Certificate of Analysis - Amended

Product Description	ES03	
Cell Line Provider	ES Cell International	
MCB Lot Number	ES03-MCB-01	
Date Vialed	30-May-2007	
Passage Number	P61	
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[1]
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	WuXi Apptec	37000	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	BioReliance	105010	Negative	Pass
HTLV 1&2 by PCR	BioReliance	105013	Negative	Pass
HBV by PCR	BioReliance	105042	Negative	Pass
HCV by PCR	BioReliance	105025	Negative	Pass
CMV by PCR	BioReliance	105012	Negative	Pass
EBV by PCR	BioReliance	105011	Negative	Pass
HHV-6 by PCR	BioReliance	105020	Negative	Pass
HHV-7 by PCR	BioReliance	105029	Negative	Pass

Certificate of Analysis - Amended

HHV-8 by PCR	BioReliance	105056	Negative	Pass
HP B19 by PCR	BioReliance	105037	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	O+

[1]This test was the first HLA performed for this cell line and therefore it establishes the HLA identity for this cell line.

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, and removed text regarding technical services and distribution of MCBs	22-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	14-July-2010
Original CoA	26-November-2007

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

UW Health
University of Wisconsin
Hospital and Clinics

Histocompatibility/Molecular Diagnostics Laboratory
Madison, WI 53792-2472

Short Tandem Repeat Analysis*

Sample Report: NSCB# 1590 UW HLA#: 56748 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) **Description:** DNA Extracted by WiCell

NSCB# 1590 WiCell DNA038

Locus	Repeat #	STR Genotype
D16S539	5, 8-15	9,9
D7S820	6-14	11,12
D13S317	7-15	9,12
D5S818	7-15	10,12
CSF1PO	6-15	12,12
TPOX	6-13	8,8
Amelogenin	NA	X,X
TH01	5-11	7,9
vWA	11, 13-21	17,18

105 ug/mL; 260/280 = 2.25

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 ES03 comprising 13 allelic polymorphisms across the 8 STR loci analyzed. No STR polymorphisms other than those corresponding to the human ES03 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 ES03 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 Cytogenetics Department and J.J. of WI Cell Research Institute on Friday, August 10, 2007.

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

UW Health

University of Wisconsin
Hospital and Clinics

Histocompatibility/Molecular Diagnostics Laboratory

Date: 08/13/2007 12:29:13

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, DNA038 1590	DQB SSP		1101	4001/49	0102	1202				
56748 /	A,B,C Seq	08/01/2007	3303	5502/16	0702					
08/01/2007	DRB Seq	08/01/2007								

Manager
HLA/Molecular Diagnostics Laboratory

Date

PhD, Director
HLA/Molecular Diagnostics Laboratory

Date

This test was developed and its performance characteristics determined by the UWHC Clinical Laboratory. It has not been cleared or approved by the U.S. Food and Drug Administration. However, the FDA does not require licensure of analyte specific reagents since the laboratory is approved, under CLIA, for high complexity testing.

Test Facility:

WiCell Research Institute

June 25, 2007
P.O. #:

STERILITY TEST REPORT

Sample Information: 2: Cryopreserved Human Embryonic Stem Cell Line ESI03 grown on Mouse Feeders, ESI03-MCB.1

Date Received: June 05, 2007
Date in Test: June 08, 2007
Date Completed: June 22, 2007

Test Information: Test Codes: 30744, 30744A
Immersion, USP / 21 CFR 610.12
Procedure #: BS210WCR.02

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

QA Reviewed: Page 1 signed

Reviewed: Page 1 signed

Testing conducted in accordance with current Good Manufacturing Practices.

Test Facility:

WiCell Research Institute

June 25, 2007
P.O. #:

STERILITY TEST REPORT

Sample Information:

1: Cryopreserved Human Embryonic Stem Cell Line HSF1.14 grown on Mouse Feeders, HSF1.14-MCB.1

2: Cryopreserved Human Embryonic Stem Cell Line ESI03 grown on Mouse Feeders, ESI03-MCB.1

Date Received: June 05, 2007
Date in Test: June 08, 2007
Date Completed: June 22, 2007

Test Information:

Test Codes: 30744, 30744A
Immersion, USP / 21 CFR 610.12
Procedure #: BS210WCR.02

QA Reviewed:

Reviewed:

Testing conducted in accordance with current Good Manufacturing Practices.

Test Facility:

AppTec

Report Number
748921
Page 1 of 1

WiCell Research Institute

June 26, 2007
P.O. #:

STERILITY TEST VALIDATION (B/F) REPORT

Sample Information: Cryopreserved Human Embryonic Stem Cell Line ESI03 grown on Mouse Feeders, ESI03-MCB.1

Date Received: June 05, 2007
Date in Test: June 20, 2007
Date Completed: June 25, 2007

Test Information:
Test Code: 30736
Immersion, USP / 21 CFR 610.12
Procedure #: BS210WCR.02
Media Volume: 20 mL
Volume Tested: 0.05 mL

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)	46	41	16
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)	50	39	38
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): 748572.

QA Reviewed: **Reviewed:**

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:	31216B
TEST ARTICLE IDENTIFICATION:	7565-APT, ES03-MCB.1
SPONSOR:	WiCell Research Institute
PERFORMING LABORATORY:	AppTec Laboratory Services
STUDY NUMBER:	61644
RESULT SUMMARY:	Considered **negative** for mycoplasma contamination and **non-inhibitory** for the detection of mycoplasma

Reference PO #

WCR01

61644

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
Staining of Coverslips	07/27/07	07/27/07	09/06/07
Final Report	09/05/07	09/05/07	09/06/07

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

Quality Assurance Auditor:_____ Date: 9-6-07

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: 9/6/07

Professional Personnel Involved:

Chief Executive Officer
Vice President of St. Paul Operations
Supervisor, Mycoplasma Testing Laboratory
Client Relations Manager

1.0 PURPOSE

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

2.0 SPONSOR: WiCell Research Institute

3.0 TEST FACILITY: AppTec Laboratory Services, Inc.

4.0 SCHEDULING

DATE SAMPLE RECEIVED: 07/17/07
STUDY INITIATION DATE: 07/23/07
STUDY COMPLETION DATE: 09/06/07

5.0 TEST ARTICLE IDENTIFICATION: WiCell Research Institute 7565-APT, ES03-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:	7565-APT, ES03-MCB.1
Lot/Batch #:	Not Given
Stability (Expiration):	Not Given
Storage Conditions:	Ultracold (≤ -60°C)
Safety Precautions:	BSL-1
Intended Use/Application:	Distribution lot cells from MCB cells

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 two (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 two 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).

The assay will be repeated in part or in total if a control failure occurs.

13.4 Negative Results

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

13.5 Positive Results

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

13.6 Mycoplasmastasis (Test Article Inhibition) Results Interpretation

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

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

13.6.1 Direct Assay

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

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

13.6.2 Indirect Assay

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

13.6.3 Repeat Testing for Products Containing Inhibitory Substances

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

14.0 RESULTS

14.1 Mycoplasmastasis (Test Article Inhibition)

14.1.1 Indirect assay

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

14.1.2 Direct assay – Day 0 Agar Plates

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

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

If TA Spike Ratio:

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

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

TABLE 2 – DAY 0 AGAR PLATES – POSITIVE CONTROLS

POSITIVE CONTROL	AVE. CFU / PLATE
M. orale	**39.7**
M.pneumoniae	**46.3**

TABLE 3 – DAY 0 AGAR PLATES – SPIKED TEST ARTICLES

		AVE. CFU / PLATE	TA SPIKE RATIO	INHIBITORY / NON-INHIBITORY
Test Article: 7565-APT, ES03-MCB.1	*M. orale* spike	**29.0**	**0.7**	Non-Inhibitory
	M. pneumoniae spike	**47.0**	**1.0**	Non-Inhibitory

14.1.3 Direct assay – Subculture Plates

The subculture plates for the test article spiked with *M. orale* at ≤ 100 CFU per inoculum and those spiked with *M. pneumoniae* at ≤ 100 CFU per inoculum yielded a positive result on the same subculture day as 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: 7565-APT, ES03-MCB.1	Negative	Negative	Negative	Negative
7565-APT, ES03-MCB.1, Spiked with *M. orale*	Non-inhibitory Positive	Non-inhibitory Positive	Non-inhibitory Positive	Non-inhibitory Positive
7565-APT, ES03-MCB.1, Spiked with *M. hyorhinis*	Non-inhibitory Positive			Non-inhibitory Positive
7565-APT, ES03-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, 7565-APT, ES03-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: None.

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 #1590

Report Date: July 26, 2007

Case Details:

Cell Line: *ES03*

Passage #: *68*

Date Completed: *7/26/2007*

***Cell Line Gender*:** female

Investigator: *NSCB*

Specimen: *hESC on MEF feeder*

Date of Sample: *7/20/2007*

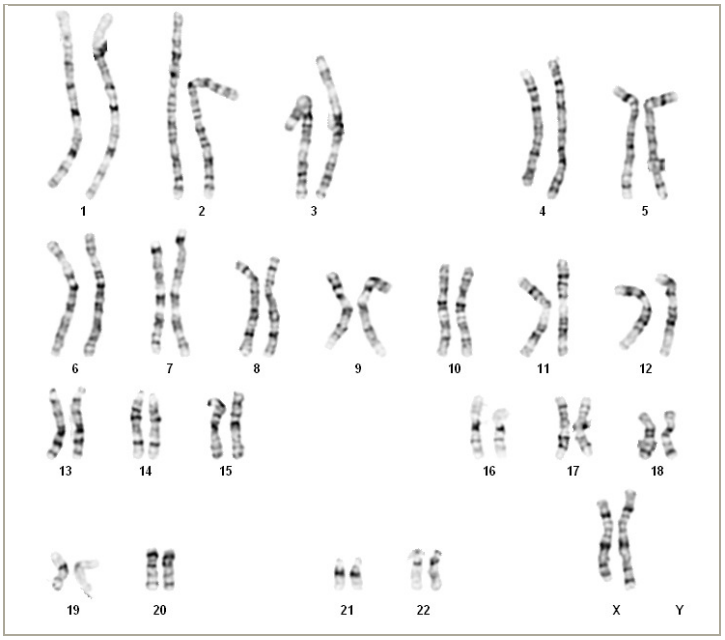
Tests,Reason for: *Confirmation of normal karyotype for MCB release*

Results: *46,XX*

Completed by , CLSp(CG), on 7/26/2007

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

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



Cell: *S01-05*

Slide: *B*

Slide Type: *Karyotyping*

Cell Results: *Karyotype: 46,XX*

# of Cells Counted: 20

# of Cells Karyotyped: 5

# of Cells Analyzed: 9

Band Level: 450-575

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: AC06AE.032901.BSV

Test Article ID: ES03-MCB.1 / 1590-BIR

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

Bovine viruses were not detected when the test article, ES03-MCB.1 / 1590-BIR, 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: ES03-MCB.1 / 1590-BIR was received by BioReliance on 25-Jul-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: 26-Jul-2007

Lab Initiation: 31-Jul-2007

Lab Completion: 22-Aug-2007

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

Study Director: Ph. D.

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, ES03-MCB.1 / 1590-BIR. 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.

_____ _____ 31Aug07

Ph. D Date

Study Director

TABLE 1

Observations for Cytopathic Effects in Cultures of BT and Vero Cells Inoculated with ES03-MCB.1 / 1590-BIR

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 ES03-MCB.1 / 1590-BIR

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 [b]	+

[a] Positive control tested on day 17
[b] Positive control tested on day 21
- CPE not observed
+ CPE observed

Table 3

Observations for Hemadsorption in BT and Vero Cultures Inoculated with ES03-MCB.1 / 1590-BIR

	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 21
- Hemadsorption not observed
+ Hemadsorption observed

TABLE 4

Immunofluorescent Staining Results for BT and Vero Cultures Inoculated with ES03-MCB.1 / 1590-BIR

	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 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]	+

[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
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: AC06AE.032901.BSV

Study Director: Ph.D.

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	31-Aug-07 - 31-Aug-07 To Study Dir 31-Aug-07 To Mgmt 31-Aug-07
	Phase	Final Report and data audit
*	Inspect On	12-Jun-07 - 20-Jun-07 To Study Dir 20-Jun-07 To Mgmt 20-Jun-07
	Phase	Systems Inspection - Test System Preparation
*	Inspect On	12-Jun-07 - 20-Jun-07 To Study Dir 20-Jun-07 To Mgmt 20-Jun-07
	Phase	Systems Inspection - Administration of Test Substance to Test System
*	Inspect On	12-Jun-07 - 20-Jun-07 To Study Dir 20-Jun-07 To Mgmt 20-Jun-07
	Phase	Systems Inspection - Manipulation of Test System
*	Inspect On	12-Jun-07 - 20-Jun-07 To Study Dir 20-Jun-07 To Mgmt 20-Jun-07
	Phase	Systems Inspection - Observation of Test System/Data Collection and/or Analysis

** 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.

B.S.
QUALITY ASSURANCE

31 Aug 07
DATE

Final Report

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

Study Number: AC06AE.033901.BSV

Test Article ID: ES03-MCB.1 / 1590-BIR

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

Porcine viruses, BVDV, reovirus and Rabies were not detected when the test article ES03-MCB.1 / 1590-BIR 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: ES03-MCB.1 / 1590-BIR was received by BioReliance on 25-Jul-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: 26-Jul-2007

Lab Initiation: 31-Jul-2007

Lab Completion: 22-Aug-2007

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

Study Director: Ph. D

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. The cells were fixed for immunofluorescent staining when the monolayers exhibited ≥10% CPE. 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 ES03-MCB.1 / 1590-BIR . 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.

_____ 31Aug07

Ph. D Date

Study Director

TABLE 1

Observations for Cytopathic Effects in Cultures of PT-1 Cells Inoculated with ES03-MCB.1 / 1590-BIR

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 ES03-MCB.1 / 1590-BIR

	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 ES03-MCB.1 / 1590-BIR

	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: AC06AE.033901.BSV

Study Director: Ph.D.

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	31-Aug-07 - 31-Aug-07 To Study Dir 31-Aug-07 To Mgmt 31-Aug-07
	Phase	Final Report and data audit
*	Inspect On	12-Jun-07 - 20-Jun-07 To Study Dir 20-Jun-07 To Mgmt 20-Jun-07
	Phase	Systems Inspection - Test System Preparation
*	Inspect On	12-Jun-07 - 20-Jun-07 To Study Dir 20-Jun-07 To Mgmt 20-Jun-07
	Phase	Systems Inspection - Administration of Test Substance to Test System
*	Inspect On	12-Jun-07 - 20-Jun-07 To Study Dir 20-Jun-07 To Mgmt 20-Jun-07
	Phase	Systems Inspection - Manipulation of Test System
*	Inspect On	12-Jun-07 - 20-Jun-07 To Study Dir 20-Jun-07 To Mgmt 20-Jun-07
	Phase	Systems Inspection - Observation of Test System/Data Collection and/or Analysis

** 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.

B.S.
QUALITY ASSURANCE

31Aug17
DATE

Final Report

MOUSE ANTIBODY PRODUCTION (MAP) TEST

Study Number: AC06AE.004000.BSV

Test Article ID: ES03-MCB.1 / 1590-BIR

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

Based on the data obtained in the assays performed, the test article, ES03-MCB.1 / 1590-BIR , has been shown to be free of all of the seventeen murine viruses for which it was examined.

BioReliance®

STUDY INFORMATION

Test Article Receipt: ES03-MCB.1 / 1590-BIR was received at BioReliance on 07/25/2007. 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: 07/31/2007

Lab Initiation: 08/01/2007

Lab Completion: 08/31/2007

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

Study Director:

Technical Support Staff: Ph.D., Laboratory Manager, Serology

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

BioReliance

Negative Control:	Eagle's Minimum Essential Medium with Penicillin/Streptomycin
LCM Challenge Virus:	Lymphocytic Choriomeningitis (CA1371 Strain)
Test System:	Mice, HSD:ICR twelve males, 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.

One of the three test article injected animals challenged with LCM virus was found dead at 48 hours post injection. The cause of death of this animal may have resulted from trauma associated with intracranial injection. The remaining two 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. One of the three LCM virus control animals was found dead at 24 hours post challenge. The cause of death of this animal most likely resulted from trauma associated with intracranial injection. The remaining two LCM virus control animals from the same source and shipment as the test group exhibited a rate of mortality, after challenge, which confirmed virulence of the challenge virus. (See Evaluation of Test Results/Criteria for a Valid Test.)

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.

_____ 14 Sep 07

Study Director 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 ES03-MCB.1 / 1590-BIR

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	.01	0	.01	0	.15	0	0	0	0	.01	0	0	.10	-	-	-
	.04	0	0	0	0	.02	.03	0	0	0	0	0	.03	-	-	-
	.05	.02	.01	0	0	.05	.08	0	0	.01	0	0	.04	-	-	-
	.08	0	.05	0	0	I[4]	I[4]	.02	0	.01	0	0	I[4]	-	-	-
Negative Control	0	.01	0	0	0	.01	0	.02	0	0	0	0	.02	-	-	-
	0	0	.01	0	0	0	0	.01	0	0	0	0	.02	-	-	-
Serology Positive Control	1.14	1.15	1.06	1.03	1.11	1.16	1.15	.81	.92	.96	1.03	1.13	1.14	+	640	+

[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 ES03-MCB.1 / 1590-BIR

Plasma from Animals Injected with	LDH Titer[a]
Test Article (1:10) (Group II)	249 286 362
Negative Control (Group III)	456 369
LDV Control	1341 1603

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

Quality Assurance Statement

Study Title: MOUSE ANTIBODY PRODUCTION (MAP) TEST

Study Number: AC06AE.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	10-Sep-07 - 10-Sep-07 To Study Dir 10-Sep-07 To Mgmt 14-Sep-07
	Phase	Final Report and data audit
*	Inspect On	24-Jul-07 - 25-Jul-07 To Study Dir 25-Jul-07 To Mgmt 25-Jul-07
	Phase	Systems Inspection - Administration of Test Substance to Test System
*	Inspect On	10-Aug-07 - 10-Aug-07 To Study Dir 10-Aug-07 To Mgmt 10-Aug-07
	Phase	Systems Inspection - Observation of Test System/Data Collection and/or Analysis
*	Inspect On	30-Aug-07 - 30-Aug-07 To Study Dir 30-Aug-07 To Mgmt 30-Aug-07
	Phase	Systems Inspection - Manipulation of Test System
*	Inspect On	14-Sep-07 - 14-Sep-07 To Study Dir 14-Sep-07 To Mgmt 14-Sep-07
	Phase	Systems Inspection - Test System Preparation

** Inspection specific for this study
* Systems Inspection

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

14 Sep 07

A.A.S. DATE

QUALITY ASSURANCE

E-FR-1-07-001589.37000

FINAL STUDY REPORT

STUDY TITLE: **Custom *In Vitro* Assays for Adventitious Viral Contaminants**

TEST PROTOCOL NUMBER: **37000.03**

TEST ARTICLE IDENTIFICATION	APPTEC ACCESSION NUMBER
ES03-MCB.1 1590-APT	07-001589

SPONSOR:

WiCell

PERFORMING LABORATORY: AppTec, Inc.

