

DATE

QUALITY ASSURANCE

WiCell CGH Report: 000143 NSCB# 6185

Report Date: *March 13, 2009*

Case Details:

Cell Line: *WA09 (O) p24 MCB (Female)*

Reference: *WA01 (N) p37 (Male)*

Investigator: *National Stem Cell Bank*

Specimen: *hES cells on MEFs*

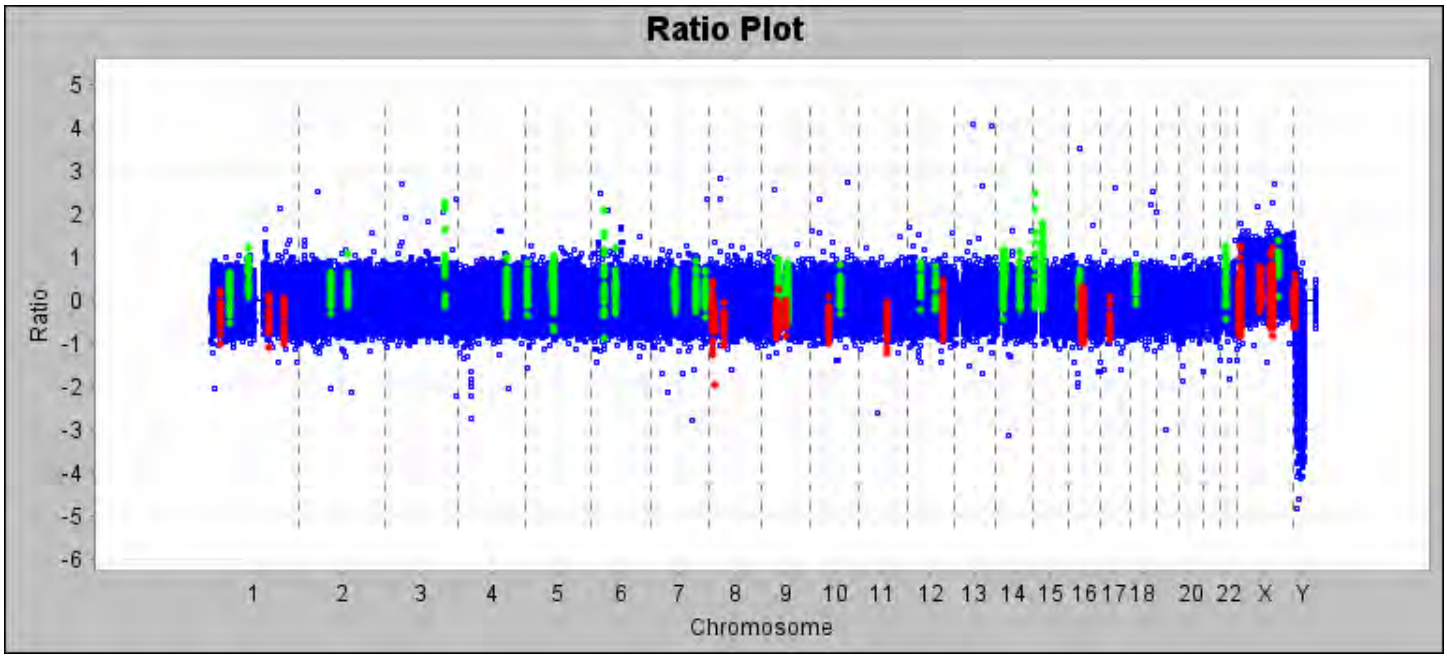
Date of Sample: *7/23/2007*

Reason for Testing: *NSCB MCB Testing*

GEO Accession #: *GSM347606*

Results:

Results are given in the attached excel spreadsheet labeled „report.' There were 61 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]	*2 of 2*
Published Copy Number Changes[5,6]	*1 of 8*
Reference DNA Copy Number Changes[2]	*12 of 18*
Select Differentially Expressed Genes	*0 of 45*

These results are consistent with karyotype results [46,XX] as reported in 6185-KAR.

Test sample gain or loss is consistent with the opposite gender reference standard.

Results Completed By: *CLSp(CG)*
Reviewed and Interpreted By: *, PhD, FACMG*

***aCGH Specifications**:*

- *Platform: NimbleGen 385K array (HG18 CGH 385K WG Tiling v2 X1)*
- *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 spanning non-repetitive regions of the human genome*
- *Average probe spacing = 6270bp*
- *Analysis software: NimbleScan™ , SignalMap™, OneClickCGH™, OneClickFusion™*
- *Analysis is based on examination of unaveraged and/or 60Kbp (10X) averaged data tracks as noted.*
- *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:_____**

