Product Information and Testing - Amended

Product Information

Product Name	TE04
Alias	I-4
Lot Number	TE04-MCB-01
Depositor	Technion
Banked by	WiCell
Thaw Recommendation	Thaw 1 vial into 1 well of a 6 well plate.
Culture Platform	Feeder Dependent
	Medium: hES Medium
	Matrix: MEF
Protocol	WiCell Feeder Dependent Protocol
Passage Number	p36 These cells were cultured for 35 passages prior to freeze. WiCell adds +1 to the passage number at freeze so that the number on the vial best represents the overall passage number of the cells at thaw.
Date Vialed	27-July-2008
Vial Label	TE04-MCB-1 p36 MW 27 JULY 2008 SOPCC035D
Biosafety and Use Information	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. Cells distributed by WiCell are intended for research purposes only and are not intended for use in humans.

Testing Performed by WiCell

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[1]
	[1]An anomalous band pattern has been observed in this TE04 cell lot for STR. See additional information regarding similar anomalies at: "A Genetic Basis for Anomalous Band Patterns Encountered During DNA STR Profiling", Clayton, T.M., et al. J. Forensic Sci, Nov. 2004, Vol. 49, No. 6. The STR anomalies were verified by 2 independent laboratories. Based on results from the standard G-band analysis, the karyotype of the cell line appears normal at the corresponding STR location.			
HLA profile	UW Molecular Diagnostics Laboratory	AlleleSEQR Kits by Abbott	Consistent with known profile	Pass[2]
	[2]This test was the first HLA performed for this cell line and therefore it establishes the HLA identity for this cell line.			
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 WuXi Apptec	105020 30863	Inconclusive results	See reports[3]
	[3]Cell line TE04 displays inconsistent results for HHV6 virus testing. Initial positive HHV-6 results based on a PCR assay could not be confirmed by an independent testing lab. As with all cell lines, universal precautions are required.			
HHV-7 by PCR	BioReliance	105029	Negative	Pass
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	[4]
	[4]Gene Expression Profile testing was part of the National Stem Cell Bank contract; it was for research/informational purposes only and was not completed for this lot.			
ABO and rH typing	American Red Cross	ABO/rH System	Report Blood type	O+

Product Information and Testing - Amended

Amendment(s):

Reason for Amendment	Date
22-September-2010	See signature
CoA updated for format changes, including adding fields of thaw recommendation, vial label, protocol, and banked by, and removal of footnotes.	09-JUL-2013
CoA updated for clarification of test specifications, test description, corrected sterility – direct transfer method test method, corrected date vialed and passage number, added HHV6 test method, and removed text regarding technical services and distribution of MCBs	23-SEP-2010
CoA updated for format changes, clarification of test specifications, test method, addition of test provider, culture platform, updated HHV6 footnote, electronic signature, and reference to WiCell instead of the NSCB	30-JUL-2010
Original CoA	30-OCT-2009

Date of Lot Release	Quality Assurance Approval
30-October-2009	9/30/2013 X AMC AMC Quality Assurance Signed by: [redacted]

UW Health

University of Wisconsin
Hospital and Clinics

Histocompatibility/Molecular Diagnostics Laboratory

Short Tandem Repeat Analysis*

Sample Report: 9113-STR UW HLA#: 61023 Sample Date: 05/28/09
Received Date: 05/28/09

Requestor: WiCell Research Institute
Test Date: 06/02/09 File Name: 090603 Report Date: 06/06/09

Sample Name: (label on tube)
9113-STR

Description: DNA Extracted by WiCell

247.15 ug/mL; 260/280 = 1.88

Locus	Repeat #	STR Genotype
D16S539	5, 8-15	11,12
D7S820	6-14	12,13
D13S317	7-15	12,14,15
D5S818	7-15	11,12
CSF1PO	6-15	12,12
TPOX	6-13	8,8
Amelogenin	NA	X,X
TH01	5-11	7,9
vWA	11, 13-21	16,18

Comments: Based on the 9113-STR DNA submitted by WI Cell dated 05/28/09 and received on 05/28/09, this sample (UW HLA# 61023) generally matches the STR profile of the human stem cell line TE04 comprising 14 allelic polymorphisms across the 8 STR loci analyzed. However, at the D13S317 loci, the 9113-STR DNA sample displays a reduced amplification efficiency of the expected 14 allele and strong amplification of an aberrant 15 allele. Other than this anomaly, no STR polymorphisms other than those corresponding to the human TE04 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 9113-STR DNA sample submitted corresponds to the TE04 stem cell line and while it does not appear to be contaminated with any other human stem cells or a significant amount of mouse feeder layer cells, this TEO4 cell line may be exhibiting some instability as noted by the unique findings at the D13S317 loci. Sensitivity limits for detection of STR polymorphisms unique to either this or other human stem cell lines is ~5%. These results were communicated via phone to of WiCell Research Institute on Friday, June 5, 2009.

6-10-09
Manager Date
HLA/Molecular Diagnostics Laboratory

06/08/09
PhD, Director Date
HLA/Molecular Diagnostics Laboratory

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

File: Final STR Report

UW Health
University of Wisconsin
Hospital and Clinics

Histocompatibility/Molecular Diagnostics Laboratory

Date: 09/12/2008 10:23:51

To: Cytogenetics, WiCell Research Institute

Re: High-resolution HLA results

Patient

Name HLA / MR# received	Method / Test date		HLA DNA-based typing* Method: PCR-SSP			Direct Sequencing				PCR-SSP
			A*	B*	C*	DRB1*	DRB3*	DRB4*	DRB5*	DQB1*
WICELL, 1962-HLA TE04	DQB SSP		0301/7/8/15/17	3502	0401/09N	1301				
59443 /	A,B,C Seq	09/08/2008	2402/7/21/53/56	3801	1203					
09/08/2008	DRB Seq	09/08/2008								

Manager
HLA/Molecular Diagnostics Laboratory

9-12-08 1025
Date

PhD, Director
HLA/Molecular Diagnostics Laboratory

Sept 16 2008
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.

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Test Facility:

WuXi AppTec

Report Number
786463
Page 1 of 1

WiCell Research Institute

September 15, 2008
P.O. #:

STERILITY TEST REPORT

Sample Information: hES Cells
TE04-MCB-1

Date Received: August 26, 2008
Date in Test: August 28, 2008
Date Completed: September 11, 2008

Test Information: Test Codes: 30744, 30744A
Immersion, USP / 21 CFR 610.12
Procedure #: BS210WCR.201

TEST PARAMETERS	PRODUCT	
Approximate Volume Tested	0.45 mL	0.45 mL
Number Tested	2	2
Type of Media	SCD	FTM
Media Volume	400 mL	400 mL
Incubation Period	14 Days	14 Days
Incubation Temperature	20 °C to 25 °C	30 °C to 35 °C
RESULTS	2 NEGATIVE	2 NEGATIVE

QA Reviewed: _____ **Reviewed:** _____

Testing conducted in accordance with current Good Manufacturing Practices.