APPTEC ACCESSION NUMBER	RESULTS
07-001589	No evidence of viral contamination was detected.

QUALITY ASSURANCE UNIT SUMMARY

STUDY: Custom In Vitro Assays for Adventitious Viral Contaminants

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 37100.02 Step 4.13.3 Handling each cell line separately, remove medium from the plates for test article #07-001589.	September 11, 2007	September 24, 2007	September 24, 2007

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

Quality Assurance — Date: 3 Oct 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 as set forth in 21 CFR Part 58. Test article characterization is the responsibility of the Sponsor.

Study Director — Date: 030CT07

Professional Personnel involved in study:

1.0 PURPOSE

The purpose of this study is to detect the presence of adventitious viral agents in a test article.

2.0 SPONSOR: WiCell

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

4.0 SCHEDULING

DATE SAMPLES RECEIVED: July 25, 2007
STUDY INITIATION DATE: August 3, 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: ES03-MCB.1 1590-APT

7.0 TEST SYSTEM DESCRIPTION

Utilization of mammalian cells in the manufacture of biologicals carries a potential risk of contamination of the product with adventitious viruses.[1] Many viruses, both of human and animal origin, can potentially contaminate biologically-derived products. These viruses can vary widely in their pathogenicity and account for significant morbidity and mortality.[2,3] The choice of cell lines used in this assay is dictated by the 1993 Points To Consider directive from the FDA.[4]

Introduction of test article cells and/or culture fluids derived from such cells or other types of test articles, such as monoclonal antibodies and gene therapy vectors, to the indicator cell monolayers allows the detection of a wide range of animal and human viruses, including picornaviruses, orthomyxoviruses, paramyxoviruses, herpesviruses, adenoviruses, and reoviruses.[5]

Inoculated indicator cell cultures are examined at least twice a week for at least 14 days, with one subculture typically on day 7. If a human virus is suspected, a 28-day assay is recommended, with subcultures typically on days 7, 14, and 21. The cells are examined for the presence of replicating viruses, typically manifested as changes in morphology of the cells, cell death, fusion of the cells, etc. (cytopathic effects or CPE). The test article-inoculated cultures are also compared to positive control cultures inoculated with low levels of selected viruses. Since orthomyxo- and paramyxoviruses may replicate in cells in the absence of cytopathic effects,[1] the presence of these viruses may be detected by their ability to adsorb erythrocytes to the surface of infected cells.[2] This hemadsorption assay is performed at the conclusion of the observation period, day 14 or later or day 28 or later, depending on the duration of the assay.

8.0 EXPERIMENTAL DESIGN

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

8.1 MRC-5, VERO, and NIH/3T3 indicator cell monolayers were inoculated with Eagle's Minimum Essential Medium (EMEM) and served as the negative controls.

8.2 MRC-5, VERO, and NIH/3T3 indicator cell monolayers were inoculated with 0.2 mL per well of disrupted, clarified test article lysate. Six wells per cell line were inoculated.

8.3 Indicator cell monolayers were inoculated with viruses as appropriate for each cell line chosen to serve as the positive controls. MRC-5 cells were inoculated with encephalomyocarditis virus (EMC), VERO cells were inoculated with adenovirus type 5 (Ad 5), and NIH/3T3 cells were inoculated with herpes simplex virus (HSV).

8.4 Cultures were incubated at 37±2°C in a humidified atmosphere of 5±2% CO_2. Cultures were observed for cytopathic changes over the course of 28 days. Specifically, cultures were monitored for macroscopic changes in the monolayer, such as plaques, foci, or areas lacking uniformity, as well as microscopic changes in cell morphology.[4,5]

8.5 Cultures were fed on days 4, 11, 18 and 25. Subcultivation was performed on days 7, 14 and 21.

8.6 Two days prior to hemadsorption, one set of VERO negative cultures was infected with parainfluenza type 3 (PI3) virus to serve as the hemadsorption positive control.[3]

8.7 On day 28, the hemadsorption assay was performed. The monolayers were rinsed, and suspensions of chicken, human, and guinea pig erythrocytes were added separately to the monolayers. Replicate cultures were incubated at 2 - 8°C or 15 - 30°C for 30 to 45 minutes, washed, and examined macroscopically and microscopically for adsorption of erythrocytes to the monolayers.

9.0 TEST ARTICLE PREPARATION

On July 25, 2007, AppTec, Inc. received 2 tubes each containing 8 mL of "hES cells grown with mouse embryonic fibroblast feeder cells," frozen on dry ice and designated for use in this assay. The test article was stored at or below -60°C and placed on hold pending the receipt of further information from the client. On July 26, 2007, the test article was released from hold and continued to be stored at ≤ -60°C until the assay was initiated.

On August 17,2007, the test article was thawed using a 37±2°C waterbath and subjected to an additional freeze/thaw cycle using a dry ice/ethanol bath and a 37±2°C waterbath. The test article was clarified by low-speed centrifugation and was placed on ice until the time of inoculation.

10.0 POSITIVE CONTROLS

10.1 Positive control inoculum was derived from virus stocks that have met the criteria set forth in an internal SOP. The viruses were inoculated at 100-300 PFU per well.

Positive controls for CPE:

1. MRC-5 cultures infected with EMC
2. VERO cultures infected with Ad 5
3. NIH/3T3 cultures infected with HSV-1

10.2 The positive control for hemadsorption was one set of VERO negative control cultures infected with PI3.

11.0 NEGATIVE CONTROLS

Negative controls for CPE and hemadsorption were indicator cell cultures inoculated with EMEM.

12.0 ASSAY VALIDITY

The test is considered valid if characteristic cytopathic changes and/or hemadsorption are detected in the positive control cell cultures, and the negative control cell cultures are both free of viral cytopathic changes and do not hemadsorb erythrocytes.

13.0 TEST EVALUATION

A positive result, as judged by the development of cytopathic changes during the course of 28 days and/or the adsorption of erythrocytes in cultures inoculated with the test article, would indicate the presence of adventitious viral agents.

A negative result would indicate that the test article is free of detectable adventitious viruses. However, it does not indicate that the culture is free of persistent or latent virus infection. Detection of the latter agents may require further studies.

14.0 RESULTS

The test was valid. The MRC-5 culture infected with EMC virus evidenced +4 viral CPE on day 3. The VERO culture infected with Ad 5 evidenced +4 viral CPE on day 7 and were discarded. The NIH/3T3 culture infected with HSV-1 evidenced +3 viral CPE on day 4. Cell line negative control cultures showed no morphologic changes over the 28-day test period (Table 1).

One set of VERO negative cultures infected with PI3 virus were positive for hemadsorption when used as positive controls for the assay run on day 28 (Table 2).

The test article did not induce cytopathic changes in the MRC-5, VERO and NIH/3T3 cultures (Table 1). The test article-inoculated MRC-5, VERO, and NIH/3T3 cultures did not induce hemadsorption activity (Table 2). Thus, the presence of adventitious viruses was not detected in the test article.

TABLE 1: Observation of Cytopathic Effects in Indicator Cell Lines

Culture Inoculum	Cell Line		
	MRC-5	VERO	NIH/3T3
Accession # 07-001589	**0**	**0**	**0**
EMEM (Negative Control)	0	0	0
Encephalomyocarditis Virus (Positive Control)	+4	NA	NA
Adenovirus Type 5 (Positive Control)	NA	+4	NA
Herpes Simplex Type 1 (Positive Control)	NA	NA	+3

Legend:

0	No viral cytopathic changes observed during the 28-day test period
+1	Up to 50% of the cells in culture showed viral cytopathic changes
+2	50 - 75% of the cells in culture showed viral cytopathic changes
+3	75 - 90% of the cells in culture showed viral cytopathic changes
+4	90 - 100% of the cells in culture showed viral cytopathic changes
NA	Not applicable

TABLE 2: Hemadsorption Activity of Indicator Cell Lines

Culture Inoculum	Cell Line	Day of Test	2 - 8°C			15 – 30°C		
			C	GP	H	C	GP	H
Accession #07-001589	**MRC-5**	**28**	-	-	-	-	-	-
	VERO	**28**	-	-	-	-	-	-
	NIH/3T3	**28**	-	-	-	-	-	-
EMEM (Negative Control)	MRC-5	28	-	-	-	-	-	-
	VERO	28	-	-	-	-	-	-
	NIH/3T3	28	-	-	-	-	-	-
Positive Control[1] 1:10	VERO	28	+	+	+	+	+	+
Positive Control[1] 1:20		28	+	+	+	+	+	+

Legend:
C Chicken erythrocytes
GP Guinea pig erythrocytes
H Human type O erythrocytes
\- Negative reaction indicating the absence of viral agent
\+ Positive reaction indicating presence of viral agent
[1] PI3 virus inoculated onto one set of VERO negative control cultures two days prior to hemadsorption; results are indicative of both portions of testing

15.0 CONCLUSION

No evidence of adventitious virus contamination was detected in the test article when tested on MRC-5, VERO and NIH/3T3 indicator cell monolayers.

16.0 STATISTICAL DATA ANALYSIS

Statistical analysis of the data was not required.

17.0 DEVIATIONS / AMENDMENTS

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

No amendments to the protocol were generated.

18.0 RECORD RETENTION

The testing facility will retain all records involving the study for seven (7) years including, but not limited to: the signed 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.

19.0 REFERENCES

1. Jacobs JP, McGrath DI, Garrett AJ and Schild GC (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 Stand* **9**: 331-342.

2. Belshe RB, ed. (1984). Textbook of Human Virology. PSG Publishing Company, Inc., Littleton, MA.

3. Poiley JA (1990). "Methods for the detection of adventitious viruses in cell cultures used in the production of biotechnology products," in Large-scale Mammalian Cell Culture Technology. Marcel Dekker, Inc., New York, NY.

4. "Points to Consider in the Characterization of Cell Lines Used To Produce Biologicals" (1993). Center for Biologics Evaluation and Research Food and Drug Administration.

5. Hay RJ (1994). ATCC Quality Control Methods for Cell Lines. American Type Culture Collection, Rockville, MD.

Final Report

TEST FOR THE PRESENCE OF INAPPARENT VIRUSES

Study Number: AC06AE.005002.BSV

Test Article ID: ES03-MCB.1 / 1590-BIR

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

No evidence of contamination with adventitious viral agents was observed due to the test article, ES03-MCB.1 / 1590-BIR.

BioReliance®

STUDY INFORMATION

Test Article Receipt:	ES03-MCB.1 / 1590-BIR was received by BioReliance on 07/25/2007. 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 Date:	08/06/2007
Lab Initiation Date:	08/07/2007
Lab Completion Date:	09/07/2007
Study Completion Date:	See Study Director's signature date in the "Approval" section.
Study Director:	Ph.D.
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
Negative Control:	Hank's Balanced Salt Solution (HBSS) Lot No.: 76K2334 Source: Sigma St. Louis, Missouri

Test System:

Mice Suckling litters (Primary Injection):HSD:ICR, four adult females, each with ten one day old suckling pups

Source: Harlan Sprague Dawley
Frederick, Maryland

Suckling litters (Blind Passage):HSD:ICR, four adult females, each with ten two 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 (BE Eggs)
York Springs, Pennsylvania

Embryonated Hens' Eggs (yolk sac route): forty, seven days old

Source: Sunrise (BE Eggs)
York Springs, Pennsylvania

OBJECTIVE

The study objective was 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.

PROCEDURES

Experimental Design

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. 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.

Test System Identification and Randomization

Each animal cage was assigned a number and labeled with the appropriate test material information. Guinea pigs were housed separately and 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 hen's eggs were labeled 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.

Methods

Mice and Guinea Pigs

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 HBSS (containing 1.0 mg/ml of gentamicin sulfate) 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 Hen's Eggs

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

The fluids from each group were pooled and these pooled allantoic fluids were then passaged to a new group of 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 $4^{\circ}C \pm 2^{\circ}C$ and $25^{\circ}C \pm 2^{\circ}C$.

Each of ten 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 at least nine days of incubation post-injection, 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 embryonated eggs per group. Each egg was candled for viability at 24 and 48 hours post-injection. After at least nine days post-injection, 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 Teklad. 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.

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 test 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.

RESULTS

Mice and Guinea Pigs

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 and nineteen of the twenty suckling mice injected with the negative control article homogenate appeared normal and healthy after 14 days. One of the suckling mice injected with the negative control article homogenate was missing and presumed cannibalized on day 5 post injection. (See Criteria for a Valid Test.)

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.

Embryonated Hens' Eggs: Allantoic Route

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.

Embryonated Hens' Eggs: Yolk Sac Route

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. All eggs appeared viable at 24 hours post injection. At 48 hours post injection, nine of the test article injected eggs and seven of the negative control article injected eggs appeared viable. At examination on day 10 post injection, nine of the ten test article

injected eggs and seven of the ten negative control article injected eggs contained viable embryos. One of the test article injected eggs and three of the negative control article injected eggs contained non-viable embryos. Growth identified as *Proteus mirabilis* was observed on blood agar plates streaked with fluid from the non-viable eggs. The cause of death of these embryos most likely resulted from the bacterial contamination. Although less than 80% of the control article injected eggs contained viable embryos, the assay is considered valid. Greater than 80% of the test article injected eggs contained viable embryos. (See Evaluation of Test Results/Criteria for a Valid Test.)

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 24and 48 hours post injection. At examination on day 9 post injection, eight of the ten test article subpassage eggs and seven of ten negative control article subpassage eggs contained viable embryos. Two of the test article subpassage eggs and three of the negative control article subpassage eggs contained non-viable embryos. Growth identified as *Streptococcus sp* was observed on blood agar plates streaked with fluid from the non-viable test article subpassage egg. The cause of death of this embryo most likely resulted from the bacterial contamination. No growth was observed on blood agar plates streaked with fluid from the non-viable negative control article subpassage eggs. The cause of death of these embryos could not be determined. Although less than 80% of the control article injected eggs contained viable embryos, the assay is considered valid. Eighty percent of the test article injected eggs contained viable embryos. (See Criteria for a Valid Test.) See Table 4 for a summary of the data.

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 Organization for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice.

Ph.D.

Study Director

14 Sep 07

Date

TABLE 1

Suckling Mice

Group No.	Number of Animals	Sex	Route(s) of Injection[a]	Volume of Test Material	Test Material	Treatment After Injection
SM 1	10	various[b]	i.p. p.o. i.c.	0.1 ml 0.01 ml 0.01 ml	Test Article	Suckling mice were observed for illness. After 14 days, a single pool of emulsified tissue (minus skin and gastrointestine) of all surviving mice was passaged into 10 additional suckling mice. Same routes and volumes of test material as injected in the primary injection were used.
SM 2						
SM 3	10				Sham Control	
SM 4						

[a] i.p. = Intraperitoneal injection; p.o. = Per os injection (by mouth); i.c. = Intracranial injection.
[b] Each cage contained one adult female lactating mouse. No testing was performed on the adult lactating female.

Adult Mice

Group No.	Number of Animals	Sex	Route(s) of Injection[a]	Volume of Test Material	Test Material	Treatment After Injection
AM 1	5	male	i.p. p.o. i.n. i.c.	0.5 ml 0.05 ml 0.05 ml 0.03 ml	Test Article	Observe for illness.
AM 2	5	female				
AM 3	5	male			Sham Control	
AM 4	5	female				

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

TABLE 1 (Continued)

Guinea Pigs

Group No.	Number of Animals	Sex	Route(s) of Injection[a]	Volume of Test Material	Test Material	Treatment After Injection
GP 1	1	Male	i.p. i.c.	5.0 ml 0.1 ml	Test Article	Observe for illness.
GP 2	1					
GP 3	1					
GP 4	1	Female				
GP 5	1					
GP 6	1					
GP 7	1	Male			Sham Control	
GP 8	1					
GP 9	1	Female				
GP 10	1					

[a] i.p. = Intraperitoneal injection; i.c. = Intracranial injection.

TABLE 2

Survival Summary for ES03-MCB.1 / 1590-BIR

	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	19/20

[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 ES03-MCB.1 / 1590-BIR

Guinea Pigs

Test Material	Animal Number	Clinical Signs	Day of Onset (Post-Injection)	Day of Death/Sacrifice (Post-Injection)
Test Article	12683	Normal		
	12684	Normal		
	12685	Normal		
	12686	Normal		
	12687	Normal		
	12688	Normal		
Negative Control	12667	Normal		
	12668	Normal		
	12669	Normal		
	12670	Normal		

TABLE 3 (Continued)

Summary of Daily Observations for ES03-MCB.1 / 1590-BIR

Adult Mice

Test Material	Animal Number	Clinical Signs	Day of Onset (Post-Injection)	Day of Death/Sacrifice (Post-Injection)
Test Article	12641	Normal		
	12642	Normal		
	12643	Normal		
	12644	Normal		
	12645	Normal		
	12646	Normal		
	12647	Normal		
	12648	Normal		
	12649	Normal		
	12650	Normal		
Negative Control	12611	Normal		
	12612	Normal		
	12613	Normal		
	12614	Normal		
	12615	Normal		
	12616	Normal		
	12617	Normal		
	12618	Normal		
	12619	Normal		
	12620	Normal		

TABLE 3 (Continued)

Summary of Daily Observations for ES03-MCB.1 / 1590-BIR

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) (9)[c]	Normal	5	5
		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.
[c] One suckling mouse was missing and presumed cannibalized.