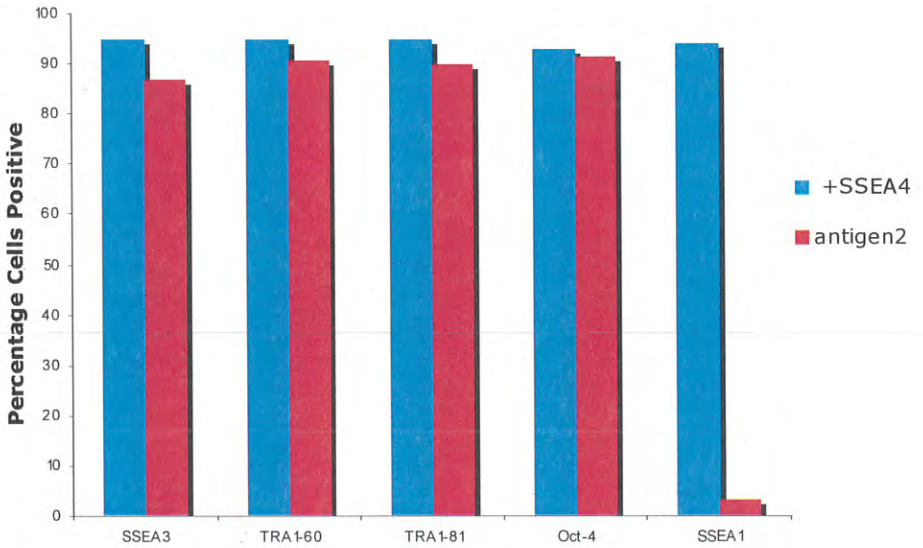
NSCB National Stem Cell Bank

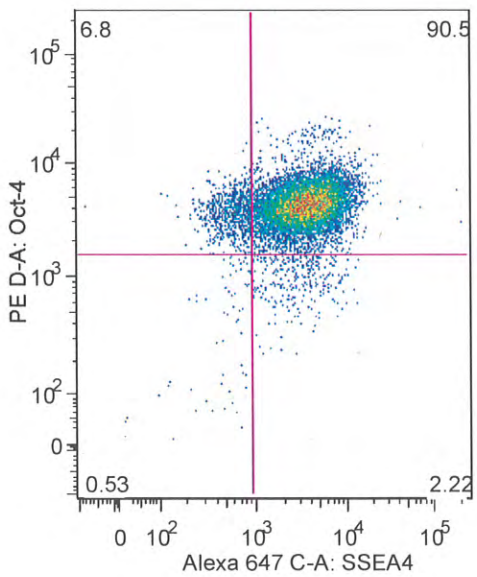
Characterization Report-
Flow Cytometry (FACS)

SOP-CH-101 B
SOP-CH-102 B
SOP-CH-103 B
SOP-CH-105 B

Cell Line: H9	**Date of:** *(mm/dd/yy)*
Passage: p24	Acquisition: 08/01/07
Sample ID: 6185-FAC	File Creation: 08/01/07
File created by: EP	File Submission: 08/07/07

antigen2:	SSEA4 - antigen2 +	SSEA4 + antigen2 +	SSEA4 + antigen2 -	SSEA4 - antigen2 -	ALL SSEA4 +	ALL antigen2 +
SSEA3	0	93.5	6.27	0.2	99.77	93.5
TRA1-60	0.01	92.6	7.14	0.26	99.74	92.61
TRA1-81	0.016	92.2	7.56	0.24	99.76	92.216
Oct-4	3.06	94.9	1.58	0.46	96.48	97.96
SSEA1	0.38	3.05	95.3	1.31	98.35	3.43





Sample RNA: R00384	Reference DNA: D00073	Date of report: 111807
Sample Cell Line: H9	Reference Cell Line: H1	Report prepared by: CY
Passage: 24	Passage:	QA reviewed: 11/20/07 EM
Lot: H9-MCB-1		Date sent to WiCell Iceland:
Sample ID: 6185-GEP		GEO accession #: GSM239975

1. Chip design: 2007-03-02_WiCell_HG18

2. Sample labeling:
   Cy5: 6185-GEP 2ug (Barcode: LR00350);
   Cy3: Sonicated H1 gDNA 4.5ug (Barcode: LD00123);

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			
DNMT3B	NM_006892	23.14153439	Y
GABRB3	NM_000814	0.980085349	Y
GDF3	NM_020634	2.252013809	Y
NANOG	NM_024865	1.07320442	Y
POU5F1	NM_002701	28.94645441	Y
TDGF1	NM_003212	8.615674454	Y
Non-core ES markers			
BXDC2	NM_018321	4.047692308	Y
CD9	NM_001769	12.50317797	Y
FGF4	NM_002007	0.693959732	Y
FOXD3	NM_012183	2.131372549	Y
GAL	NM_015973	3.439886846	Y
GRB7	NM_001030002	1.523809524	Y
IFITM1	NM_003641	7.159600998	Y
LEFTY1	NM_020997	2.941402498	Y
LEFTY2	NM_003240	1.976082005	Y
LIN28	NM_024674	15.62807296	Y
PODXL	BC093730	24.1592233	Y
SOX2	NM_003106	12.38373425	Y
TERT	NM_003219	0.792349727	Y
UTF1	NM_003577	0.57078926	Y

5. Expression of differentiation markers:

Gene Symbol	Accession	Ratio	Expression
ACTC	NM_005159	6.096733668	Y
AFP	NM_001134	0.542613636	Y
CDX2	NM_001265	0.389240506	Y
CGB	NM_000737	0.103205629	N
COL1A1	NM_000088	1.318756074	Y
COL2A1	NM_001844	0.491137793	Y
EOMES	NM_005442	1.617312073	Y
FLT1	NM_002019	0.549492386	Y
FN1	NM_002026	26.98188875	Y
FOXA2	NM_021784	0.646473779	Y
GATA4	NM_002052	1.308610401	Y
GATA6	NM_005257	0.610241821	Y
GCM1	NM_003643	0.207042254	N
IPF1	NM_000209	0.221512247	N
LAMA1	NM_005559	3.325285896	Y
NEUROD1	NM_002500	0.228787879	N
NKX2-5	NM_004387	0.131455399	N
PAX6	NM_000280	0.26993865	N
PDHX	NM_003477	2.364583333	Y
SOX17	NM_022454	1.050070522	Y
SYP	NM_003179	0.476944253	Y
TNNI3	NM_000363	0.981514085	Y

American Red Cross

Blood Services
Penn-Jersey Region
Musser Blood Center

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.

10/25/2007
PhD

Scientific Director

10/25/07

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.