Test Facility:

WuXi AppTec

Report Number
786464
Page 1 of 1

WiCell Research Institute

September 05, 2008
P.O. #:

GENERAL MICROBIOLOGY TEST REPORT

Sample Information: TE04-MCB-1, hES Cells

Date Received: August 26, 2008
Date in Test: August 29, 2008
Date Completed: September 02, 2008

Test Information:
Test Code: 30736
Sterility Test Validation (Bacteriostasis / Fungistasis)
Immersion, USP / 21 CFR 610.12
Procedure #: BS210WCR.201
Media Volume: 20 mL
Volume Tested: 0.025 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)	73	39	31
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)	96	75	47
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): 786463

QA Reviewed: _____ Reviewed: _____

Testing conducted in accordance with current Good Manufacturing Practices.

St. Paul

Report

WuXi AppTec

Copy of Original

FINAL STUDY REPORT

STUDY TITLE:	**MYCOPLASMA DETECTION: "Points to Consider" with Mycoplasmastasis**
PROTOCOL NUMBER:	31216C
TEST ARTICLE IDENTIFICATION:	TE04-MCB-1 #7856
SPONSOR:	WiCell Research Institute
PERFORMING LABORATORY:	WuXi AppTec, Inc.
STUDY NUMBER:	120300
RESULT SUMMARY:	Considered **negative** for mycoplasma contamination and **non-inhibitory** for the detection of mycoplasma

Reference PO #

WCR01

120300

QUALITY ASSURANCE UNIT SUMMARY

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

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

Critical Phase	Date	Study Director	Management
Inoculation of Coverslips	05/05/09	05/05/09	06/10/09
Final Report	06/09/09	06/09/09	06/10/09

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

Quality Assurance Auditor:___ _____ Date: 6/10/09

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 WuXi AppTec, Inc., are exempt from this Good Laboratory Practice Statement and include characterization and stability of the test compound(s)/test article.

Study Director:_____ _____ Date: 6-10-09

Professional Personnel Involved:

Vice President of St. Paul Operations
Manager, Mycoplasma Testing Laboratory
Study Director
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: WuXi AppTec, Inc.

4.0 SCHEDULING

DATE SAMPLE RECEIVED:	04/28/09
STUDY INITIATION DATE:	05/04/09
STUDY COMPLETION DATE:	06/10/09

5.0 TEST ARTICLE IDENTIFICATION: WiCell Research Institute
TE04-MCB-1 #7856

6.0 SAMPLE STORAGE

Upon receipt by the Sample Receiving Department, the test samples were placed in a designated, controlled access storage area ensuring proper temperature conditions. Test and control article storage areas are designed to preclude the possibility of mix-ups, contamination, deterioration or damage. The samples remained in the storage area until retrieved by the technician for sample preparation and/or testing. Unused test samples remained in the storage area until the study was completed. Once completed, the remaining samples were discarded or returned as requested by the Sponsor.

7.0 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 WuXi AppTec's Cell Production Laboratory.

8.0 EXPERIMENTAL DESIGN

8.1 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.

8.2 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."

9.0 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. 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.

10.0 TEST MATERIAL PREPARATION

10.1 Test Article Identification:

Test Article Name:	TE04-MCB-1 #7856
General Description:	hES Cells
Number of Aliquots used:	1 x 45 mL
Stability (Expiration):	Not Given
Storage Conditions:	Ultracold (< –60 °C)
Safety Precautions:	BSL-1
Intended Use/Application:	Not Given

10.2 Test Sample Preparation

The test article was thawed in a water bath at 37 ± 2 °C. 1:5 and 1:10 dilutions were prepared in sterile phosphate buffered saline (PBS). 1.0 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 Vero cells. The coverslips were incubated in incubator E770 for 1-2 hours at 36 ± 1 °C / 5 - 10% CO_2 and then 2.0 mL of EMEM, 8% fetal bovine serum (FBS) was added to each coverslip. The coverslips were returned to incubator E770 at 36 ± 1 °C / 5 - 10% 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 10.0 mL was inoculated into a 75 cm^2 flask containing 50 mL of SP-4 broth. The plates were incubated anaerobically at 36 ± 1 °C for a minimum of 14 days.

The broth flask was incubated aerobically at 36 ± 1 °C, and subcultured onto each of two SP-4 agar plates (0.2 mL/plate) on Days 3, 7, and 14. These subculture plates were incubated anaerobically at 36 ± 1 °C for a minimum of 14 days. The broth 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.

10.3 Preparation of Spiked Test Articles

10.3.1 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.

10.3.2 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.

10.3.3 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.

10.3.4 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.

10.3.5 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.

10.3.6 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.

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

10.4 Controls and Reference Materials

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

10.4.2 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.

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.

10.4.3 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. These coverslips served as the positive controls in the indirect assay.

b.2 The coverslips were incubated in incubator E950 for 1-2 hours at 36 ± 1 °C / 5 - 10% CO_2 and then 2 mL of EMEM, 8% Fetal Bovine Serum (FBS) was added to each coverslip. The coverslips were returned to incubator E950 at 36 ± 1 °C / 5 - 10% 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 incubated anaerobically 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 incubated anaerobically 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 15.0, Results, for the results of these controls.

11.0 DATA ANALYSIS

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

12.0 STATISTICAL METHODS

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

13.0 EVALUATION CRITERIA

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

13.1 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)	+/-

13.1.1 Mycoplasma fluorescence must be observed for the strongly cyto-adsorbing mycoplasma species *(M. hyorhinis)* and for the poorly cyto-adsorbing mycoplasma species *(M. orale)*.

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

13.2 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	-	+	+

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

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

14.0 TEST EVALUATION

14.1 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.

14.2 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.

14.3 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.

14.4 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.

14.5 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.

14.6 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 CFU	+/-	+*	+
M. pneumoniae ≤ 100 CFU	NA	+*	+
THEN: Overall Inhibitory Result	Non-Inhibitory	Non-Inhibitory	Non-Inhibitory

*See section 14.6.1 for additional criteria.

14.6.1 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).

14.6.2 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.

14.6.3 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).

15.0 RESULTS

15.1 Mycoplasmastasis (Test Article Inhibition)

15.1.1 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. See Table 1.

15.1.2 Direct assay – Day 0 Agar Plates

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

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

If TA Spike Ratio:

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

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

TABLE 1 – DAY 0 AGAR PLATES – POSITIVE CONTROLS

POSITIVE CONTROL	AVE. CFU / PLATE
M. orale	16.3
M. pneumoniae	33.7

TABLE 2 – DAY 0 AGAR PLATES – SPIKED TEST ARTICLES

TEST ARTICLE		AVE. CFU / PLATE	TA SPIKE RATIO	INHIBITORY / NON-INHIBITORY
Test Article: TE04-MCB-1 #7856	*M. orale* spike	15.3	0.9	Non-Inhibitory
	M. pneumoniae spike	28.0	0.8	Non-Inhibitory

15.1.3 Direct assay – Subculture Plates

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

15.2 Overall Result

TABLE 3 – Indirect Assay and Direct Assay Results

SAMPLE	INDIRECT	DIRECT		OVERALL
		BROTH FLASKS	AGAR PLATES	
Test Article: TE04-MCB-1 #7856	Negative	Negative	Negative	Negative
TE04-MCB-1 #7856 Spiked with *M. orale*	Non-inhibitory Positive	Non-inhibitory Positive	Non-inhibitory Positive	Non-inhibitory Positive
TE04-MCB-1 #7856 Spiked with *M. hyorhinis*	Non-inhibitory Positive	NA	NA	Non-inhibitory Positive
TE04-MCB-1 #7856 Spiked with *M. pneumoniae*	NA	Non-inhibitory Positive	Non-inhibitory Positive	Non-inhibitory Positive
Negative Control	Negative	Negative	Negative	Negative
M. hyorhinis	Positive	NA	NA	Positive
M. orale	Positive	Positive	Positive	Positive
M. pneumoniae	NA	Positive	Positive	Positive

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

16.0 ANALYSIS AND CONCLUSION

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

16.2 These findings indicate that the test article, TE04-MCB-1 #7856, is considered negative for the presence of mycoplasma contamination and non-inhibitory to the detection of mycoplasma. The indirect assay was negative at the undiluted.