TABLE 4

Embryonated Hens' Eggs
Allantoic Route Survival Summary and Hemagglutination Results for ES03-MCB.1 / 1590-BIR

Test Material	Primary Injection							
	24 Hour Viability	Viability[a] Harvest (Day 3)	Hemagglutination Results[b]					
			4°C			25°C		
			C	GP	H	C	GP	H
Test Article	10/10	10/10	0/10	0/10	0/10	0/10	0/10	0/10
Negative Control	10/10	10/10	0/10	0/10	0/10	0/10	0/10	0/10

Test Material	Blind Passage							
	24 Hour Viability	Viability[a] Harvest (Day 3)	Hemagglutination Results[b]					
			4°C			25°C		
			C	GP	H	C	GP	H
Test Article	10/10	10/10	0/10	0/10	0/10	0/10	0/10	0/10
Negative Control	10/10	10/10	0/10	0/10	0/10	0/10	0/10	0/10

[a] Number of viable eggs/number examined.
[b] Fluids from all eggs were tested for hemagglutinins using chicken (C), guinea pig (G) and human type O (H) erythrocytes.

NOTE: Hemagglutination positive control (Parainfluenza 3, SF-4 strain, batch PI3062702V) and erythrocyte negative controls were satisfactory.

TABLE 4 (Continued)

Summary of Observations for ES03-MCB.1 / 1590-BIR

Embryonated Hens' Eggs - Yolk Sac Route

Primary Injection				
Test Material	Number of Eggs Injected	Viability Observations[a]		
		24 Hours	48 Hours	Harvest Day 10
Test Article	10	10/10	9/10	9/10
Negative Control Article	10	10/10	7/10	7/10

[a] Number of viable eggs/number examined.

Blind Passage				
Test Material	Number of Eggs Injected	Viability Observations[a]		
		24 Hours	48 Hours	Harvest Day 9
Test Article Homogenate	10	10/10	10/10	8/10
Negative Control Article Homogenate	10	10/10	10/10	7/10

[a] Number of viable eggs/number examined.

Quality Assurance Statement

Study Title: TEST FOR THE PRESENCE OF INAPPARENT VIRUSES

Study Number: AC06AE.005002.BSV

Study Director: Ph.D.

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	11-Sep-07 - 12-Sep-07 To Study Dir 12-Sep-07 To Mgmt 14-Sep-07
	Phase	Final Report and data audit
*	Inspect On	24-Jul-07 - 25-Jul-07 To Study Dir 25-Jul-07 To Mgmt 25-Jul-07
	Phase	Systems Inspection - Administration of Test Substance to Test System
*	Inspect On	10-Aug-07 - 10-Aug-07 To Study Dir 10-Aug-07 To Mgmt 10-Aug-07
	Phase	Systems Inspection - Observation of Test System/Data Collection and/or Analysis
*	Inspect On	30-Aug-07 - 30-Aug-07 To Study Dir 30-Aug-07 To Mgmt 30-Aug-07
	Phase	Systems Inspection - Manipulation of Test System
*	Inspect On	14-Sep-07 - 14-Sep-07 To Study Dir 14-Sep-07 To Mgmt 14-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.

B.S

14 Sep07

DATE

QUALITY ASSURANCE

E-FR-1-07-001920.30610

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
ES03 MCB.1 5 passages in TeSR1	07-001920
HSF 1 MCB.1 5 passages in TeSR1	07-001921
HSF1 MCB.1 10 passages in TeSR1	07-001923

SPONSOR: WiCell

PERFORMING LABORATORY: AppTec, Inc.

APPTEC ACCESSION NUMBER	RESULTS
07-001920	Transmission electron microscopic examination revealed no identifiable virus.
07-001921	Transmission electron microscopic examination revealed no identifiable virus.
07-001923	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 Articles 07-001920, 07-001921 and 07-001923	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
07-001920	ES03 MCB.1 5 passages in TeSR1
07-001921	HSF 1 MCB.1 5 passages in TeSR1
07-001923	HSF1 MCB.1 10 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 07-001920

13.1 Cellular Ultrastructure

Cells in the section were of moderate size and polygonal to irregular in shape (L22846). Cells had microvilli (MV: L22846)[1] unevenly distributed on the surface. Nuclei (N: L22846) tended to be polygonal to irregular, with chromatin relatively evenly dispersed or clumped along the periphery. Nuclei often had one or more nucleoli (NS: L22846) that were variably located, and nuclear pores (NP: L22842) were seen.

The cytoplasm of most cells contained varying numbers of dense mitochondria (MI: L22847). Profiles of rough endoplasmic reticulum (RER: L22845) were seen among the mitochondria. Ribosomes (RB: L22841) were abundant in the cytoplasm of most cells. Cells were observed to contain centrioles (CN: L22844), glycogen (G: L22848) and filaments (F: L22841, L22843). Myelin figures (MF: L22848), desmosomes (D: L22840), and cleft-like spaces (CS: L22847) were also seen.

13.2 General Viral Particle Evaluation

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

13.3 Retrovirus-like Particle Evaluation and Tabulation

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

Test Article: 07-001920
PAI EM Number: 07.475-1

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%

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

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

Test Article 07-001921

13.4 Cellular Ultrastructure

Cells in the section were of moderate size, polygonal to irregular in shape, and often closely packaged (J57709). Cells that were not closely packed had microvilli (MV: J57713) unevenly distributed on the surface. Nuclei (N: J57709) tended to be polygonal to irregular, with chromatin relatively evenly dispersed or clumped along the periphery. Nuclei often had one or more nucleoli (NS: J57709) that were variably located.

The cytoplasm of most cells contained varying numbers of dense mitochondria (MI: J57710). Profiles of rough endoplasmic reticulum (RER: J57711) were seen among the mitochondria. Ribosomes (RB: J57708) were abundant in the cytoplasm of most cells. Cells were observed to contain autophagic vacuoles (AV: J57714), many filled with clumps of an electron-dense material.

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: 07-001921
PAI EM Number: 07.476-1

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-three percent of the cells were necrotic.

Test Article 07-001923

13.7 Cellular Ultrastructure

Cells in the section were of moderate size and polygonal to irregular in shape (L22866). Cells had microvilli (MV: L22871) unevenly distributed on the surface. Nuclei (N: L22866) tended to be polygonal to irregular, with chromatin relatively evenly dispersed or clumped along the periphery. Nuclei often had one or more nucleoli (NS: L22866) that were variably located, and nuclear pores (NP: L22870) were seen.

The cytoplasm of most cells contained varying numbers of dense mitochondria (MI: L22870). Ribosomes (RB: L22867) were abundant in the cytoplasm of most cells. Cells were observed to contain centrioles (CN: L22867), glycogen (G: L22870), desmosomes (D: L22868) and annulate lamellae (AL: L22869).

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: 07-001923
PAI EM Number: 07.477-1

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 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.

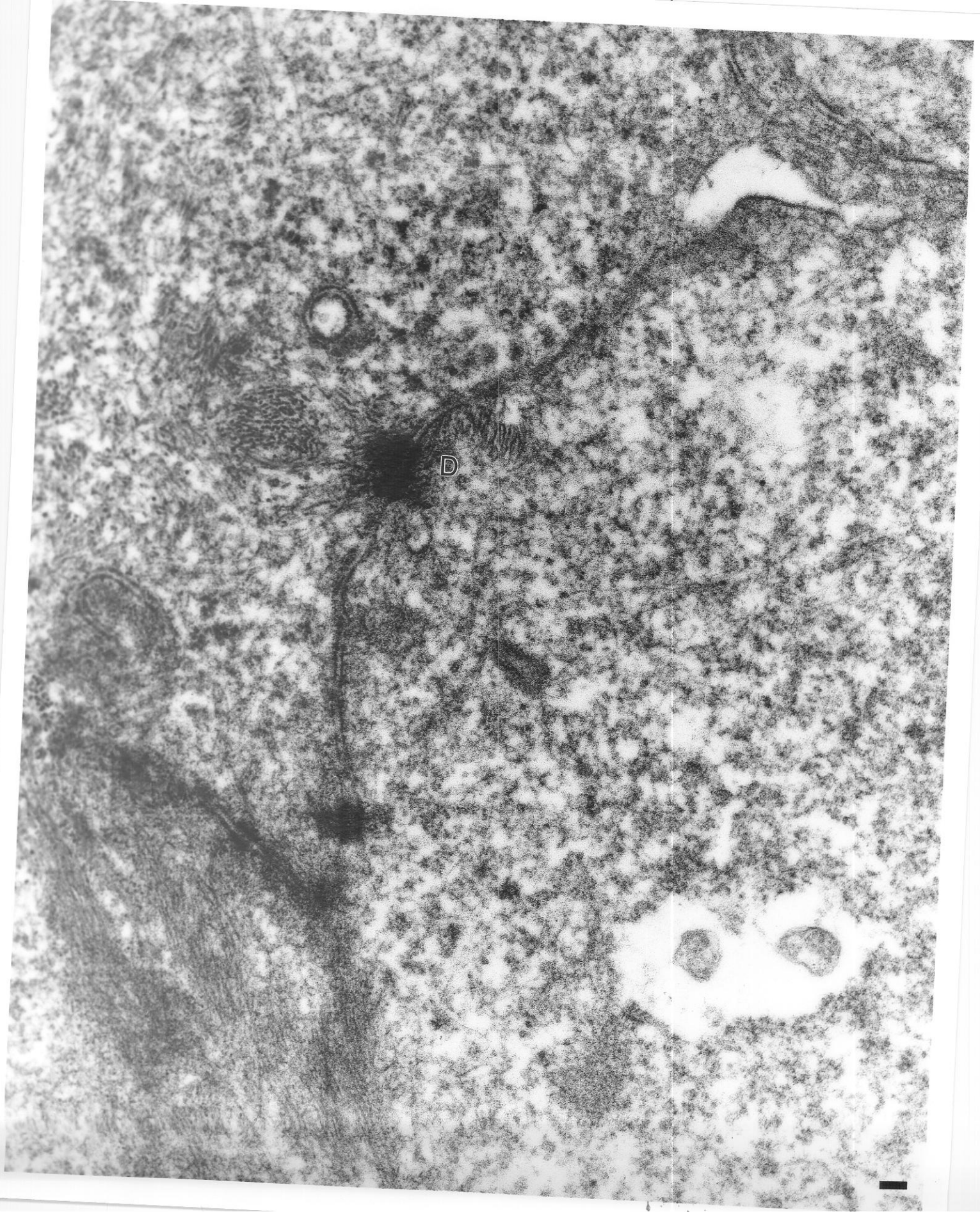
18.0 REFERENCES

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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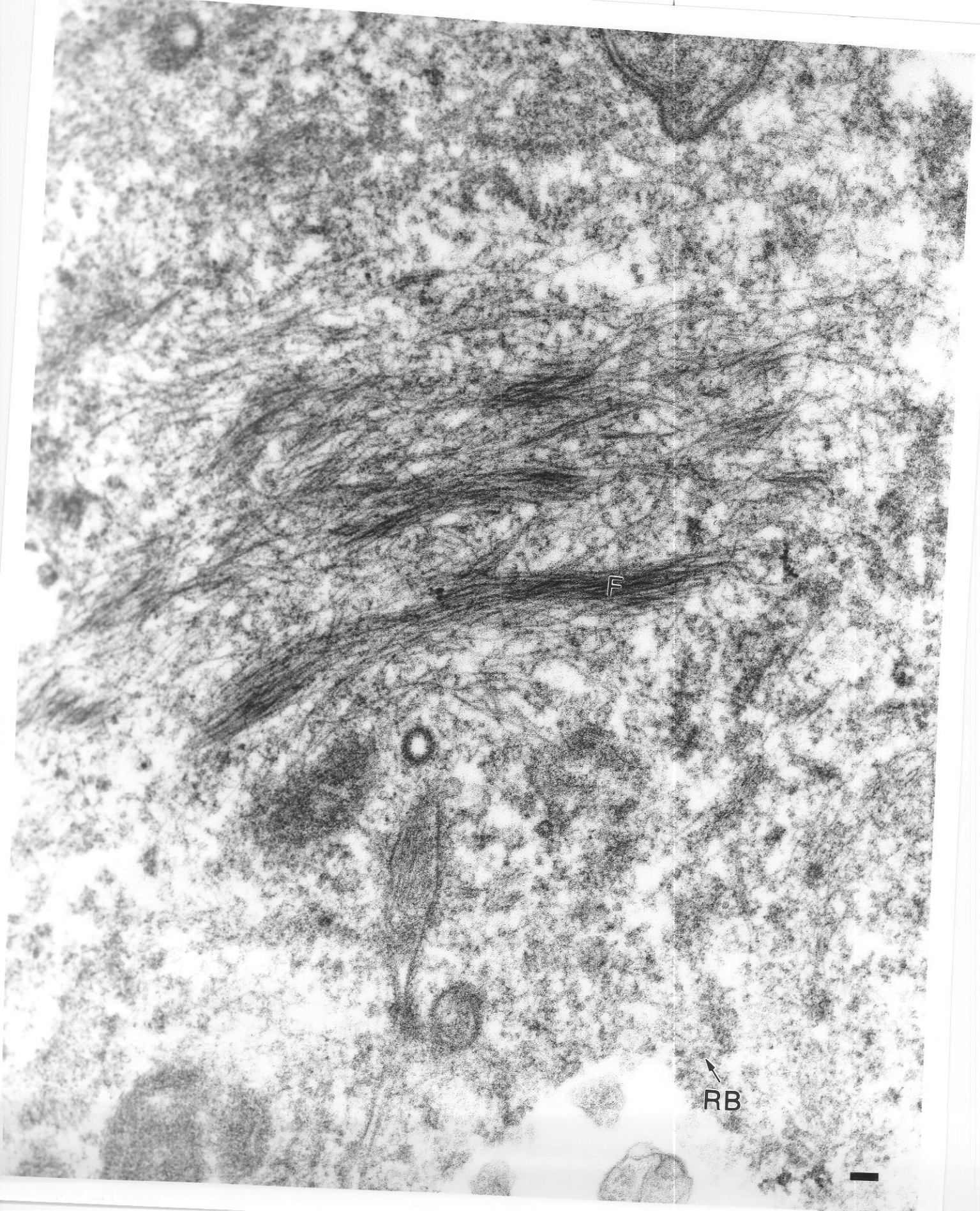
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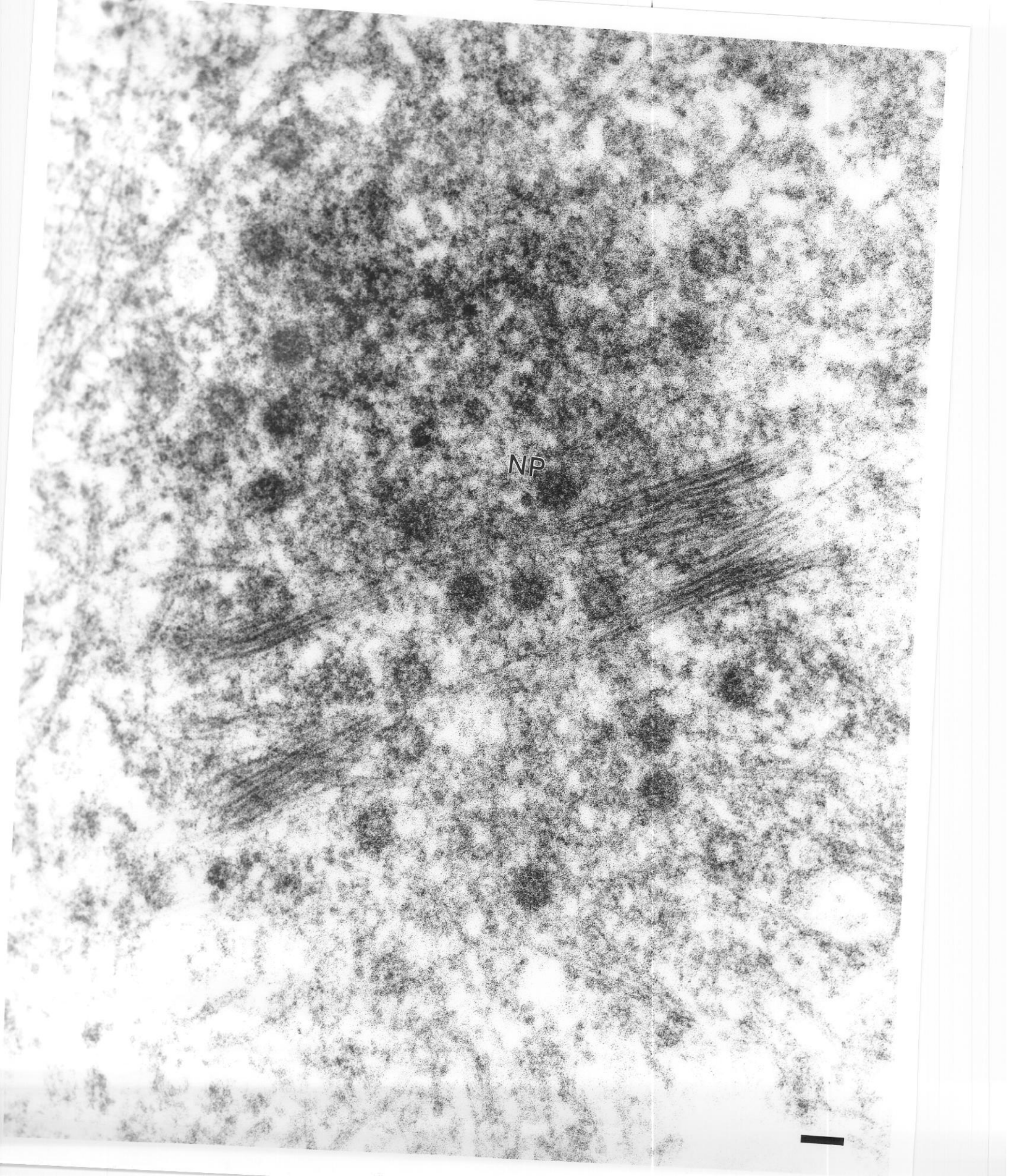
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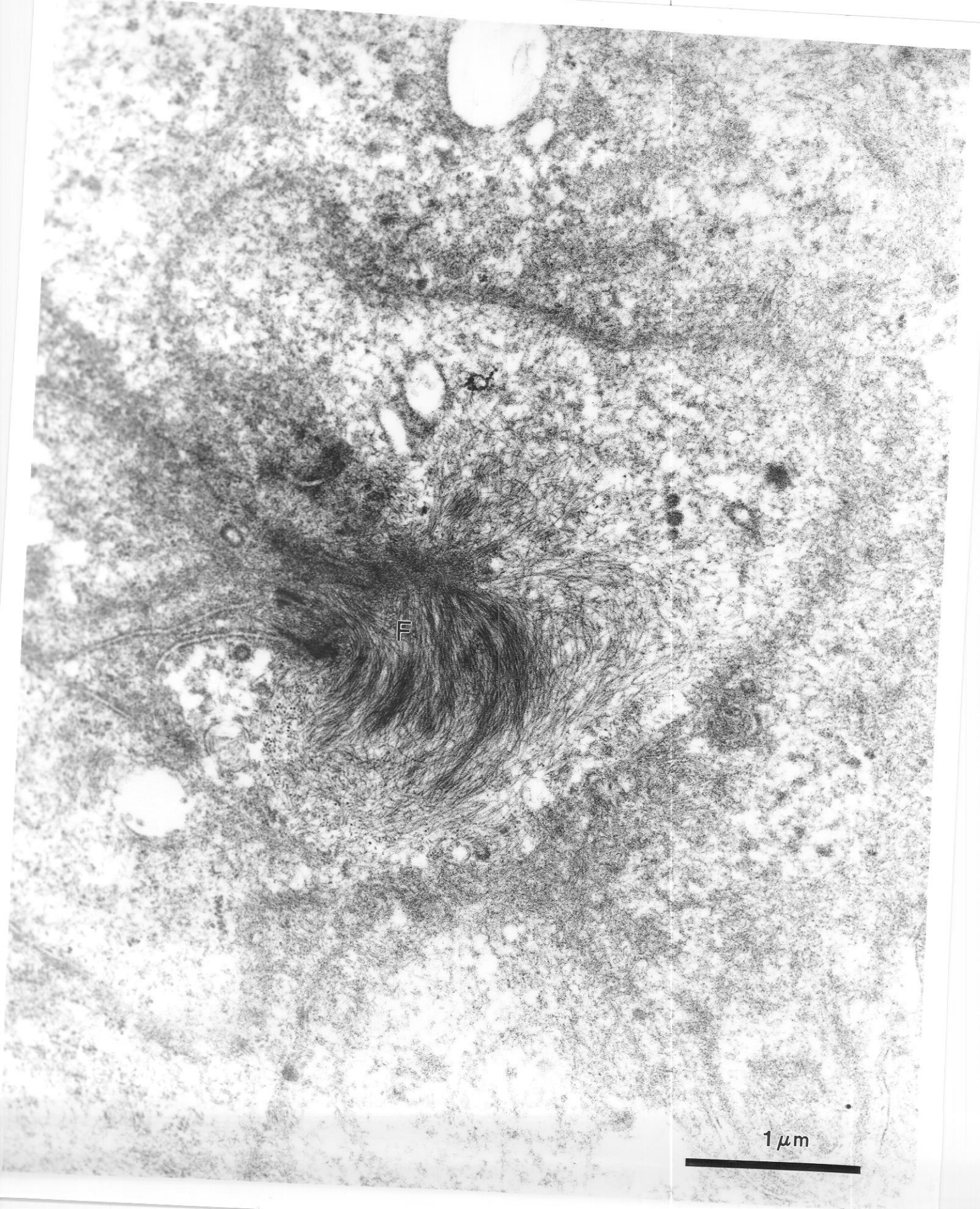
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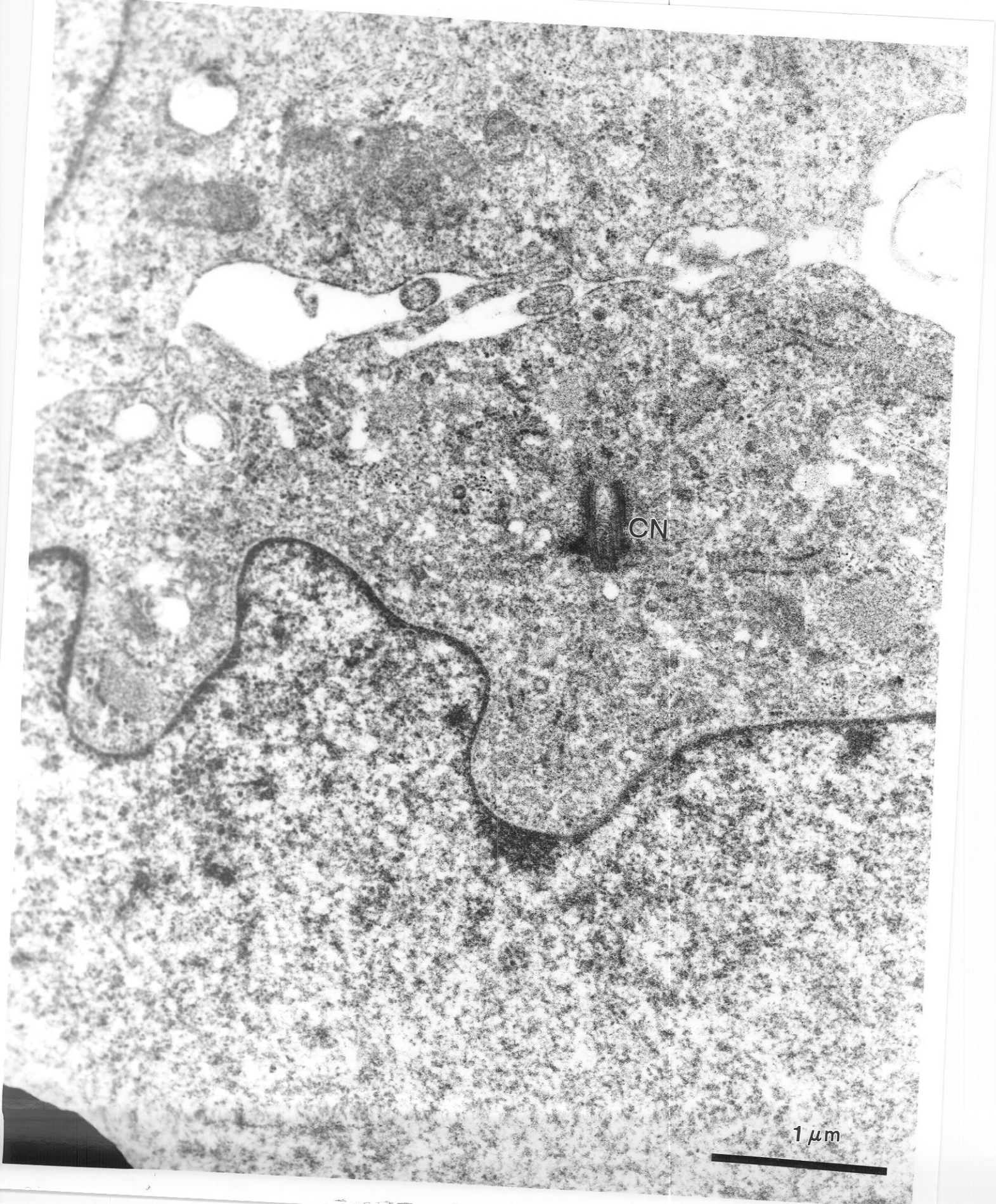
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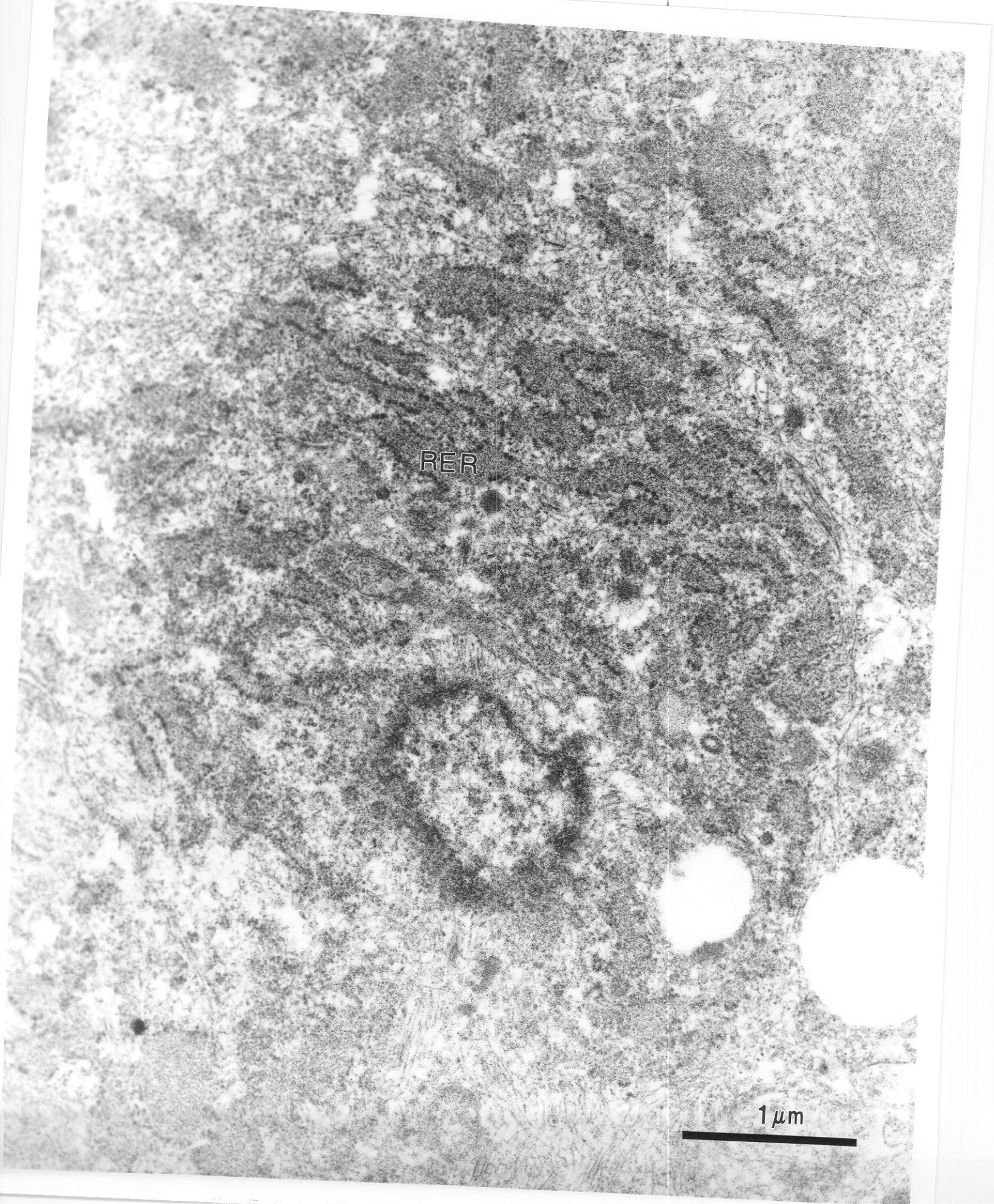
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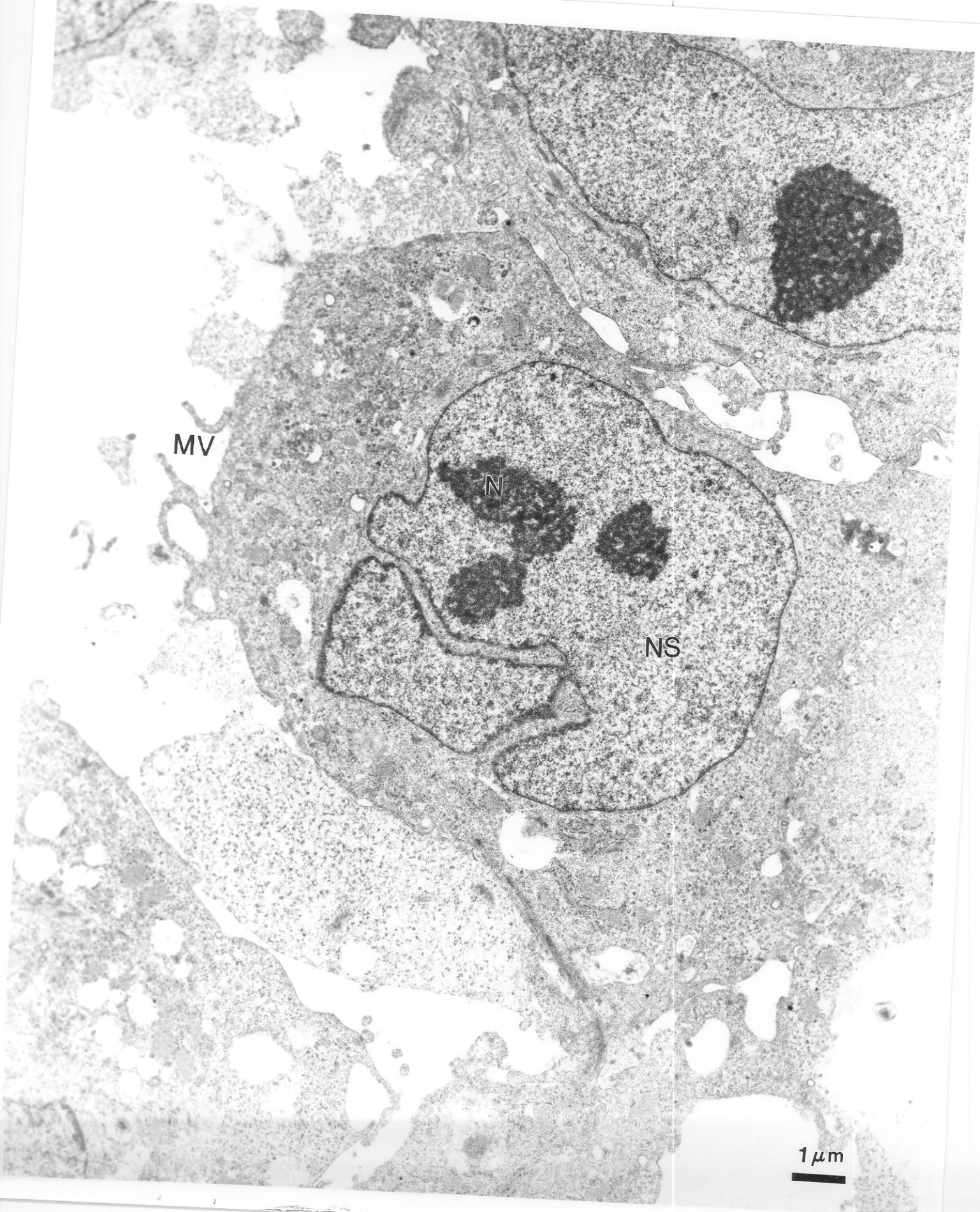
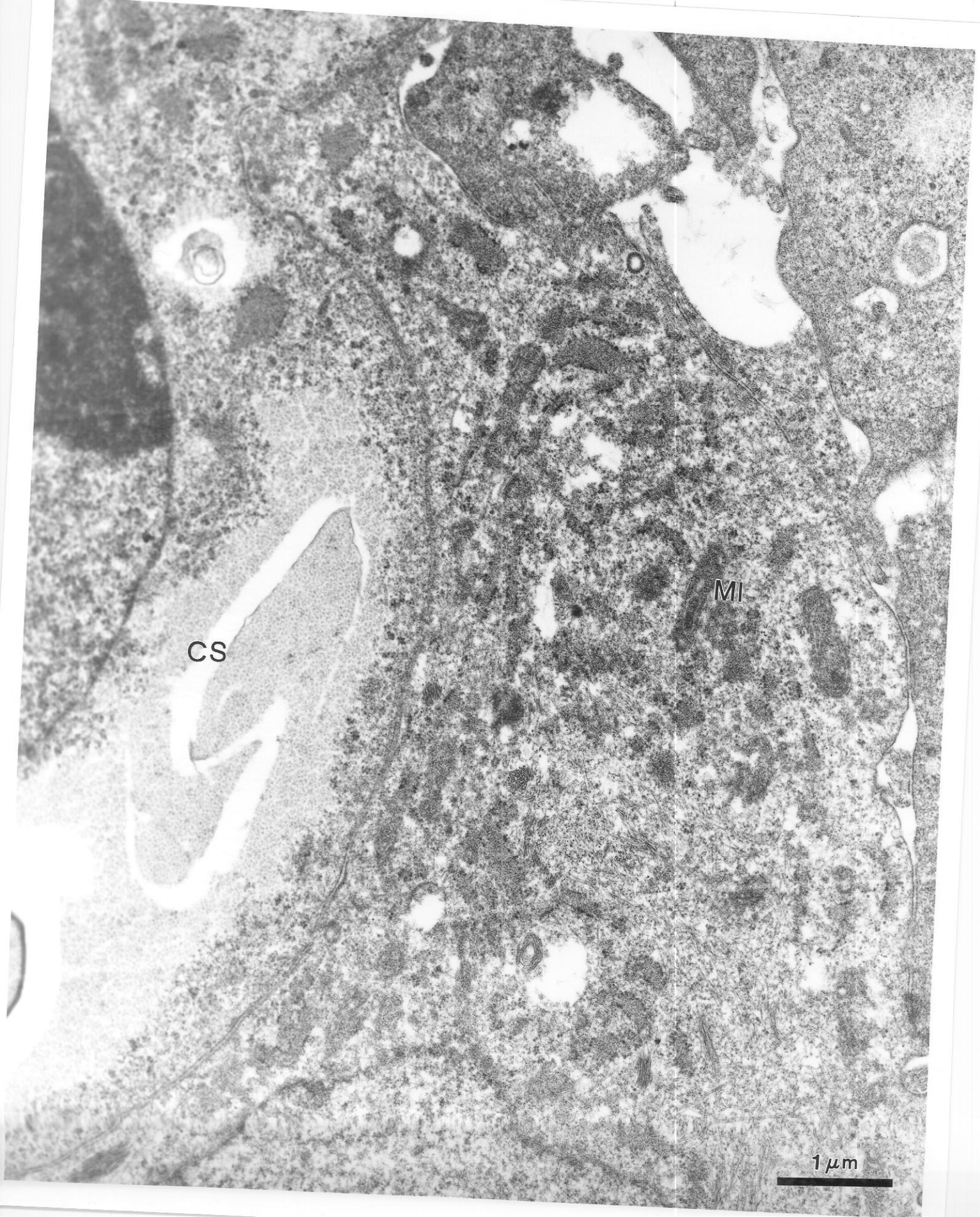
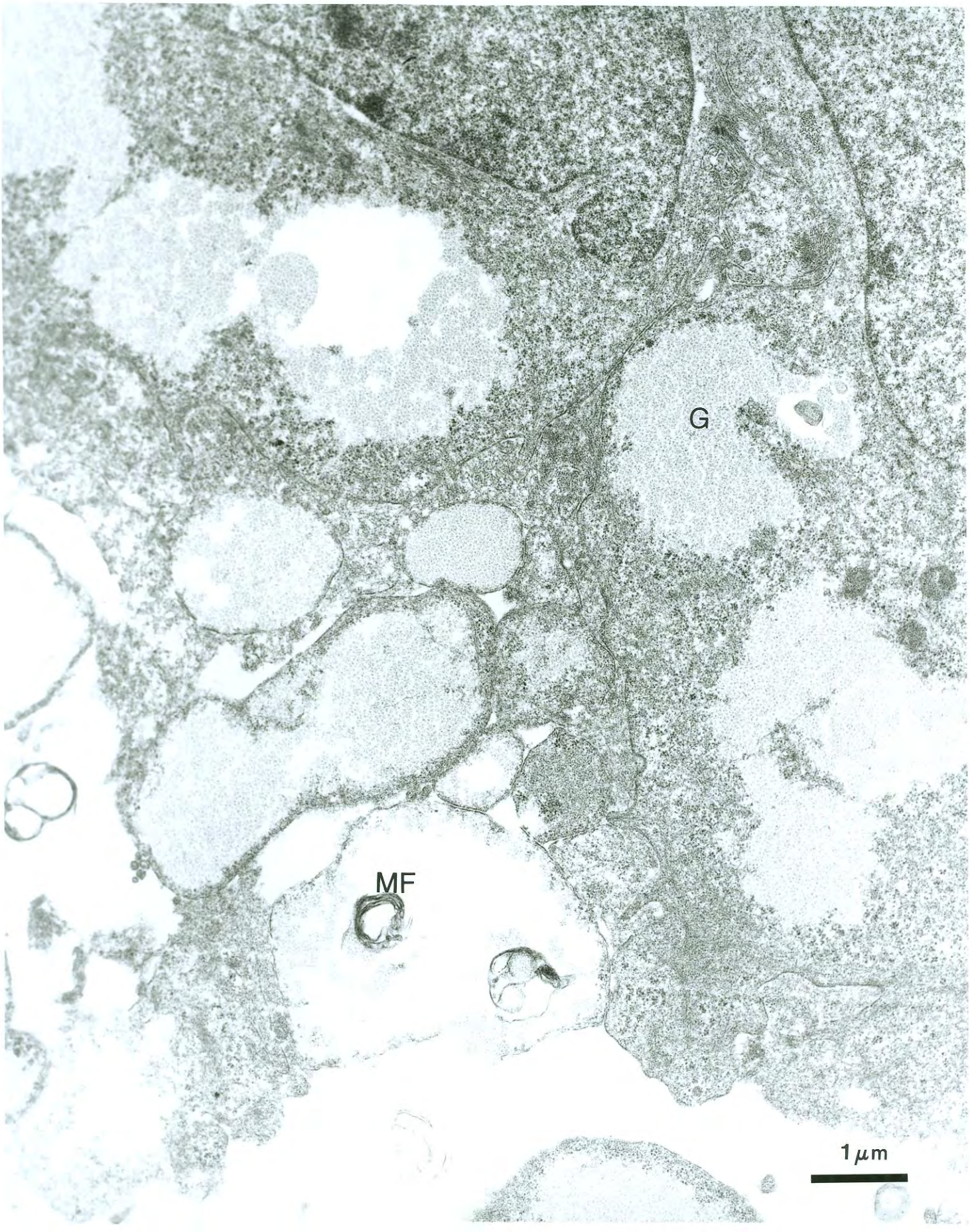