17.0 DEVIATIONS: None.

18.0 AMENDMENT: None.

19.0 RECORD RETENTION

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

20.0 TECHNICAL REFERENCES

20.1 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.

20.2 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).

20.3 European Pharmacopoeia, 6th Edition, Section 2.6.7. Mycoplasmas.

20.4 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).

20.5 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).

20.6 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).

20.7 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: 000983-040109
NSCB 4016

Report Date: April 06, 2009

Case Details:

Cell Line: *TE04-MCB-1 (4016)*

Passage #: *48*

Date Completed: *4/6/2009*

Cell Line Gender: *female*

Investigator: National Stem Cell Bank

Specimen: *hESC on MEF feeder*

Date of Sample: *4/1/2009*

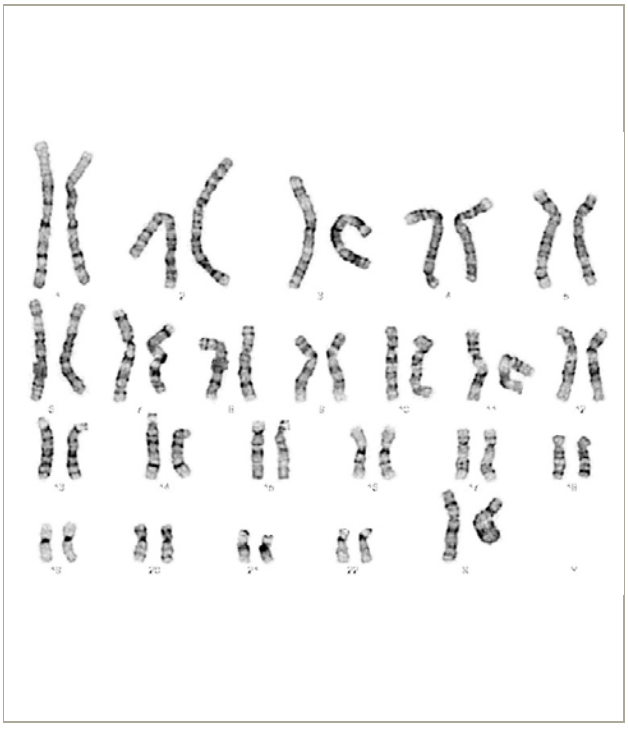
Test, Reason for: *not given*

Results: *46,XX*

Completed by , CLSp(CG), on 4/6/2009

Reviewed and interpreted by PhD, FACMG, on 4/6/2009

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



Cell: *S01-01*

Slide: *A*

Slide Type: *Karyotyping*

Cell Results: *46,XX*

# of Cells Counted: *20*

# of Cells Karyotyped: *4*

# of Cells Analyzed: *8*

Band Level: *450-600*

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

PROTOCOL AMENDMENT NUMBER 2

QA Reviewed
GLA 24Sep09
Init. Date

BIORELIANCE STUDY NO: AC31CH.032901.BSV

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

SECTION TO BE AMENDED: 6.2.2 Guinea-Pig erythrocytes
Source: BioReliance

AMENDMENT: Change Source to Guinea-Pig erythrocytes
Source: Lonza

REASON FOR AMENDMENT: To reflect the correct source for the erythrocyte used

APPROVAL:

_____ _____ 26 Aug 09
STUDY DIRECTOR DATE

_____ _____ 06 OCT 09
SPONSOR REPRESENTATIVE DATE

BioReliance®

Final Report

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

Study Number: AC31CH.032901.BSV

Test Article ID: TE04-MCB-01

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

Bovine viruses were not detected when the test article, TE04-MCB-01, 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: TE04-MCB-01 was received by BioReliance on 19-Aug-2009. 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: 20-Aug-2009

Lab Initiation: 21-Aug-2009

Lab Completion: 14-Sep-2009

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 + 110 mg/L Sodium Pyruvate + 15% foal serum, 2% 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: Lonza Walkersville, Inc.
Walkersville, MD

Guinea-pig erythrocytes
Source: Lonza Walkersville, Inc.

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.

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 or on day 21 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 and at 20 - 25°C for approximately 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, TE04-MCB-01. 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.

DEVIATION

Event Record #45930. Expired Assay medium was used to refeed BT cells following their inoculation with the positive controls. Since the medium was only one day past its expiration date, and since the positive controls performed as expected, this deviation did not affect the integrity of the study or interpretation of the results of the 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.

____ Ph. D. _____ 01Oct09

Study Director Date

TABLE 1

Observations for Cytopathic Effects in Cultures of BT and Vero Cells Inoculated with TE04-MCB-01

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 TE04-MCB-01

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 18
[b] Positive control tested on day 21
- CPE not observed
+ CPE observed

TABLE 3

Observations for Hemadsorption in BT and Vero Cultures Inoculated with TE04-MCB-01

	Hemadsorption Results			
	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[c]	+	+	+	+

[a] Erythrocytes used in these assays: C = chicken, G = guinea pig
[b] Positive control tested on day 18
[c] Positive control tested on day 21
- Hemadsorption not observed
+ Hemadsorption observed

TABLE 4

Immunofluorescent Staining Results for BT and Vero Cultures Inoculated with TE04-MCB-01

	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 18										
Negative Control	-	-	-	-	-	-	NA	NA	-	-
Test Article	-	-	-	-	-	-	NA	NA	-	-
Slides Prepared Day 21	PBS [a, b]	αBAV5 [a]	αBPV [a]	αBRSV [a]	αBTV [a]	αBVDV [a]	αREO3 [a, b]	αRabies [a, b]	αIBR [a]	αPI3 [a]
Negative Control	-	-	-	-	-	-	-	-	-	-
Test Article	-	-	-	-	-	-	-	-	-	-
	PBS [a, b]	αBAV5 [a]	αBPV [a]	αBRSV [a]	αBTV [a]	αBVDV [a]	αREO3 [b]	αRabies [c]	αIBR [a]	αPI3 [a]
Positive Control	-	+[d]	+[d]	+[d]	+[d]	+[d]	+	+	+[d]	+[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 18
NA = Not Applicable

BioReliance®

Quality Assurance Statement

Study Information

Number: AC31CH.032901.BSV

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

Compliance

Procedures, documentation, equipment and other records were examined in order to assure this study was performed in accordance with the regulation(s) listed below and conducted according to the protocol and relevant Standard Operating Procedures. Verification of the study protocol was performed and documented by Quality Assurance.

US FDA Good Laboratory Practice Regulations (21CFR 58)
UK GLP Regulations
Japanese GLP Standard
OECD Principles of Good Laboratory Practice

Inspections

Quality Assurance performed the inspections(s) below for this study.