MV
N
NS
1 μm


CS
MI
1 μm


G
MF
1 μm

E-FR-1-07-001589.30201

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
ES03-MCB.1 1590-APT	07-001589

SPONSOR: WiCell Research Institute

PERFORMING LABORATORY: AppTec, Inc.

APPTEC ACCESSION NUMBER	RESULTS
07-001589	Evidence for xenotropic, amphotropic, or MCF MuLV retroviral contamination was found in the test article. Following co-cultivation the test article demonstrated a positive 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 4.9.4 Remove the growth medium from all test article flasks.	August 6, 2007	August 29, 2007	August 29, 2007

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

[redacted]
Quality Assurance

01 Nov 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.

[redacted]
Study Director

11-1-07
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 Research Institute

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

4.0 SCHEDULING

DATE SAMPLES RECEIVED: July 24, 2007
STUDY INITIATION DATE: July 24, 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: ES03-MCB.1 1590-APT

7.0 TEST SYSTEM DESCRIPTION

In the generation of retroviral vectors for gene therapy, it has become necessary to assay for replication-competent retroviruses (RCRs) 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 sensitive to various classes of the murine retroviruses is the method of choice to detect any potential RCRs 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, however, will not replicate in *Mus dunni* cells; if an RCR is suspected to 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 2 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 RCRs 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 Three aliquots of the test article supernatant were saved for testing in the PG4 S+L- assay as a T_0 time point.

8.1.3 Equal numbers of the *Mus dunni* cells ($5x10^5$ cells) and the test article cells ($5x10^5$ cells) were mixed to initiate the co-cultivation.

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 On day 14, cell culture supernatants were collected from the negative control, test article, and positive control cultures. 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 3 dilutions (1:10, 1:100, and 1:1000).

8.2.3 The assay positive control was inoculated onto PG4 cells, utilizing a few dilutions of the virus (1:1000 and 1:10,000). The 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 July 24, 2007, AppTec, Inc. received 1 flask of "hES cells grown with mouse embryonic fibroblast feeder cells," at room temperature and designated for use in this assay. The test article was stored at 37±2°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) 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 is considered valid if supernatant samples derived from negative control co-cultivation cultures are negative for retroviral growth in the PG4 S^+L^- and if the positive cultures inoculated with A-MuLV demonstrate a positive reaction in the PG4 S^+L^- assay.

12.2 Validity Criteria for PG4 S^+L^- Assay

The test is considered valid if no foci are observed in the negative control and the positive control displays viral-specific focus formation.

13.0 TEST EVALUATION

Co-cultivation of the test article cells with detector cells is considered positive if cell culture supernatants harvested after day 14 demonstrate 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 supernatants 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 positive PG4 S^+L^- result.

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

	Culture Inoculum	Time	FFU/mL
Co-Cultivation Samples	**Accession # 07-001589[1] (diluted 1:2)**	**T_0**	**ND**
	Accession # 07-001589 (diluted 1:2)	**PP2**	**4**
	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	$1.3x10^5$

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

Evidence of xenotropic, amphotropic, or MCF MuLV retroviral contamination was detected in the test article.

16.0 STATISTICAL DATA ANALYSIS

Statistical analysis of the data was not required.

17.0 DEVIATIONS / AMENDMENTS

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

No amendments to the protocol were generated.

18.0 RECORD RETENTION

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

19.0 REFERENCES

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

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

POLYMERASE CHAIN REACTION ASSAY FOR THE DETECTION OF HUMAN IMMUNODEFICIENCY VIRUS TYPES 1 AND 2 (HIV-1/2) IN BIOLOGICAL SAMPLES

Study Number: AC06AE.105010.BSV

Test Article ID: ES03-MCB.1 / 1590-BIR

Sponsor:

Authorized Representative:

CONCLUSION

One-half (0.5) μg of DNA (representing approximately 7.5 x 10^4 cells) isolated from test article ES03-MCB.1 / 1590-BIR was analyzed for the presence of human immunodeficiency virus types 1 and 2 (HIV-1/2) proviral DNA by the polymerase chain reaction (PCR)[1] technique. The assay can detect 100 copies of HIV-1/2 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 HIV-1/2 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: ES03-MCB.1 / 1590-BIR was received by BioReliance on 07/25/2007. 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: 07/26/2007

Lab Initiation: 07/31/2007

Lab Completion: 08/03/2007

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

Study Director: Ph.D.

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 HIV-1/2 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

The test system consists of two independent PCR assays for the detection of HIV-1 or HIV-2, respectively. For detection of HIV-1 proviral DNA, PCR amplification is performed on test article extract using HIV-1-specific primers. In the presence of HIV-1 proviral sequences, these primers produce a 115 bp amplification product. For detection of HIV-2 proviral DNA, PCR amplification is performed on test article extract using HIV-2-specific primers. In the presence of HIV-2 proviral sequences, these primers produce a 196 bp amplification product. The amplification products are analyzed by agarose gel electrophoresis in the presence of ethidium bromide. The following controls are included in each assay:

Negative Control: Genomic DNA from MRC5 human fetal lung fibroblasts.
Source: BioReliance.

Positive Control:

HIV-1: Negative control DNA spiked with 100 copies of pSYC1857, a plasmid containing the complete genome of HIVZ6 with an interruption in the protease coding region.
Source: Perkin-Elmer

HIV-2: Negative control DNA spiked with 100 copies of pMAHIV2, a plasmid containing a 963 bp fragment from the HIV-2 proviral genome.
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.

HIV-1: Test article extract spiked with 100 copies of pSYC1857.

HIV-2: Test article extract spiked with 100 copies of pMAHIV2.

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 either primers SK38 and SK39, specific for the core protein coding region of HIV-1, or primers OG63 and OG81, specific for the core protein coding region of HIV-2, 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 (SOP OPBT0922 and OPBT0933).

RESULTS

Test article DNA (0.5 µg), representing approximately 7.5×10^4 test article cells, was analyzed for the presence of HIV-1/2 proviral DNA by PCR amplification and agarose gel electrophoresis in the presence of ethidium bromide. The results are shown in Figure 1a (HIV-1) and 1b (HIV-2).

In Figure 1a, the No DNA control (NO) and Negative control (NC) showed no bands at 115 bp. The positive control (PC) produced a 115 bp band. The test article spiked with 100 copies of pSYC1857 (TAS) produced a 115 bp band, demonstrating that the test article did not inhibit the PCR reaction. The test article (TA) produced no bands at 115 bp.

In Figure 1b, the No DNA control (NO) and Negative control (NC) showed no bands at 196 bp. The positive control (PC) produced a 196 bp band. The test article spiked with 100 copies of pMAHIV2 (TAS) produced a 196 bp band, demonstrating that the test article did not inhibit the PCR reaction. The test article (TA) produced no bands at 196 bp.

These results provide evidence that the test article ES03-MCB.1 / 1590-BIR tested negative for the presence of HIV-1/2 proviral DNA.

APPROVAL

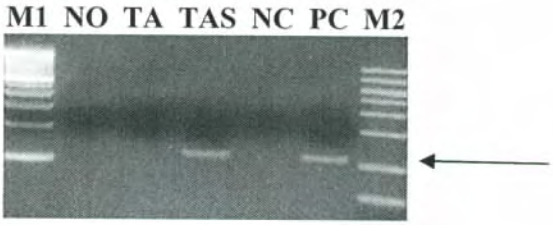
I accept responsibility for the conduct of this study which was performed in compliance with the U.S. FDA Good Laboratory Practice regulations (21 CFR 58), the UK GLP Regulations, the Japanese GLP Standard and the Organization for Economic Cooperation and Development (OECD) Principles of Good Laboratory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

Study Director
Ph.D.

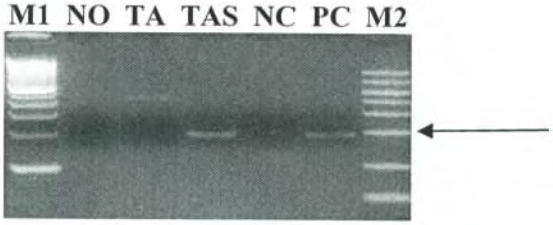
16-Aug-07
Date

FIGURE 1

a. HIV-1



b. HIV-2



Detection of HIV-1 (**a.**) or HIV-2 (**b.**) proviral sequences in the test article ES03-MCB.1 / 1590-BIR utilizing agarose gel electrophoresis in the presence of ethidium bromide. Arrows indicate specific amplification products.

M1: 100 bp DNA ladder
NO: No DNA control
TA: Test Article
TAS: Test article spiked with 100 copies pSYC1857 (**a.**) or pMAHIV2 (**b.**)
NC: Negative control (MRC5 genomic DNA)
PC: Positive control (negative control DNA spiked with 100 copies pSYC1857 (**a.**) or pMAHIV2 (**b.**)
M2: Biomarker low DNA size marker.

Quality Assurance Statement

Study Title: POLYMERASE CHAIN REACTION ASSAY FOR THE DETECTION OF HUMAN IMMUNODEFICIENCY VIRUS TYPES 1 AND 2 (HIV-1/2) IN BIOLOGICAL SAMPLES

Study Number: AC06AE.105010.BSV

Study Director: Ph.D.

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	03-Aug-07 - 03-Aug-07 To Study Dir 03-Aug-07 To Mgmt 03-Aug-07
	Phase	Data Audit
**	Inspect On	16-Aug-07 - 16-Aug-07 To Study Dir 16-Aug-07 To Mgmt 17-Aug-07
	Phase	Final Report and data audit
*	Inspect On	28-Jun-07 - 28-Jun-07 To Study Dir 28-Jun-07 To Mgmt 28-Jun-07
	Phase	Systems Inspection - Test System Preparation
*	Inspect On	28-Jun-07 - 28-Jun-07 To Study Dir 28-Jun-07 To Mgmt 28-Jun-07
	Phase	Systems Inspection - Administration of Test Substance to Test System
*	Inspect On	28-Jun-07 - 28-Jun-07 To Study Dir 28-Jun-07 To Mgmt 28-Jun-07
	Phase	Systems Inspection - Manipulation of Test System
*	Inspect On	28-Jun-07 - 28-Jun-07 To Study Dir 28-Jun-07 To Mgmt 28-Jun-07
	Phase	Systems Inspection - Observation of Test System/Data Collection and/or Analysis

** 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.

B.S.
QUALITY ASSURANCE

August 17, 2007
DATE

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: AC06AE.105013.BSV

Test Article ID: ES03-MCB.1 / 1590-BIR

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 ES03-MCB.1 / 1590-BIR 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: ES03-MCB.1 / 1590-BIR was received by BioReliance on 07/25/2007. 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: 07/26/2007

Lab Initiation: 07/31/2007

Lab Completion: 08/01/2007

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

Study Director: Ph.D.

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 0.5µg 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 (0.5µg), 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.

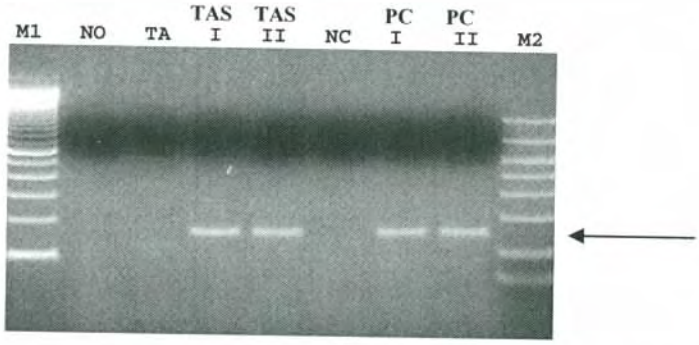
These results provide evidence that the test article ES03-MCB.1 / 1590-BIR tested negative for the presence of HTLV-I/II proviral DNA.

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.

Ph.D. _____ 22-Aug-07
Study Director Date

FIGURE 1



Detection of HTLV-I/II proviral sequences in test article ES03-MCB.1 / 1590-BIR utilizing agarose gel electrophoresis in the presence of ethidium bromide

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.

The arrow indicates specific amplification products.

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: AC06AE.105013.BSV

Study Director: Ph.D.

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	03-Aug-07 - 03-Aug-07 To Study Dir 03-Aug-07 To Mgmt 03-Aug-07
	Phase	Data Audit
**	Inspect On	22-Aug-07 - 22-Aug-07 To Study Dir 22-Aug-07 To Mgmt 22-Aug-07
	Phase	Final Report and data audit
*	Inspect On	28-Jun-07 - 28-Jun-07 To Study Dir 28-Jun-07 To Mgmt 28-Jun-07
	Phase	Systems Inspection - Test System Preparation
*	Inspect On	28-Jun-07 - 28-Jun-07 To Study Dir 28-Jun-07 To Mgmt 28-Jun-07
	Phase	Systems Inspection - Administration of Test Substance to Test System
*	Inspect On	28-Jun-07 - 28-Jun-07 To Study Dir 28-Jun-07 To Mgmt 28-Jun-07
	Phase	Systems Inspection - Manipulation of Test System
*	Inspect On	28-Jun-07 - 28-Jun-07 To Study Dir 28-Jun-07 To Mgmt 28-Jun-07
	Phase	Systems Inspection - Observation of Test System/Data Collection and/or Analysis

** 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.

_____ 8/22/07

QUALITY ASSURANCE DATE

Final Report

POLYMERASE CHAIN REACTION ASSAY FOR THE DETECTION OF HEPATITIS B VIRUS (HBV) IN BIOLOGICAL SAMPLES

Study Number: AC06AE.105042.BSV

Test Article ID: ES03-MCB.1 / 1590-BIR

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

One-half (0.5) µg of DNA isolated from test article ES03-MCB.1 / 1590-BIR (representing approximately 7.5 x 10^4 cells) was analyzed for the presence of Hepatitis B virus (HBV) DNA by the polymerase chain reaction (PCR)[1] technique. The assay can detect 100 copies of HBV 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 HBV DNA.

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1 The Polymerase Chain Reaction (PCR) process is covered by U.S. patents Nos. 4,683,195 and 4,683,202 owned by Roche Molecular Systems, Inc. and F. Hoffman-LaRoche Ltd.

STUDY INFORMATION

Test Article: ES03-MCB.1 / 1590-BIR was received by BioReliance on 07/25/2007. 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: 07/26/2007

Lab Initiation: 07/31/2007

Lab Completion: 08/03/2007

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

Study Director: Ph.D.

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 HBV DNA in the test article by PCR amplification, an *in vitro* procedure that can generate millions of copies of a sequence from very low levels of template DNA.

Test System:

PCR amplification is performed on test article DNA using HBV-specific primers. In the presence of HBV, these primers produce a 347 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 HBV negative cells Source: BioReliance
Positive Control:	Negative control DNA spiked with 100 copies of HBV185, a plasmid containing a 1850 bp fragment from the HBV core antigen sequence Source: BioReliance
No DNA Control:	Nuclease-free water Source: USB or other commercial source.
Spiked Control:	Test article extract spiked with 100 copies of HBV185, 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 HBV-C2 and HBV-C3 specific for the HBV core antigen sequence, employing conditions optimized to achieve detection of 100 copies of HBV. 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 OPBT0953).

RESULTS

Test article DNA (0.5µg), representing approximately 7.5 x 10^4 test article cells, was analyzed for the presence of HBV 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 347 bp. The positive control (PC) produced a 347 bp band. The test article spiked with 100 copies of HBV185 (TAS) produced a 347 bp band, demonstrating that the test article did not inhibit the PCR reaction. The test article (TA) produced no bands at 347 bp.

These results provide evidence that the test article ES03-MCB.1 / 1590-BIR tested negative for the presence of HBV DNA.

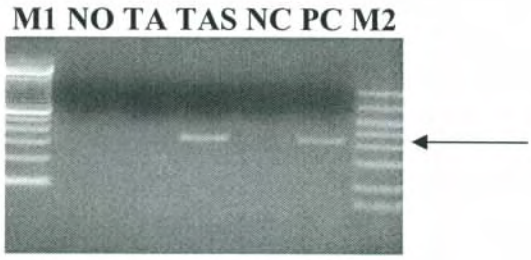
APPROVAL

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.

16-Aug-07
Date

Study Director

FIGURE 1



Detection of HBV specific sequences in the test article ES03-MCB.1 / 1590-BIR 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 HBV185
NC: Negative control
PC: Positive control (NC spiked with 100 copies HBV185)
M2: Biomarker low DNA size marker

Arrow indicates the specific amplification product.

Quality Assurance Statement

Study Title: POLYMERASE CHAIN REACTION ASSAY FOR THE DETECTION OF HEPATITIS B VIRUS IN BIOLOGICAL SAMPLES

Study Number: AC06AE.105042.BSV

Study Director: Ph.D.

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	03-Aug-07 - 03-Aug-07 To Study Dir 03-Aug-07 To Mgmt 03-Aug-07
	Phase	Data Audit
**	Inspect On	16-Aug-07 - 16-Aug-07 To Study Dir 16-Aug-07 To Mgmt 16-Aug-07
	Phase	Final Report and data audit
*	Inspect On	28-Jun-07 - 28-Jun-07 To Study Dir 28-Jun-07 To Mgmt 28-Jun-07
	Phase	Systems Inspection - Test System Preparation
*	Inspect On	28-Jun-07 - 28-Jun-07 To Study Dir 28-Jun-07 To Mgmt 28-Jun-07
	Phase	Systems Inspection - Administration of Test Substance to Test System
*	Inspect On	28-Jun-07 - 28-Jun-07 To Study Dir 28-Jun-07 To Mgmt 28-Jun-07
	Phase	Systems Inspection - Manipulation of Test System
*	Inspect On	28-Jun-07 - 28-Jun-07 To Study Dir 28-Jun-07 To Mgmt 28-Jun-07
	Phase	Systems Inspection - Observation of Test System/Data Collection and/or Analysis

** Inspection specific for this study
* Systems Inspection

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

_____ 14 Aug 07
DATE

QUALITY ASSURANCE

Final Report

RT-PCR ASSAY FOR THE DETECTION OF HEPATITIS C VIRUS (HCV)

Study Number: AC06AE.105025.BSV

Test Article ID: ES03-MCB.1 / 1590-BIR

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

RNA isolated from test article ES03-MCB.1 / 1590-BIR was analyzed for the presence of Hepatitis C virus (HCV) RNA by the reverse-transcriptase polymerase chain reaction (RT-PCR)[1] technique. The assay can detect 100 copies of HCV in the presence of 0.5 µg of genomic RNA.

The results presented herein indicate that the test article tested negative for the presence of HCV RNA.

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[1] The Polymerase Chain Reaction (PCR) process is covered by U.S. patents Nos. 4,683,195 and 4,683,202 owned by Roche Molecular Systems, Inc. and F. Hoffman-LaRoche Ltd.

STUDY INFORMATION

Test Article: ES03-MCB.1 / 1590-BIR was received by BioReliance on 07/25/2007. 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: 07/26/2007

Lab Initiation: 07/30/2007

Lab Completion: 08/06/2007

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

Study Director: Ph.D.

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 HCV viral RNA sequences in the test article by RT-PCR amplification. RT-PCR is a standard procedure in which a specific fragment of RNA is reverse transcribed to generate cDNA, which is then amplified *in vitro* by PCR to generate many more DNA copies of the fragment.

Test System:

PCR amplification is performed on 0.5 µg of test article RNA using HCV-specific primers. In the presence of wild type HCV RNA sequences, these primers produce a 257 bp amplicon. In the presence of positive control RNA sequences, these primers produce a 144 bp amplicon. The amplification products are analyzed by agarose gel electrophoresis in the presence of ethidium bromide. The assay is performed according to SOP OPBT0956. The following controls are included in the assay:

Negative Control:	RNA (0.5 µg) from HCV negative cells. Source: BioReliance
Positive Control:	Negative control RNA spiked with 100 copies of p19-100D RNA transcript. Source: BioReliance
No RNA Control:	Nuclease-free water to verify the absence of contamination in the RT-PCR reagents. Source: USB or other commercial source.
Spiked Control:	Test article extract spiked with 100 copies of p19-100D, to verify the absence of PCR inhibitors in the test article RNA (amplification suitability control).

METHODS

Sample Preparation

The test article was received at BioReliance and provided to the laboratory for testing. RNA was isolated from the test article sample using the RNeasy ® RNA Isolation Kit (Qiagen) as outlined in the kit procedure and SOP OPBT0968.

RT-PCR

RT-PCR amplification was performed on the test article using primers HCV-F3 and HCV-R2 specific to the highly conserved fragment of the 5' non-coding region of the HCV RNA genome, employing conditions optimized to achieve detection of 100 copies of HCV. 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 (SOPs BPBT0784 and OPBT0956).

REPEAT

The first performance of the assay was invalid as the No RNA control did not meet the acceptance criteria. The assay was repeated and produced a valid test with a negative result. The results are presented in the results section below.

RESULTS

The test article was analyzed for the presence of HCV viral RNA by RT-PCR amplification and agarose gel electrophoresis in the presence of ethidium bromide. The results are shown in Figure 1. The No RNA control (NO) and Negative control (NC) showed no bands at 144 or 257 bp. The positive control (PC) produced a 144 bp band. The test article spiked with 100 copies of p19-100D (TAS) produced a 144 bp band, demonstrating that the test article did not inhibit the PCR reaction. The test article (TA) produced no bands at 144 or 257 bp.

These results provide evidence that the test article ES03-MCB.1 / 1590-BIR tested negative for the presence of HCV RNA.

APPROVAL

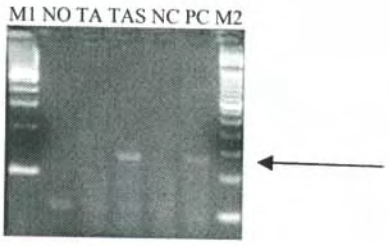
I accept responsibility for the conduct of this study which was performed in compliance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the UK GLP Regulations, the Japanese GLP Standard and the Organization for Economic Cooperation and Development (OECD) Principles of Good Laboratory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

_____ 16-Aug-07

Ph.D. Date

Study Director

FIGURE 1



Detection of HCV RNA in the test article ES03-MCB.1 / 1590-BIR utilizing agarose gel electrophoresis in the presence of ethidium bromide.

M1: 100 bp DNA ladder
NO: No RNA control
TA: Test Article
TAS: Test article spiked with 100 copies p19-100D
NC: Negative control
PC: Positive control (NC spiked with 100 copies p19-100D)
M2: 50 bp DNA ladder

Arrow indicates the specific amplification product of the positive control.

Quality Assurance Statement

Study Title: RT-PCR ASSAY FOR THE DETECTION OF HEPATITIS C VIRUS (HCV)

Study Number: AC06AE.105025.BSV

Study Director: Ph.D.

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	06-Aug-07 - 06-Aug-07 To Study Dir 06-Aug-07 To Mgmt 06-Aug-07
	Phase	Data Audit
**	Inspect On	16-Aug-07 - 16-Aug-07 To Study Dir 16-Aug-07 To Mgmt 17-Aug-07
	Phase	Final Report and data audit
*	Inspect On	28-Jun-07 - 28-Jun-07 To Study Dir 28-Jun-07 To Mgmt 28-Jun-07
	Phase	Systems Inspection - Test System Preparation
*	Inspect On	28-Jun-07 - 28-Jun-07 To Study Dir 28-Jun-07 To Mgmt 28-Jun-07
	Phase	Systems Inspection - Administration of Test Substance to Test System
*	Inspect On	28-Jun-07 - 28-Jun-07 To Study Dir 28-Jun-07 To Mgmt 28-Jun-07
	Phase	Systems Inspection - Manipulation of Test System
*	Inspect On	28-Jun-07 - 28-Jun-07 To Study Dir 28-Jun-07 To Mgmt 28-Jun-07
	Phase	Systems Inspection - Observation of Test System/Data Collection and/or Analysis

** 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.

August 17, 2007
DATE

QUALITY ASSURANCE

Final Report

POLYMERASE CHAIN REACTION ASSAY FOR THE DETECTION OF CYTOMEGALOVIRUS (CMV) IN BIOLOGICAL SAMPLES

Study Number: AC06AE.105012.BSV

Test Article ID: ES03-MCB.1 / 1590-BIR

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

One-half (0.5) μg of DNA isolated from test article ES03-MCB.1 / 1590-BIR (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.

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1 The Polymerase Chain Reaction (PCR) process is covered by U.S. patents Nos. 4,683,195 and 4,683,202 owned by Roche Molecular Systems, Inc. and F. Hoffman-LaRoche Ltd.

STUDY INFORMATION

Test Article: ES03-MCB.1 / 1590-BIR was received by BioReliance on 07/25/2007. 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: 07/26/2007

Lab Initiation: 07/31/2007

Lab Completion: 08/01/2007

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

Study Director: Ph.D.

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 commerical 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 0.5 µg 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).

RESULTS

Test article DNA (0.5 µg), representing approximately 7.5 x 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 ES03-MCB.1 / 1590-BIR tested negative for the presence of CMV DNA.

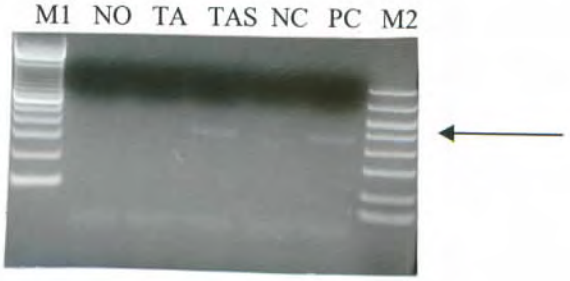
APPROVAL

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.

_____ 16-Aug-07

Study Director Ph.D. Date

FIGURE 1



Detection of CMV specific sequences in the test article ES03-MCB.1 / 1590-BIR 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: AC06AE.105012.BSV

Study Director: Ph.D.

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	03-Aug-07 - 03-Aug-07 To Study Dir 03-Aug-07 To Mgmt 03-Aug-07
	Phase	Data Audit
**	Inspect On	16-Aug-07 - 16-Aug-07 To Study Dir 16-Aug-07 To Mgmt 17-Aug-07
	Phase	Final Report and data audit
*	Inspect On	28-Jun-07 - 28-Jun-07 To Study Dir 28-Jun-07 To Mgmt 28-Jun-07
	Phase	Systems Inspection - Test System Preparation
*	Inspect On	28-Jun-07 - 28-Jun-07 To Study Dir 28-Jun-07 To Mgmt 28-Jun-07
	Phase	Systems Inspection - Administration of Test Substance to Test System
*	Inspect On	28-Jun-07 - 28-Jun-07 To Study Dir 28-Jun-07 To Mgmt 28-Jun-07
	Phase	Systems Inspection - Manipulation of Test System
*	Inspect On	28-Jun-07 - 28-Jun-07 To Study Dir 28-Jun-07 To Mgmt 28-Jun-07
	Phase	Systems Inspection - Observation of Test System/Data Collection and/or Analysis

** 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.

August 17, 2007
DATE

QUALITY ASSURANCE

Final Report

POLYMERASE CHAIN REACTION ASSAY FOR THE DETECTION OF EPSTEIN BARR VIRUS (EBV) IN BIOLOGICAL SAMPLES

Study Number: AC06AE.105011.BSV

Test Article ID: ES03-MCB.1 / 1590-BIR

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

One-half (0.5) μg of DNA isolated from test article ES03-MCB.1 / 1590-BIR (representing approximately 7.5 x 10^4 cells) was analyzed for the presence of Epstein Barr virus (EBV) DNA by the polymerase chain reaction (PCR)[1] technique. The assay can detect 10 copies of EBV 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 EBV 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: ES03-MCB.1 / 1590-BIR was received by BioReliance on 07/25/2007. 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: 07/26/2007

Lab Initiation: 07/31/2007

Lab Completion: 08/02/2007

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

Study Director: Ph.D.

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 EBV DNA in the test article by PCR amplification, an *in vitro* procedure that can generate millions of copies of a sequence from very low levels of template DNA.

Test System:

PCR amplification is performed on test article DNA using EBV-specific primers. In the presence of EBV, these primers produce a 376 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 BamW, a plasmid containing the BamW fragment from the IR1 region of the EBV genome. Since the EBV genome includes approximately 10 tandem repeats of the IR1 region, 100 copies of BamW plasmid are approximately equivalent to 10 copies of EBV genome. Source: BioReliance
No DNA Control:	Nuclease-free water Source: USB or other commercial source
Spiked Control:	Test article extract spiked with 100 copies of BamW, 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 TC58 and TC61 specific for the BamW region of EBV, employing conditions optimized to achieve detection of 10 copies of EBV. 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 OPBT0934).

RESULTS

Test article DNA (0.5µg), representing approximately 7.5 x 10^4 test article cells, was analyzed for the presence of EBV 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 376 bp. The positive control (PC) produced a 376 bp band. The test article spiked with 100 copies of BamW (TAS) produced a 376 bp band, demonstrating that the test article did not inhibit the PCR reaction. The test article (TA) produced no bands at 376 bp.

These results provide evidence that the test article ES03-MCB.1 / 1590-BIR tested negative for the presence of EBV DNA.

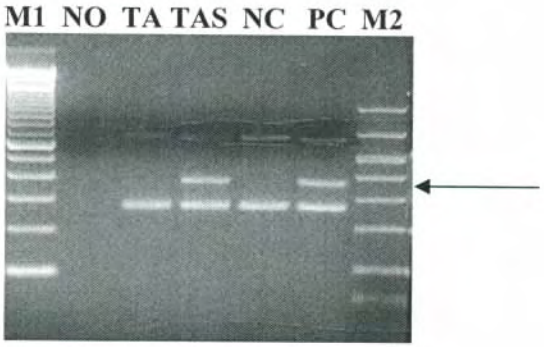
APPROVAL

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.

16-Aug-07
Date

Study Director

FIGURE 1



Detection of EBV specific sequences in the test article ES03-MCB.1 / 1590-BIR utilizing agarose gel electrophoresis in the presence of ethidium bromide

M1: 100 bp DNA ladder
NO: No DNA control
NC: Negative control (MRC5 DNA)
PC: Positive control (MRC5 DNA spiked with 100 copies BamW)
TA: Test Article
TAS: Test article spiked with 100 copies BamW
M2: Biomarker low DNA size marker

Arrow indicates specific amplification product.