Insp. Dates (From/To)		Phase Inspected	To Study Director	To Management
25-Sep-2009	25-Sep-2009	Data and Final Reporting	25-Sep-2009	25-Sep-2009
16-Sep-2009	18-Sep-2009	Admin. Of Test Substance	24-Sep-2009	24-Sep-2009 *
10-Sep-2009	18-Sep-2009	Manipulation of Test System	18-Sep-2009	18-Sep-2009 *
15-Sep-2009	18-Sep-2009	Observation of Test System	18-Sep-2009	18-Sep-2009 *
15-Sep-2009	18-Sep-2009	Test System Preparation	18-Sep-2009	18-Sep-2009 *

* Process-based Inspection

The Final Report for this study describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

E-signature

Quality Assurance: 01-Oct-2009 6:59 pm GMT

Reason for signature: QA Approval

PROTOCOL AMENDMENT NUMBER 1

QA Reviewed
BJM 01OCT09
Init. Date

BIORELIANCE STUDY NO: AC31CH.033901.BSV

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

SECTION TO BE AMENDED: 7.2.2 Guinea-Pig erythrocytes
Source: BioReliance

AMENDMENT: Change Source to Guinea-Pig erythrocytes
Source: Lonza

REASON FOR AMENDMENT: To specify the source of the erythrocyte used

APPROVAL:

_____ _____ 26Aug09
STUDY DIRECTOR DATE

_____ _____ 060CT09
SPONSOR REPRESENTATIVE DATE

BioReliance®

Final Report

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

Study Number: AC31CH.033901.BSV

Test Article ID: TE04-MCB-01

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

Porcine viruses, BVDV, reovirus and Rabies were not detected when the test article TE04-MCB-01 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: TE04-MCB-01 was received by BioReliance on 19-Aug-2009. 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: 20-Aug-2009

Lab Initiation: 21-Aug-2009

Lab Completion: 14-Sep-2009

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: Lonza Walkersville, Inc.
Walkersville, MD

Guinea-pig erythrocytes
Source: Lonza Walkersville, Inc.

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 6-well plates 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 and at 20 - 25°C for approximately 30 minutes. Cultures were examined microscopically for areas of adherent erythrocytes after each incubation.

RESULTS

Porcine viruses were not detected in the test article TE04-MCB-01. 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.

DEVIATION

No known procedural deviations from the study protocol or pertinent assay SOPs occurred during the conduct of the study.

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. _____ 24Sep09

Study Director Date

TABLE 1

Observations for Cytopathic Effects in Cultures of PT-1 Cells Inoculated with TE04-MCB-01

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 TE04-MCB-01

	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 18
- Hemadsorption not observed
+ Hemadsorption observed

TABLE 3

Immunofluorescent Staining Results for PT-1 cells Inoculated With TE04-MCB-01

	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 Information

Number: AC31CH.033901.BSV

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

Compliance

Procedures, documentation, equipment and other records were examined in order to assure this study was performed in accordance with the regulation(s) listed below and conducted according to the protocol and relevant Standard Operating Procedures. Verification of the study protocol was performed and documented by Quality Assurance.

US FDA Good Laboratory Practice Regulations (21CFR 58)
UK GLP Regulations
Japanese GLP Standard
OECD Principles of Good Laboratory Practice

Inspections

Quality Assurance performed the inspections(s) below for this study.

Insp. Dates (From/To)		Phase Inspected	To Study Director	To Management
28-Sep-2009	28-Sep-2009	Data and Final Reporting	28-Sep-2009	28-Sep-2009
16-Sep-2009	18-Sep-2009	Admin. Of Test Substance	24-Sep-2009	24-Sep-2009 *
10-Sep-2009	18-Sep-2009	Manipulation of Test System	18-Sep-2009	18-Sep-2009 *
15-Sep-2009	18-Sep-2009	Observation of Test System	18-Sep-2009	18-Sep-2009 *
15-Sep-2009	18-Sep-2009	Test System Preparation	18-Sep-2009	18-Sep-2009 *

* Process-based Inspection

The Final Report for this study describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

E-signature

Quality Assurance: 01-Oct-2009 3:41 pm GMT

Reason for signature: QA Approval

Printed by:

Final Report

MOUSE ANTIBODY PRODUCTION (MAP) TEST

Study Number: AC27WH.004000.BSV

Test Article ID: TE04-MCB-1 NSCB Sample #7856

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

Based on the data obtained in the assays performed, the test article, TE04-MCB-1 NSCB Sample #7856, has been shown to be free of all of the seventeen murine viruses for which it was examined.

BioReliance®

STUDY INFORMATION

Test Article Receipt: TE04-MCB-1 NSCB Sample #7856 was received at BioReliance on 04/28/2009. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor. Retention of samples of the test article is the sole responsibility of the sponsor.

Testing Facility: BioReliance

Animal Facility: BioReliance

Schedule:

Study Initiation: 04/30/2009

Lab Initiation: 05/06/2009

Lab Completion: 06/09/2009

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 females, four to ten weeks old Source: Harlan Sprague Dawley Frederick, Maryland

JUSTIFICATION

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

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

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

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

PROCEDURES

Animal Husbandry

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

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

Methods

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

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

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

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

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

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

[1] Enzyme Linked Immunosorbent Assay (OPDL0806)
[2] Testing performed using BioReliance SOP OPVM7009
[3] Indirect Fluorescent Antibody Test (OPDL0810)
[4] Hemagglutination Inhibition (OPDL0621)

CRITERIA FOR A VALID TEST

Serology Assays

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

LDV Assay

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

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

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

LCM Virus Assay

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

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

EVALUATION OF TEST RESULTS

Serology Assays

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

LDV Assay

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

LCM Virus Assay

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

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

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

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

RESULTS

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

In the original LDH bleed, plasma from two of the three test article 1:10 animals and both of the negative control animals tested for lactate dehydrogenase activity showed normal levels. Plasma from one of the three test article 1:10 animals exhibited elevated levels of lactate dehydrogenase activity. LDV injected control animals exhibited elevated levels of LDH activity. See Table 3.

Due to the elevated levels of lactate dehydrogenase activity in one of the test article animals, the animals in Groups II and III were rebled and the plasma was tested for lactate dehydrogenase activity.

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

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

REFERENCES

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

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

DEVIATIONS

No known deviations from the protocol or pertinent assay SOPs occurred during the conduct of this study.

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.

[redacted]

June 19, 2009
Date

Study Director

Table 1

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

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

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

Table 2

Serological Assays
for TE04-MCB-1 NSCB Sample #7856

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	.15	0	0	.01	.02	I[4]	I[4]	.07	.03	.07	.05	.01	.03	-	-	-
	.02	0	.03	.02	.01	I[4]	I[4]	.06	.02	.10	.10	0	.08	-	-	-
	.03	0	.01	.02	.01	I[4]	I[4]	.04	.03	.07	.02	0	.05	-	-	-
	.02	.01	.02	.02	0	I[4]	I[4]	.04	.03	.08	.02	0	.04	-	-	-
Negative Control	.01	.02	.01	.03	.01	.06	.03	.06	.03	.10	.03	0	.04	-	-	-
	0	0	0	.01	.01	.04	.04	.05	.03	.08	.03	0	.01	-	-	-
Serology Positive Control	1.07	.75	.93	.81	.87	.73	.86	.92	.61	1.01	1.08	.93	.87	+	160	+

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

Table 3

LDV Assay for TE04-MCB-1 NSCB Sample #7856

Plasma from Animals Injected with	LDH Titer[a] Original Bleed	LDH Titer[a] Rebleed
Test Article (1:10) (Group II)	269	192
	238	217
	645	286
Negative Control (Group III)	370	219
	465	377
LDV Control	1273	1211
	1443	1332

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

Quality Assurance Statement

Study Information

Number: AC27WH.004000.BSV
Protocol Title: MOUSE ANTIBODY PRODUCTION (MAP) TEST

Compliance

Procedures, documentation, equipment and other records were examined in order to assure this study was performed in accordance with the regulation(s) listed below and conducted according to the protocol and relevant Standard Operating Procedures. Verification of the study protocol was performed and documented by Quality Assurance.

US FDA Good Laboratory Practice Regulations (21CFR 58)
UK GLP Regulations
Japanese GLP Standard
OECD Principles of Good Laboratory Practice

Inspections

Quality Assurance performed the inspections(s) below for this study.

Insp. Dates (From/To)		Phase Inspected	To Study Director	To Management
18-Jun-2009	18-Jun-2009	Data and Final Reporting	18-Jun-2009	18-Jun-2009
11-Jun-2009	11-Jun-2009	Admin. Of Test Substance	12-Jun-2009	12-Jun-2009 *
11-Jun-2009	11-Jun-2009	Manipulation of Test System	12-Jun-2009	12-Jun-2009 *
11-Jun-2009	11-Jun-2009	Observation of Test System	12-Jun-2009	12-Jun-2009 *
09-Jun-2009	09-Jun-2009	Test System Preparation	11-Jun-2009	11-Jun-2009 *

* Process-based Inspection

The Final Report for this study describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

E-signature

Quality Assurance: 19-Jun-2009 6:44 pm GMT

Reason for signature: QA Approval

Printed by:

E-FR-1-09-000805.37000

FINAL STUDY REPORT

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

TEST PROTOCOL NUMBER: 37000.04

TEST ARTICLE IDENTIFICATION	WUXI APPTEC ACCESSION NUMBER
TE04-MCB-1 #7856	09-000805

SPONSOR:

WiCell Research Institute

PERFORMING LABORATORY: WuXi AppTec, Inc.

WUXI APPTEC ACCESSION NUMBER	RESULTS
09-000805	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
BR #37100.02 Step 4.3.7 Remove medium from one 6-well MRC-5 plate.	May 5, 2009

Quality Assurance

22 Jun 09
Date

GOOD LABORATORY PRACTICES STATEMENT

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

Study Director

23 JUN 09
Date

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 Research Institute

3.0 TEST FACILITY:

WuXi AppTec, Inc.

4.0 SCHEDULING

DATE SAMPLES RECEIVED: April 28, 2009
STUDY INITIATION DATE: May 4, 2009
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:

TE04-MCB-1 #7856

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 paramyxo viruses 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

8.1 Experimental Procedure

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

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

8.1.2 Indicator cell monolayers MRC-5, Vero and NIH/3T3 were inoculated with 0.2 mL disrupted, clarified test article per well. A total of 6 wells were inoculated per cell line.

8.1.3 Indicator cell monolayers, MRC-5, Vero and NIH/3T3 were inoculated with viruses as appropriate for each cell line chosen to serve as the positive controls.

8.1.4 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]

8.1.5 Cultures were fed on days 3, 10, 17 and 24. Subcultivation was performed on days 7, 14 and 21.

8.1.6 One (1) day 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]

8.1.7 On day 28, the hemadsorption assay was performed. The monolayers were rinsed and suspensions of chicken, human and guinea pig erythrocytes were then added separately to the monolayers. Replicate cultures were then 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 April 28, 2009, WuXi AppTec, Inc. received 2 tubes, each containing 8.0 mL of "hES Cells," frozen on dry ice and designated for use in this assay. The test article was stored at ≤ -60°C until the assay was initiated.

On the day of inoculation (May 5, 2009), the test article was thawed using a 37±2°C waterbath and was subjected to one 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 placed on ice until being inoculated. The supernatant was inoculated as per step 8.1.2.

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 plaque forming units (PFU) per well. Each virus was appropriate to the cell lines chosen for the assay and as listed below.

10.2 Positive controls for CPE:

1. MRC-5 cultures infected with Encephalomyocarditis Virus (EMC)
2. VERO cultures infected with Adenovirus type 5 Virus (Ad 5)
3. NIH/3T3 cultures infected with Herpes Simplex Type 1 (HSV-1)

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

11.0 NEGATIVE CONTROLS

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

12.0 ASSAY VALIDITY

The test is considered valid when characteristic cytopathic changes and 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 viral cytopathic changes during the course of at least 14 days or 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

14.1 Validity

The test was valid. MRC-5 cultures infected with EMC virus evidenced +4 CPE on day 1, and were discarded. VERO cultures infected with Ad 5 evidenced +4 CPE on day 7 and were discarded. NIH/3T3 cultures infected with HSV-1 evidenced +3 CPE on day 7 and were discarded. 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).

14.2 Test Results

The test article did not induce cytopathic changes in the MRC-5, VERO and NIH/3T3 cultures (Table 1).

The test article inoculated 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 # 09-000805	**0**	**0**	**0**
EMEM (Negative Control)	0	0	0
EMC (Positive Control)	+4	NA	NA
Ad 5 (Positive Control)	NA	+4	NA
HSV-1 (Positive Control)	NA	NA	+3

Legend:

0	No cytopathic changes observed during the 28-day test period.
+1	25 - 50% of the cells in culture show cytopathic changes indicating the presence of a viral agent.
+2	50 - 75% of the cells in culture show cytopathic changes indicating the presence of a viral agent.
+3	75 - 90% of the cells in culture show cytopathic changes indicating the presence of a viral agent.
+4	90 - 100% of the cells in culture show cytopathic changes indicating the presence of a viral agent.
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 #09-000805	**MRC-5**	**28**	-	-	-	-	-	-
	VERO	**28**	-	-	-	-	-	-
	NIH-3T3	**28**	-	-	-	-	-	-
EMEM (Negative Control)	MRC-5	28	-	-	-	-	-	-
	VERO	28	-	-	-	-	-	-
	NIH/3T3	28	-	-	-	-	-	-
Positive Control[1] (1:2)	VERO	28	+	+	+	+	+	+
Positive Control[1] (1:5)	VERO	28	+	+	+	+	+	+

Legend:
C Chicken red blood cells
GP Guinea pig red blood cells
H Human type O erythrocytes
\- Negative reaction indicating absence of viral agent
\+ Positive reaction indicating presence of viral agent
1 Parainfluenza type 3 (PI3) inoculated onto one set of VERO negative cultures 1 day prior to hemadsorption.

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 is 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 ten (10) years including, but not limited to: the signed testing 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 and amendments.

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). In "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). In "ATCC Quality Control Methods for Cell Lines", American Type Culture Collection, Rockville, MD

Final Report

TEST FOR THE PRESENCE OF INAPPARENT VIRUSES

Study Number: AC27WH.005002.BSV

Test Article ID: TE04-MCB-1 NSCB Sample #7856

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

No evidence of contamination with adventitious viral agents was observed due to the test article, TE04-MCB-1 NSCB Sample #7856.