Quality Assurance Statement

Study Title: POLYMERASE CHAIN REACTION ASSAY FOR THE DETECTION OF EPSTEIN BARR VIRUS (EBV) IN BIOLOGICAL SAMPLES

Study Number: AC06AE.105011.BSV

Study Director: Ph.D.

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	03-Aug-07 - 03-Aug-07 To Study Dir 03-Aug-07 To Mgmt 03-Aug-07
	Phase	Data Audit
**	Inspect On	15-Aug-07 - 16-Aug-07 To Study Dir 16-Aug-07 To Mgmt 17-Aug-07
	Phase	Final Report and data audit
*	Inspect On	28-Jun-07 - 28-Jun-07 To Study Dir 28-Jun-07 To Mgmt 28-Jun-07
	Phase	Systems Inspection - Test System Preparation
*	Inspect On	28-Jun-07 - 28-Jun-07 To Study Dir 28-Jun-07 To Mgmt 28-Jun-07
	Phase	Systems Inspection - Administration of Test Substance to Test System
*	Inspect On	28-Jun-07 - 28-Jun-07 To Study Dir 28-Jun-07 To Mgmt 28-Jun-07
	Phase	Systems Inspection - Manipulation of Test System
*	Inspect On	28-Jun-07 - 28-Jun-07 To Study Dir 28-Jun-07 To Mgmt 28-Jun-07
	Phase	Systems Inspection - Observation of Test System/Data Collection and/or Analysis

** 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.

August 17, 2007
DATE

QUALITY ASSURANCE

Final Report

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

Study Number: AC06AE.105020.BSV

Test Article ID: ES03-MCB.1 / 1590-BIR

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 ES03-MCB.1 / 1590-BIR 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.

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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:	ES03-MCB.1 / 1590-BIR was received by BioReliance on 07/25/2007. 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:	07/26/2007
Lab Initiation:	07/31/2007
Lab Completion:	08/02/2007
Study Completion:	See Study Director's signature date in "Approval" Section.
Study Director:	Ph.D.
Archives:	All raw data, the protocol, and a copy of the final report will be maintained according to Standard Operating Procedure OPQP3040 by the BioReliance Quality Assurance Unit headquartered at: BioReliance

OBJECTIVE

The objective of the study is to detect the presence of HHV-6 viral sequences in the test article by PCR amplification, an *in vitro* procedure that can generate millions of copies of a sequence from very low levels of template DNA.

TEST SYSTEM

For detection of HHV-6 viral DNA, PCR amplification is performed on test article DNA using HHV-6-specific primers according to SOP BPBT0924. In the presence of HHV-6A viral sequences, these primers produce a 328 bp amplification product, while the HHV-6A positive control plasmid (pU1102MOD) generates a 299 bp amplification product. In the presence of HHV-6B viral sequences, the primers produce a 553 bp amplification product, while the HHV-6B positive control plasmid (pZ29MOD) generates a 524 bp amplification product. The following controls are included in the assay:

Negative Control: Genomic DNA from MRC5 human fetal lung fibroblasts
Source: BioReliance

Positive controls:

HHV-6A: Negative control DNA spiked with 100 copies of plasmid pU1102MOD. Plasmid pU1102MOD contains a 2.3 Kb region from the HHV-6A (strain U1102) genome. The 2.3 Kb region contains a 29 bp internal deletion to distinguish it from the wild type HHV-6A sequence.
Source: BioReliance

HHV-6B: Negative control DNA spiked with 100 copies of plasmid pZ29MOD. Plasmid pZ29MOD contains a 2.3 Kb region from the HHV-6B (strain Z29) genome. The 2.3 Kb region contains a 29 bp internal deletion to distinguish it from the wild type HHV-6B sequence.
Source: BioReliance

No DNA Control: Nuclease free water
Source: USB or other commercial supplier

Spiked Controls: The spiked controls (amplification suitability controls) verify the absence of PCR inhibitors in the test article DNA.

HHV-6A: Test article spiked with 100 copies of plasmid pU1102MOD

HHV-6B: Test article spiked with 100 copies of plasmid pZ29MOD

Following amplification, samples will be run on a 1.5 - 2.5% Metaphor or Agarose gel containing ethidium bromide and visualized by photography under ultraviolet light.

METHODS

Sample Preparation

The test article was received at BioReliance and provided to the laboratory for testing. DNA was isolated using the Easy DNA™ Kit (Invitrogen) as outlined in the kit procedure and SOP OPBT0920.

DNA Amplification

PCR amplification was performed on 0.5µg 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 ES03-MCB.1 / 1590-BIR tested negative for the presence of HHV-6 (variants A and B) viral DNA.

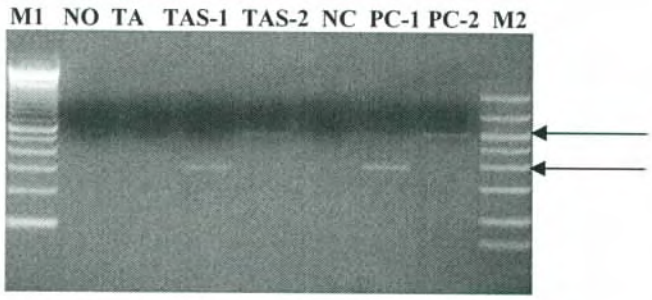
APPROVAL

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.

16-Aug-07
Date

Study Director

FIGURE 1



Detection of HHV-6 (variants A and B) viral sequences in test article ES03-MCB.1 / 1590-BIR 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: AC06AE.105020.BSV

Study Director: Ph.D.

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	03-Aug-07 - 03-Aug-07 To Study Dir 03-Aug-07 To Mgmt 03-Aug-07
	Phase	Data Audit
**	Inspect On	16-Aug-07 - 16-Aug-07 To Study Dir 16-Aug-07 To Mgmt 16-Aug-07
	Phase	Final Report and data audit
*	Inspect On	28-Jun-07 - 28-Jun-07 To Study Dir 28-Jun-07 To Mgmt 28-Jun-07
	Phase	Systems Inspection - Test System Preparation
*	Inspect On	28-Jun-07 - 28-Jun-07 To Study Dir 28-Jun-07 To Mgmt 28-Jun-07
	Phase	Systems Inspection - Administration of Test Substance to Test System
*	Inspect On	28-Jun-07 - 28-Jun-07 To Study Dir 28-Jun-07 To Mgmt 28-Jun-07
	Phase	Systems Inspection - Manipulation of Test System
*	Inspect On	28-Jun-07 - 28-Jun-07 To Study Dir 28-Jun-07 To Mgmt 28-Jun-07
	Phase	Systems Inspection - Observation of Test System/Data Collection and/or Analysis

** 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.

16Aug07

DATE

QUALITY ASSURANCE

Final Report

POLYMERASE CHAIN REACTION ASSAY FOR THE DETECTION OF HUMAN HERPESVIRUS 7 (HHV-7) IN BIOLOGICAL SAMPLES

Study Number: AC06AE.105029.BSV

Test Article ID: ES03-MCB.1 / 1590-BIR

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

One-half (0.5) µg of DNA isolated from test article ES03-MCB.1 / 1590-BIR (representing approximately 7.5 x 10^4 cells) was analyzed for the presence of Human Herpesvirus 7 (HHV-7) DNA by the polymerase chain reaction (PCR)[1] technique. The assay can detect 100 copies of HHV-7 in the presence of 0.5 µg of genomic DNA.

The results presented herein indicate that the test article tested negativefor the presence of HHV-7 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: ES03-MCB.1 / 1590-BIR was received by BioReliance on 07/25/2007. 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: 07/26/2007

Lab Initiation: 07/31/2007

Lab Completion: 08/02/2007

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

Study Director: Ph.D.

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

BioReliance

OBJECTIVE

The objective of the study is to detect HHV-7 DNA in the test article by PCR amplification, an *in vitro* procedure that can generate millions of copies of a sequence from very low levels of template DNA.

Test System:

PCR amplification is performed on test article DNA using HHV-7-specific primers. In the presence of HHV-7 DNA, these primers produce a 353 bp amplification product. The amplification products are analyzed by high resolution agarose gel electrophoresis in the presence of ethidium bromide. The following controls are included in the assay:

Negative Control:	Genomic DNA from MRC5 human fetal lung fibroblasts Source: BioReliance
Positive Control:	Negative control DNA spiked with 100 copies of pHH7, a plasmid containing a 1.2 Kb fragment of the HHV-7 genome Source: BioReliance.
No DNA Control:	Nuclease-free water Source: USB or other commercial source.
Spiked Control:	Test article extract spiked with 100 copies of pHH7, to verify the absence of PCR inhibitors in the test article DNA, (amplification suitability control)

METHODS

Sample Preparation

The test article was received at BioReliance, and provided to the laboratory for testing. DNA was isolated from the test article sample using the Easy DNA™ Kit (Invitrogen) as outlined in the kit procedure and SOP BPBT0920.

DNA Amplification

PCR amplification was performed on 0.5 µg of test article DNA using primers HHV7F and HHV7IR specific for sequences common to the capsid protein gene regions in the HHV-7 genome, employing conditions optimized to achieve detection of 100 copies of HHV-7 DNA. Aliquots of the amplification products obtained from the test article and from the control samples were analyzed by high resolution agarose gel electrophoresis in the presence of ethidium bromide. Amplification products were visualized on a UV transilluminator and photographed using Polaroid 667 film (SOP OPBT0929).

RESULTS

Test article DNA (0.5 µg), representing approximately 7.5 x 10^4 test article cells, was analyzed for the presence of HHV-7 DNA by PCR amplification and high resolution agarose gel electrophoresis in the presence of ethidium bromide. The results are shown in Figure 1. The No DNA control (NO) showed no amplification bands. The Negative Control (NC) showed no bands at 353 bp. The Positive Control (PC) produced a 353 bp band. The test article spiked with 100 copies of pHH7 (TAS) produced a 353 bp band, demonstrating that the test article did not inhibit the PCR reaction. The test article (TA) produced no bands at 353 bp.

These results provide evidence that the test article ES03-MCB.1 / 1590-BIR tested negative for the presence of HHV-7 DNA.

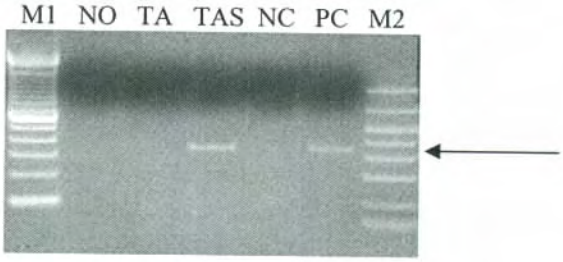
APPROVAL

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

16-Aug-07
Date

Study Director

FIGURE 1



Detection of HHV-7 specific sequences in the test article ES03-MCB.1 / 1590-BIR utilizing agarose gel electrophoresis in the presence of ethidium bromide. Arrow indicates the 353 bp amplification product.

M1: 100 bp DNA ladder.
NO: No DNA control.
TA: Test Article.
TAS: Test article spiked with 100 copies pHH7.
NC: Negative control (MRC5 genomic DNA).
PC: Positive control (negative control DNA spiked with 100 copies pHH7).
M2: Biomarker low DNA size marker.

Quality Assurance Statement

Study Title: POLYMERASE CHAIN REACTION ASSAY FOR THE DETECTION OF HUMAN HERPESVIRUS (HHV-7) IN BIOLOGICAL SAMPLES

Study Number: AC06AE.105029.BSV

Study Director: Ph.D.

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	03-Aug-07 - 03-Aug-07 To Study Dir 03-Aug-07 To Mgmt 03-Aug-07
	Phase	Data Audit
**	Inspect On	16-Aug-07 - 16-Aug-07 To Study Dir 16-Aug-07 To Mgmt 17-Aug-07
	Phase	Final Report and data audit
*	Inspect On	28-Jun-07 - 28-Jun-07 To Study Dir 28-Jun-07 To Mgmt 28-Jun-07
	Phase	Systems Inspection - Test System Preparation
*	Inspect On	28-Jun-07 - 28-Jun-07 To Study Dir 28-Jun-07 To Mgmt 28-Jun-07
	Phase	Systems Inspection - Administration of Test Substance to Test System
*	Inspect On	28-Jun-07 - 28-Jun-07 To Study Dir 28-Jun-07 To Mgmt 28-Jun-07
	Phase	Systems Inspection - Manipulation of Test System
*	Inspect On	28-Jun-07 - 28-Jun-07 To Study Dir 28-Jun-07 To Mgmt 28-Jun-07
	Phase	Systems Inspection - Observation of Test System/Data Collection and/or Analysis

** 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.

August 17, 2007
DATE

QUALITY ASSURANCE

Final Report

PCR ASSAY FOR THE DETECTION OF HUMAN HERPESVIRUS TYPE 8 (HHV-8)

Study Number: AC06AE.105056.BSV

Test Article ID: ES03-MCB.1 / 1590-BIR

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

One-half (0.5) µg of DNA isolated from test article ES03-MCB.1 / 1590-BIR (representing approximately 7.5 x 10^4 cells) was analyzed for the presence of Human Herpesvirus 8 (HHV-8) DNA by the polymerase chain reaction (PCR)[1] technique. The assay can detect 100 copies of HHV-8 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-8 DNA.

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1 The Polymerase Chain Reaction (PCR) is covered by U.S. patents Nos. 4,683,195 and 4,683,202 owned by Hoffman-LaRoche, Inc., licensed by BioReliance, from Perkin-Elmer Cetus Instruments.

STUDY INFORMATION

Test Article: ES03-MCB.1 / 1590-BIR was received by BioReliance on 07/25/2007. 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: 07/26/2007

Lab Initiation: 07/31/2007

Lab Completion: 08/03/2007

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

Study Director: Ph.D.

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

BioReliance

OBJECTIVE

The objective of the study is to detect HHV-8 DNA in the test article by PCR amplification, an *in vitro* procedure that can generate millions of copies of a sequence from very low levels of template DNA.

Test System:

PCR amplification is performed on test article DNA using HHV-8-specific primers. In the presence of HHV-8 DNA, these primers produce a 225 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 HHV-8-negative source Source: BioReliance
Positive Control:	Negative control DNA spiked with 100 copies of pHHV-8, a plasmid containing a conserved fragment from the latency associated nuclear antigen (LANA) from the HHV-8 genome Source: BioReliance
No DNA Control:	Nuclease-free water Source: USB or other commercial source
Spiked Control:	Test article extract spiked with 100 copies of pHHV-8, 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 OPBT0920.

DNA Amplification

PCR amplification was performed on 0.5µg of test article DNA using primers HHV-8F and HHV-8R specific for sequences of the latency associated nuclear antigen (LANA) in the HHV-8 genome, employing conditions optimized to achieve detection of 100 copies of HHV-8 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 (OPBT0974).

RESULTS

Test article DNA (0.5µg), representing approximately 7.5 x 10^4 test article cells, was analyzed for the presence of HHV-8 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 amplification bands and Negative Control (NC) showed no band at 225 bp. The Positive Control (PC) produced a 225 bp band. The test article spiked with 100 copies of pHHV-8 (TAS) produced a 225 bp band, demonstrating that the test article did not inhibit the PCR reaction. The test article (TA) produced no band at 225 bp.

These results provide evidence that the test article ES03-MCB.1 / 1590-BIR tested negative for the presence of HHV-8 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.

16-Aug-07
Date

Study Director

FIGURE 1

Detection of HHV-8 specific sequences in the test article ES03-MCB.1 / 1590-BIR 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 pHHV-8
NC: Negative control (genomic DNA)
PC: Positive control (negative control DNA spiked with 100 copies pHHV-8)
M2: Biomarker low DNA size marker

Arrow indicates the 225 bp amplification product.

Quality Assurance Statement

Study Title: PCR ASSAY FOR THE DETECTION OF HUMAN HERPESVIRUS TYPE 8 (HHV-8)

Study Number: AC06AE.105056.BSV

Study Director: Ph.D.

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	03-Aug-07 - 03-Aug-07 To Study Dir 03-Aug-07 To Mgmt 03-Aug-07
	Phase	Data Audit
**	Inspect On	16-Aug-07 - 16-Aug-07 To Study Dir 16-Aug-07 To Mgmt 17-Aug-07
	Phase	Final Report and data audit
*	Inspect On	28-Jun-07 - 28-Jun-07 To Study Dir 28-Jun-07 To Mgmt 28-Jun-07
	Phase	Systems Inspection - Test System Preparation
*	Inspect On	28-Jun-07 - 28-Jun-07 To Study Dir 28-Jun-07 To Mgmt 28-Jun-07
	Phase	Systems Inspection - Administration of Test Substance to Test System
*	Inspect On	28-Jun-07 - 28-Jun-07 To Study Dir 28-Jun-07 To Mgmt 28-Jun-07
	Phase	Systems Inspection - Manipulation of Test System
*	Inspect On	28-Jun-07 - 28-Jun-07 To Study Dir 28-Jun-07 To Mgmt 28-Jun-07
	Phase	Systems Inspection - Observation of Test System/Data Collection and/or Analysis

** 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.

August 17, 2007
DATE

QUALITY ASSURANCE

Final Report

POLYMERASE CHAIN REACTION ASSAY FOR THE DETECTION OF HUMAN PARVOVIRUS B19 IN BIOLOGICAL SAMPLES

Study Number: AC06AE.105037.BSV

Test Article ID: ES03-MCB.1 / 1590-BIR

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

One-half (0.5) μg of DNA isolated from test article ES03-MCB.1 / 1590-BIR (representing approximately 7.5 x 10^4 cells) was analyzed for the presence of human parvovirus B19 DNA by the polymerase chain reaction (PCR)[1] technique. The assay can detect 100 copies of B19 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 B19 DNA.

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1 PCR (Polymerase Chain Reaction) 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: ES03-MCB.1 / 1590-BIR was received by BioReliance on 07/25/2007. 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: 07/26/2007

Lab Initiation: 07/31/2007

Lab Completion: 08/02/2007

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

Study Director: Ph.D.