BioReliance®

STUDY INFORMATION

Test Article Receipt:	TE04-MCB-1 NSCB Sample #7856 was received by BioReliance on 04/28/2009. 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:	05/01/2009
Lab Initiation Date:	05/06/2009
Lab Completion Date:	06/08/2009
Study Completion Date:	See Study Director's signature date in the "Approval" section.
Study Director:	
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:	Hanks' Balanced Salt Solution (HBSS) Lot No.: 038K2378 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: Charles River Laboratories
St. Constant, Canada (males)
Kingston, New York (females)

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. On day 9 post-injection, the water bottle leaked in one of the negative control article cages and all suckling mice were wet. Suckling mice were placed in a dry cage. All animals appeared to be normal and healthy. On day 12 post-injection, animals were completely dry and appeared normal and healthy. The surviving mice of each group were homogenized and the homogenate of each group was passaged into a new group of suckling mice. The remainder of the homogenates was frozen at -70°C ± 10°C.

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

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

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

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 all viable eggs in 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 Table 4 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 11 days post-injection. Nine of the ten test article injected eggs and all of the negative control article injected eggs appeared viable at 24 and 48 hours post-injection. At examination on day 11, nine of the ten test article injected eggs and all of the negative control article injected eggs contained viable embryos. One of the test article injected eggs contained a non-viable embryo. No growth was observed on blood agar plates streaked with fluid from the non-viable egg. The cause of death of this embryo most likely resulted from injection related trauma, as the embryo appeared non-viable at 24 hours post-injection. (See Criteria for a Valid Test/Evaluation of Test Results.)

The yolk sac material from all viable eggs in each group was pooled. A 10% suspension of pooled yolk sac material was injected into ten new seven day old embryonated eggs using the same route of injection. These eggs were examined for viability at 24 and 48 hours and 9 days post-injection. All of the test article subpassage eggs and all of the negative control article subpassage eggs appeared viable at 24 and 48 hours post-injection. At examination on day 9, nine of the ten test article subpassage eggs and nine of the ten negative control article subpassage eggs contained viable embryos. One of the test article subpassage eggs and one of the negative control article subpassage eggs each contained a non-viable embryo. No growth was observed on blood agar plates streaked with fluid from the non-viable eggs. The cause of death of these embryos could not be determined. (See Criteria for a Valid Test/Evaluation of Test Results.) 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.

DEVIATIONS

No known deviations from the protocol or pertinent assay SOPs occurred during the conduct of this study.

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.

19 Jun 09
Date

Study Director

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 TE04-MCB-1 NSCB Sample #7856

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

[a] Number of surviving animals after 28 days/Number of animals injected.
[b] In the suckling mice portion of the assay, animals are injected and observed for 14 days. On day 14 post-injection a homogenate was prepared from the surviving sucklings from each group. This homogenate was used to inject another group of suckling mice which was observed for an additional 14 days.

TABLE 3

Summary of Daily Observations for TE04-MCB-1 NSCB Sample #7856

Guinea Pigs

Test Material	Animal Number	Clinical Signs	Day of Onset (Post-Injection)[a]	Day of Death/Sacrifice (Post-Injection)[a]
Test Article	1167	Normal		
	1168	Normal		
	1169	Normal		
	1170	Normal		
	1171	Normal		
	1172	Normal		
Negative Control	1157	Normal		
	1158	Normal		
	1159	Normal		
	1160	Normal		

[a] These columns will only be completed if clinical signs, moribund sacrifice, or deaths occur.

TABLE 3 (Continued)

Summary of Daily Observations for TE04-MCB-1 NSCB Sample #7856

Adult Mice

Test Material	Animal Number	Clinical Signs	Day of Onset (Post-Injection)[a]	Day of Death/Sacrifice (Post-Injection)[a]
Test Article	1131	Normal		
	1132	Normal		
	1133	Normal		
	1134	Normal		
	1135	Normal		
	1136	Normal		
	1137	Normal		
	1138	Normal		
	1139	Normal		
	1140	Normal		
Negative Control	1111	Normal		
	1112	Normal		
	1113	Normal		
	1114	Normal		
	1115	Normal		
	1116	Normal		
	1117	Normal		
	1118	Normal		
	1119	Normal		
	1120	Normal		

[a] These columns will only be completed if clinical signs, moribund sacrifice, or deaths occur.

TABLE 3 (Continued)

Summary of Daily Observations for TE04-MCB-1 NSCB Sample #7856

Suckling Mice

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

[a] Ten suckling mice injected per cage.
[b] Surviving suckling mice from the primary injection were sacrificed on day 14 for preparation of blind passage tissue homogenate.
[c] These columns will only be completed if clinical signs, moribund sacrifice, or deaths occur.

TABLE 4

Embryonated Hens' Eggs
Allantoic Route Survival Summary and Hemagglutination Results
for TE04-MCB-1 NSCB Sample #7856

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 (GP) 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 TE04-MCB-1 NSCB Sample #7856

Embryonated Hens' Eggs - Yolk Sac Route

Primary Injection				
Test Material	Number of Eggs Injected	Viability Observations[a]		
		24 Hours	48 Hours	Harvest Day 11
Test Article	10	9/10	9/10	9/10
Negative Control Article	10	10/10	10/10	10/10

[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	9/10
Negative Control Article Homogenate	10	10/10	10/10	9/10

[a] Number of viable eggs/number examined.

Quality Assurance Statement

Study Information

Number: AC27WH.005002.BSV
Protocol Title: TEST FOR THE PRESENCE OF INAPPARENT VIRUSES

Compliance

Procedures, documentation, equipment and other records were examined in order to assure this study was performed in accordance with the regulation(s) listed below and conducted according to the protocol and relevant Standard Operating Procedures. Verification of the study protocol was performed and documented by Quality Assurance.

US FDA Good Laboratory Practice Regulations (21CFR 58)
UK GLP Regulations
Japanese GLP Standard
OECD Principles of Good Laboratory Practice

Inspections

Quality Assurance performed the inspections(s) below for this study.

Insp. Dates (From/To)		Phase Inspected	To Study Director	To Management
15-Jun-2009	15-Jun-2009	Data and Final Reporting	15-Jun-2009	15-Jun-2009
11-Jun-2009	11-Jun-2009	Admin. Of Test Substance	12-Jun-2009	12-Jun-2009 *
11-Jun-2009	11-Jun-2009	Manipulation of Test System	12-Jun-2009	12-Jun-2009 *
11-Jun-2009	11-Jun-2009	Observation of Test System	12-Jun-2009	12-Jun-2009 *
09-Jun-2009	09-Jun-2009	Test System Preparation	11-Jun-2009	11-Jun-2009 *

* Process-based Inspection

The Final Report for this study describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

E-signature

Quality Assurance: 19-Jun-2009 7:18 pm GMT

Reason for signature: QA Approval

Printed by:

FINAL STUDY REPORT

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

TEST PROTOCOL NUMBER: 30610.06

TEST ARTICLE IDENTIFICATION	WUXI APPTEC ACCESSION NUMBER
TE04-MCB-1 #7856	09-000859

SPONSOR: WiCell Research Institute

PERFORMING LABORATORY: WuXi AppTec, Inc.

WUXI APPTEC ACCESSION NUMBER	RESULTS
09-000859	Transmission electron microscopic examination of 200 cells revealed no identifiable virus-like particles, nor did it reveal any other identifiable microbial agents.

QUALITY ASSURANCE UNIT SUMMARY

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

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

Phase Inspected	Date
Darkroom procedures	June 15, 2009

Quality Assurance — Date: 29 June 09

GOOD LABORATORY PRACTICES STATEMENT

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

Study Director — Date: 29 JUN 09

Professional Personnel involved in study:

1.0 PURPOSE

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

2.0 SPONSOR: WiCell Research Institute

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

4.0 SCHEDULING

DATE SAMPLES RECEIVED: May 5, 2009
STUDY INITIATION DATE: May 6, 2009
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: TE04-MCB-1 #7856

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 submitted to WuXi AppTec Laboratories were already fixed by the Client.

8.1 The cells were fixed, while in suspension, in 5% glutaraldehyde then pelleted (by the Client prior to shipping to WuXi AppTec Laboratories).

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 May 5, 2009, WuXi AppTec, Inc. received 1 vial containing "hES Cells," cold on cold packs and designated for use in this assay. The test article was stored at 2-8°C until shipment to the subcontractor. On May 6, 2009, 1 vial containing a fixed and pelleted cell culture was shipped, on ice packs, in storage conditions of 2–8°C, via overnight carrier to the subcontractor.

10.0 NEGATIVE CONTROLS

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

11.0 ASSAY VALIDITY

The following validity criteria are evaluated:

11.1 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.

Cellular Ultrastructure

Cells in the section were small to moderate in size and polygonal to irregular in shape (J61157). Cells had microvilli (MV: J61157) unevenly distributed on the surface. Nuclei (N: J61157) tended to be irregular, with chromatin relatively evenly dispersed or clumped along the periphery. Nuclei often had one or more nucleoli (NS: J61157) that were variably located.

Most cells contained condensed mitochondria (MI: J61156). Few profiles of rough endoplasmic reticulum (RER: J61155, J61158), some of which were distended with a fine granular material were seen among the mitochondria. Ribosomes (RB: J61156) were abundant in the cytoplasm of most cells. Cells were observed to contain filaments (F: J61154) and areas of granular material resembling glycogen (G: J61159).

General Viral Particle Evaluation

Transmission electron microscopic examination of 200 cells revealed no identifiable virus-like particles, nor did it reveal any other identifiable microbial agents.

Thirty percent of the cells were necrotic.

14.0 CONCLUSION

Transmission electron microscopic examination of 200 cells revealed no identifiable virus-like particles, nor did it reveal any other identifiable microbial agents.

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 ten (10) 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