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 B19 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 B19-specific primers. In the presence of B19, these primers produce a 287 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 pNPS-1, a plasmid containing a 3.6 Kb fragment from the B19 capsid gene. Source: BioReliance
No DNA Control:	Nuclease free water Source: USB or other commercial supplier.
Spiked Control:	Test article extract spiked with 100 copies of pNPS-1, 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 using the Easy DNA™ Kit (Invitrogen) as outlined in the kit procedure and SOP BPBT0920.

DNA Amplification

PCR amplification was performed on 0.5 µg of test article DNA and on the assay controls using primers B19F and B19R specific for the capsid gene of B19, employing conditions optimized to achieve detection of 100 copies of B19. 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 BPBT0936).

RESULTS

Test article DNA (0.5 µg), representing approximately 7.5×10^4 test article cells, was analyzed for the presence of B19 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 287 bp. The positive control (PC) produced a 287 bp band. The test article spiked with 100 copies of pNPS-1 (TAS) produced a 287 bp band, demonstrating that the test article did not inhibit the PCR reaction. The test article (TA) produced no bands at 287 bp.

These results provide evidence that the test article ES03-MCB.1 / 1590-BIR tested negative for the presence of B19 DNA.

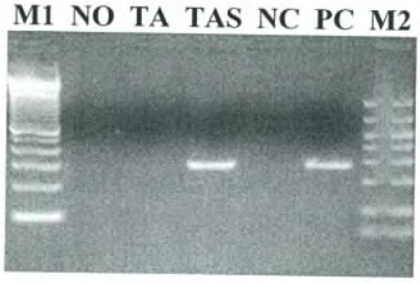
APPROVAL

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.

21-Aug-07
Date

Study Director

FIGURE 1



Detection of B19 specific sequences in the test article ES03-MCB.1 / 1590-BIR 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 of pNPS-1
NC: Negative control (MRC5 genomic DNA)
PC: Positive control (negative control DNA spiked with 100 copies of pNPS-1
M2: Biomarker low DNA size marker.

Arrow indicates the specific amplification product.

Quality Assurance Statement

Study Title: POLYMERASE CHAIN REACTION ASSAY FOR THE DETECTION OF HUMAN PARVOVIRUS B19 IN BIOLOGICAL SAMPLES

Study Number: AC06AE.105037.BSV

Study Director: Ph.D.

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	03-Aug-07 - 03-Aug-07 To Study Dir 03-Aug-07 To Mgmt 03-Aug-07
	Phase	Data Audit
**	Inspect On	21-Aug-07 - 21-Aug-07 To Study Dir 21-Aug-07 To Mgmt 21-Aug-07
	Phase	Final Report and data audit
*	Inspect On	28-Jun-07 - 28-Jun-07 To Study Dir 28-Jun-07 To Mgmt 28-Jun-07
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** 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.

21 Aug 07
DATE

QUALITY ASSURANCE

WiCell CGH Report: 000144 NSCB# 1590

Report Date: *April 7, 2009*

Case Details:

Cell Line: *ES03 p68 (Female)*

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

Investigator: *National Stem Cell Bank*

Specimen: *hES cells on MEFs*

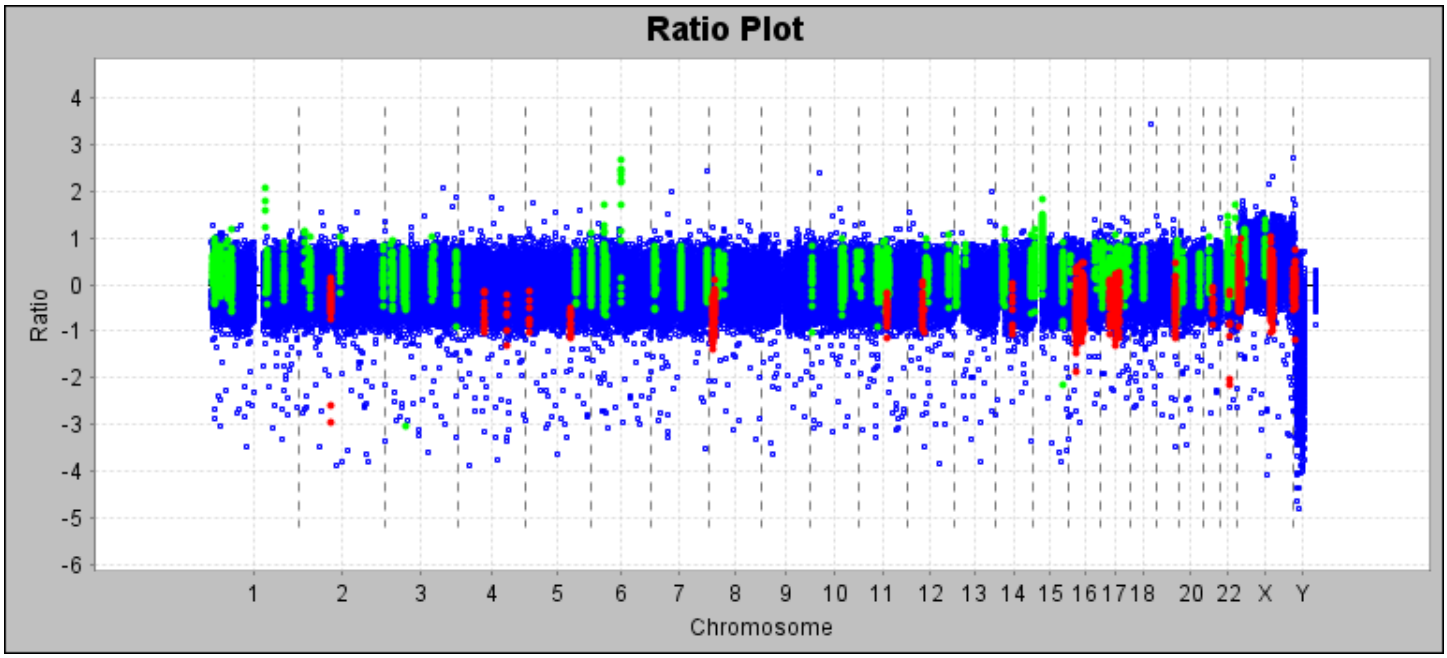
Date of Sample: *7/23/2007*

Reason for Testing: *NSCB Testing*

GEO Accession #: *GSM346758*

Results:

Results are given in the attached Excel spreadsheet labeled "report." There were 122 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]	*5 of 8*
Select Differentially Expressed Genes	*2 of 45*

These results are consistent with karyotype results [46,XX] as reported in 1590-KAR.

Test sample gain or loss is consistent with the opposite gender reference standard. Additional analysis of this data was performed using different ratio settings and different window averaging with no informative results.

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. Settings for data analysis in Infoquant include an average log-ratio threshold of 0.2 and no minimum aberration length.*
- *Raw data is deposited in GEO, accession number shown above.*
- *Reported gains and losses are based on test to reference ratios within OneClickCGH™, size of aberration, 8-9 probes per gene, and coverage of at least one reported gene or overlap with the DGV.*

Limitations: *This assay will detect aneuploidy, deletions, duplications of represented loci, but will not detect balanced alterations (reciprocal translocations, Robertsonian translocations, inversions, and insertions), point mutations, uniparental disomy or imbalances less than 30kb in size. Copy number variants can be attributable to the test or reference samples used. Exact limits of detectable mosaicism have not been determined, but >20% mosaicism is reported to be visualized by aCGH. Actual chromosomal localization of copy number change is not determined by this assay. Other mapping procedures are required for determining chromosomal localization.*

Literature Sources:

1. *Olshen, A., Venkatraman, E., Lucito, R., Wigler, M. (2004). Circular binary segmentation for the analysis of array-based DNA copy number data. Biostatistics, 5, 4, 557-572.*
2. *Internal Data, Unpublished.*
3. *Mumm, S., Molini, B., Terrell, J., Srivastava, A., Schlessinger, D. (1997). Evolutionary Features of the 4-Mb Xq21.3 XY Homology Region Revealed by a Map at 60-kb Resolution. Genome Research, 7, 307-314.*
4. *Adewumi, O., Aflatoonian A., Ahrlund-Richter L., Amit M., Andrews P., Beighton G., et al. (2007). Characterization of human embryonic stem cell lines by the International Stem Cell Initiative. Nature Biotechnology, 25, 803-816.*
5. *Werbowetski-Ogilvie, T., Bosse, M., Stewart, M., Schnerch, A., Ramos-Mejia, V., Rouleau A., et al. (2008). Characterization of human embryonic stem cells with features of neoplastic progression. Nature Biotechnology, 27, 91-97.*
6. *Wu, H., Kim, K., Mehta, K., Paxia, S., Sundstrom, A., Anantharaman, T., et al. (2008). Copy number variant analysis of human embryonic stem cells. Stem Cells, 26, 1484-1489.*

Recommendations: *For relevant findings, confirmation and localization is recommended. Contact cytogenetics@wicell.org to request further testing.*

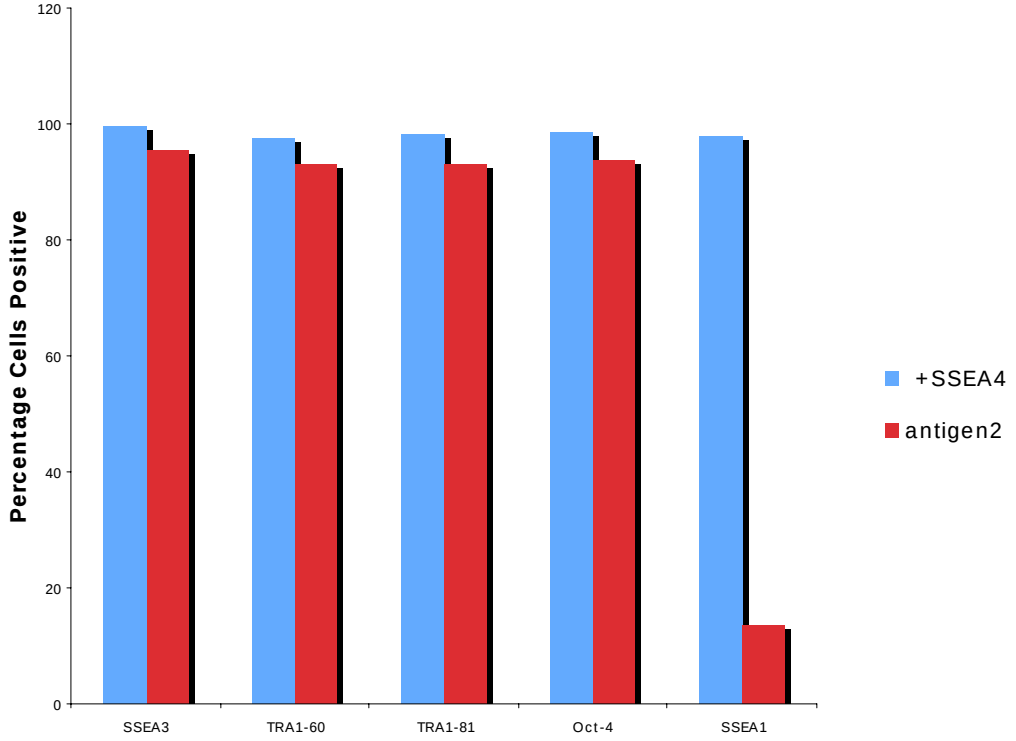
Results Transmitted by Fax / Email / Post **Date:**_____
Sent By:_____ **Sent To:**_____

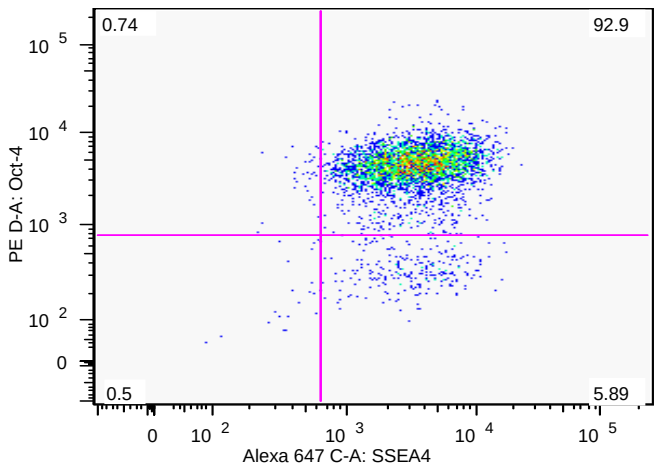
Characterization Report-
Flow Cytometry (FACS)

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

Cell Line: ES03	**Date of**: *(mm/dd/yy)*
Passage: p68	Acquisition: 07/30/07
Sample ID: 1590-FAC	File Creation: 07/30/07
File created by: EP	File Submission: 08/08/07

antigen2:	SSEA4 - antigen2 +	SSEA4 + antigen2 +	SSEA4 + antigen2 -	SSEA4 - antigen2 -	ALL SSEA4 +	ALL antigen2 +
SSEA3	0.088	95.3	4.21	0.44	99.51	95.388
TRA1-60	1.76	91.3	6.34	0.59	97.64	93.06
TRA1-81	1.28	91.8	6.38	0.56	98.18	93.08
Oct-4	0.74	92.9	5.89	0.5	98.79	93.64
SSEA1	0.089	13.5	84.6	1.81	98.1	13.589





Sample RNA: R00381	Reference DNA: D00073	Date of report: 111807
Sample Cell Line: ES03	Reference Cell Line: H1	Report prepared by: CY
Passage: 68	Passage:	QA Review: 11/20/07 EM
Lot#: ES03-MCB-1		Date sent to WiCell Iceland:
Sample ID: 1590-GEP		GEO accession #: GSM239976

1. Chip design: 2007-03-02_WiCell_HG18

2. Sample labeling:
   Cy5: 1590-GEP 2ug (Barcode: LR00351);
   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	28.53342428	Y
GABRB3	NM_000814	1.071428571	Y
GDF3	NM_020634	1.925876011	Y
NANOG	NM_024865	0.955905512	Y
POU5F1	NM_002701	40.49190647	Y
TDGF1	NM_003212	15.61355529	Y
Non-core ES markers			
BXDC2	NM_018321	5.656845754	Y
CD9	NM_001769	12.5561614	Y
FGF4	NM_002007	0.777777778	Y
FOXD3	NM_012183	3.829406986	Y
GAL	NM_015973	7.931851852	Y
GRB7	NM_001030002	2.651658768	Y
IFITM1	NM_003641	10.98809524	Y
LEFTY1	NM_020997	2.642237223	Y
LEFTY2	NM_003240	5.68907563	Y
LIN28	NM_024674	12.13896458	Y
NODAL	NM_018055	1.798726115	Y
PODXL	NM_001018111	9.686635945	Y
SOX2	NM_003106	13.18607595	Y
TERT	NM_198254	1.385405961	Y
UTF1	NM_003577	1.043543544	Y

5. Expression of differentiation markers:

Gene Symbol	Accession	Ratio	Expression
ACTC	NM_005159	2.297783934	Y
AFP	NM_001134	0.208333333	N
CDX2	NM_001265	0.219178082	N
CGB	NM_000737	0.139037433	N
COL1A1	NM_000088	0.779054917	Y
COL2A1	NM_001844	0.377652934	Y
EOMES	NM_005442	0.529829545	Y
FLT1	NM_002019	0.616935484	Y
FN1	NM_002026	12.55152395	Y
FOXA2	NM_021784	0.423413567	Y
GATA4	NM_002052	0.675995694	Y
GATA6	NM_005257	0.312399356	N
GCM1	NM_003643	0.210059172	N
IPF1	NM_000209	0.188957055	N
LAMA1	NM_005559	2.471098266	Y
NEUROD1	NM_002500	0.221674877	N
NKX2-5	NM_004387	0.358879275	Y
PAX6	NM_000280	0.28992629	N
PDHX	NM_003477	2.25281602	Y
SOX17	NM_022454	0.690414508	Y
SYP	NM_003179	0.78026534	Y
TNNI3	NM_000363	0.763416478	Y

Blood Services

American Red Cross

Together, we can save a life

10/25/07

SAMPLES: DNA from Cell Lines: Date received: 09/28/07

NSCB **1590** (TS07-0459) ES03
NSCB **6185** (TS07-0460) H9
NSCB **9592** (TS07-0461) H1
NSCB **5456** (TS07-0462) HSF1

INSTITUTION: WiCell Research Institute

TESTING REQUESTED: Genotype for *ABO* and *RH*

DNA TESTING PERFORMED: ***RH:*** PCR-multiplex analysis for *RHD* exons 4, 7, the inactivating *RHD* pseudogene and C/c genotyping. AS-PCR for *RHD-CE-D* exon 3 (455A>C). PCR-RFLP for E/e. ***ABO:*** Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) testing for nucleotide positions 261 (O^1), 467 (A^2), 703 (B), and 1096 (B and O^2).

DNA MOLECULAR RESULTS:

	Genotype	Predicted Phenotype
ES03-MCB-1 NSCB **1590:**	*ABO*O^1O^1; RHD; RHCE*Ce/Ce*	NSCB **1590:** Group O; RhD+, C+, c-, E-, e+
H9-MCB-1 NSCB **6185:**	*ABO*A^1O^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.

SBB, PhD — 10/25/07

Scientific Director — Molecular Biologist

THE MOLECULAR TEST METHODS WERE DEVELOPED, AND THEIR PERFORMANCE CHARACTERISTICS DETERMINED BY THE MOLECULAR RED CELL AND PLATELET TESTING LABORATORY AT THE AMERICAN RED CROSS PENN-JERSEY REGION. THE FDA HAS NOT REVIEWED OR APPROVED THE REAGENTS USED. THESE RESULTS ARE NOT INTENDED AS THE SOLE MEANS FOR CLINICAL DIAGNOSIS OR PATIENT MANAGEMENT DECISIONS. LIMITATIONS: The genotype may not always reflect the red cell phenotype. New mutations that inactivate gene expression or rare new variant alleles may not be identified in these assays.

Please Give Blood.