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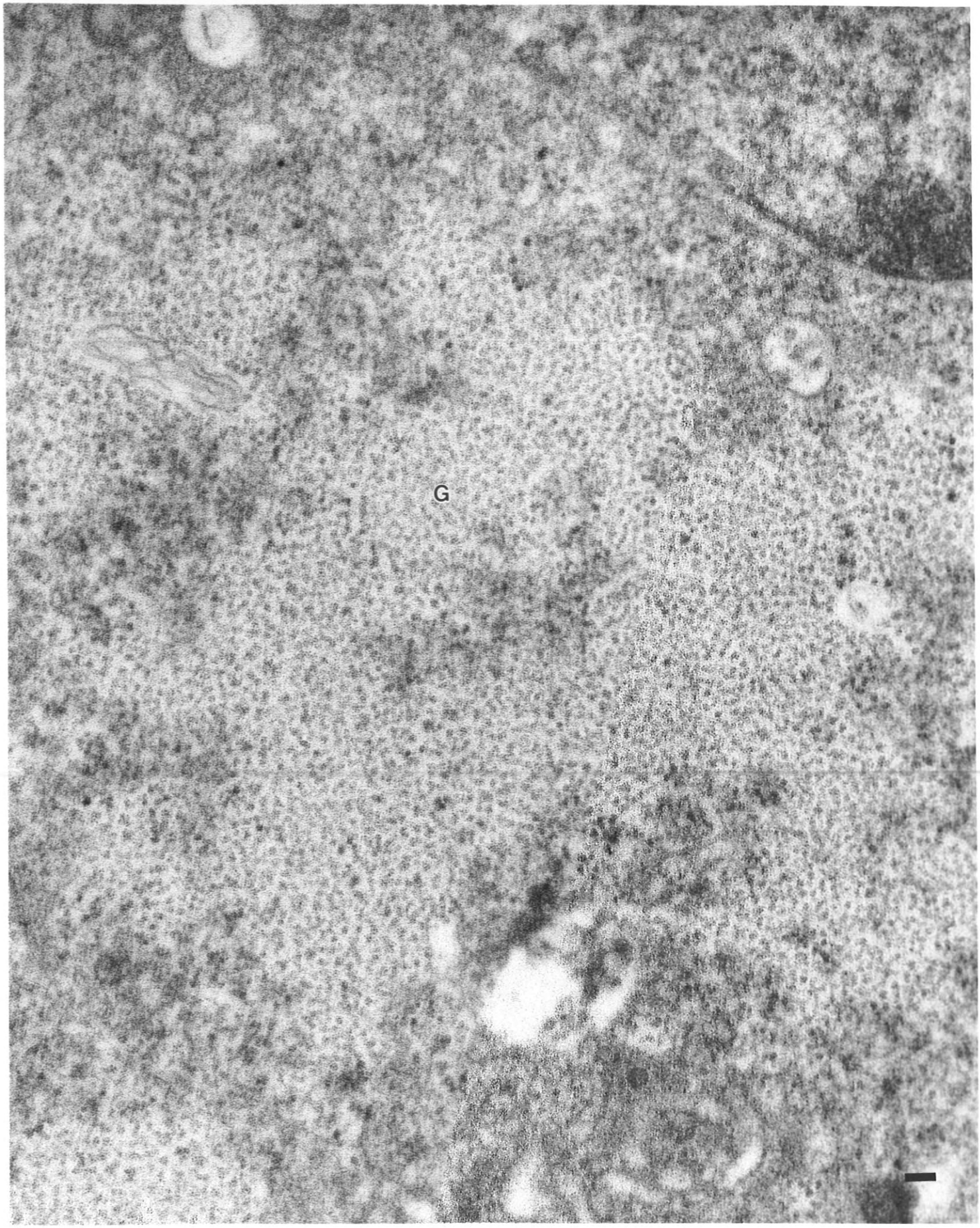
4. Jawetz E, Melnick JL, and Adelberg, EA, eds. (1984). Tumor Viruses in: "Review of Medical Microbiology," 16th Edition, Lange Medical Publications, Drawer L, Los Altos, CA, pp. 495-498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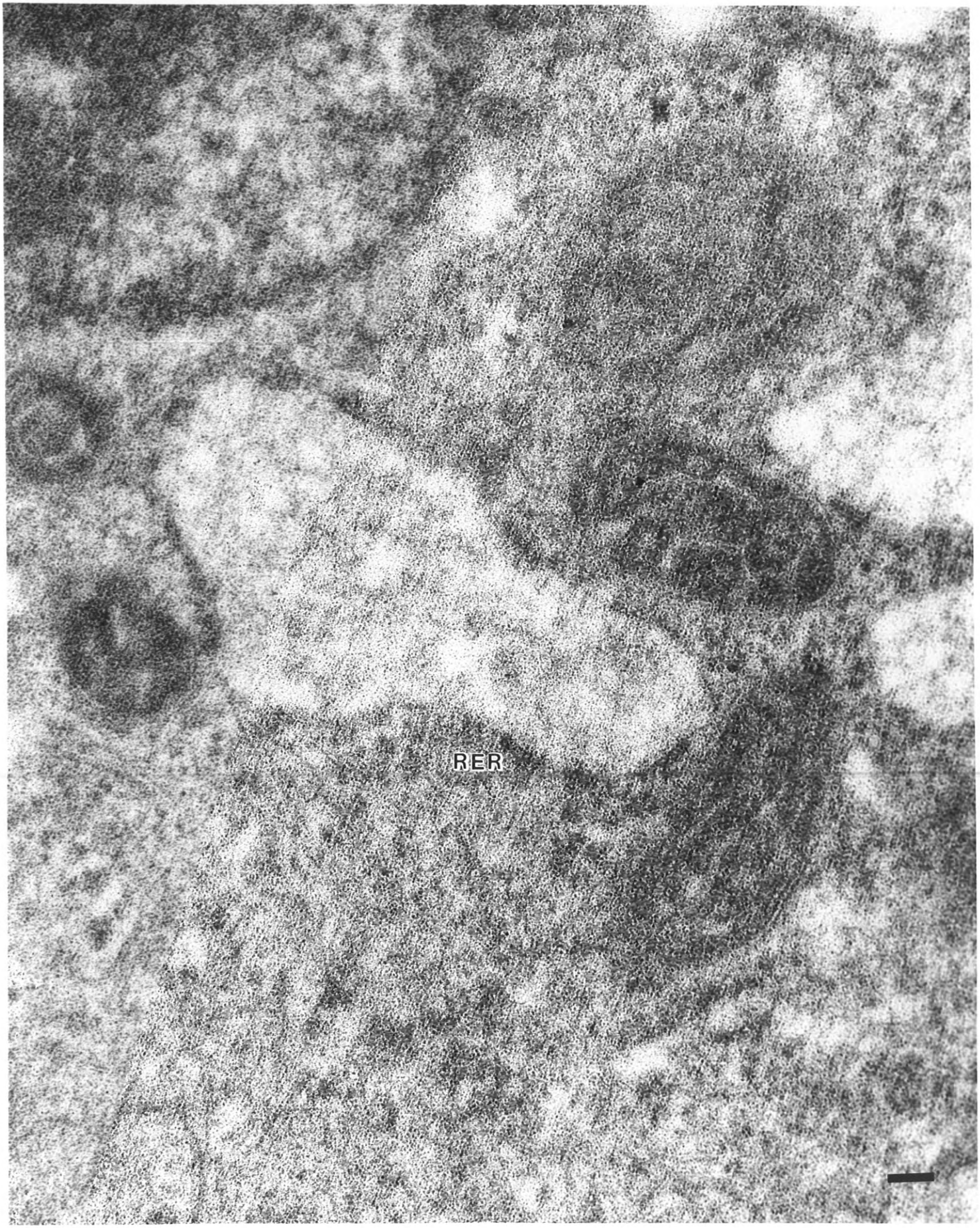
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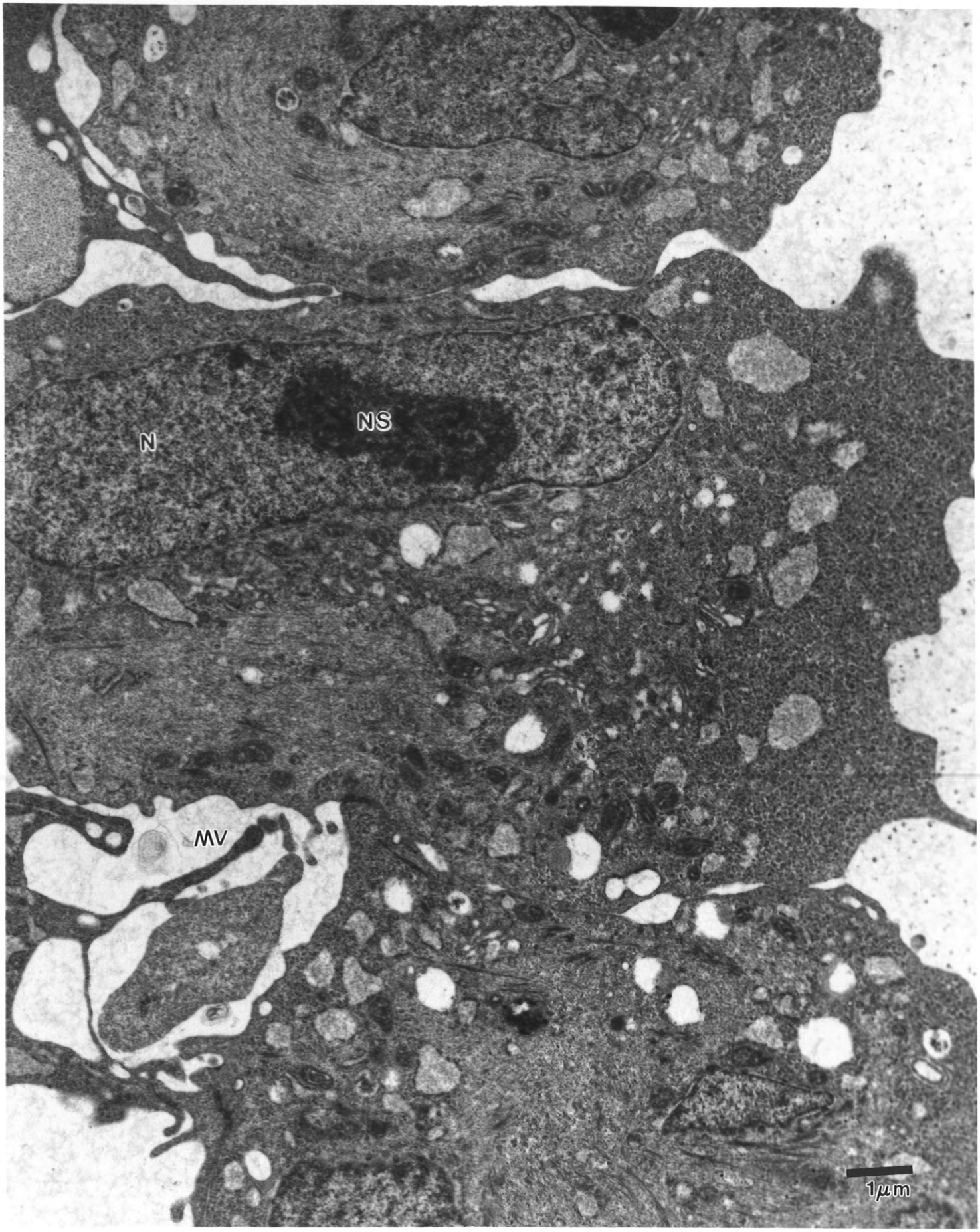
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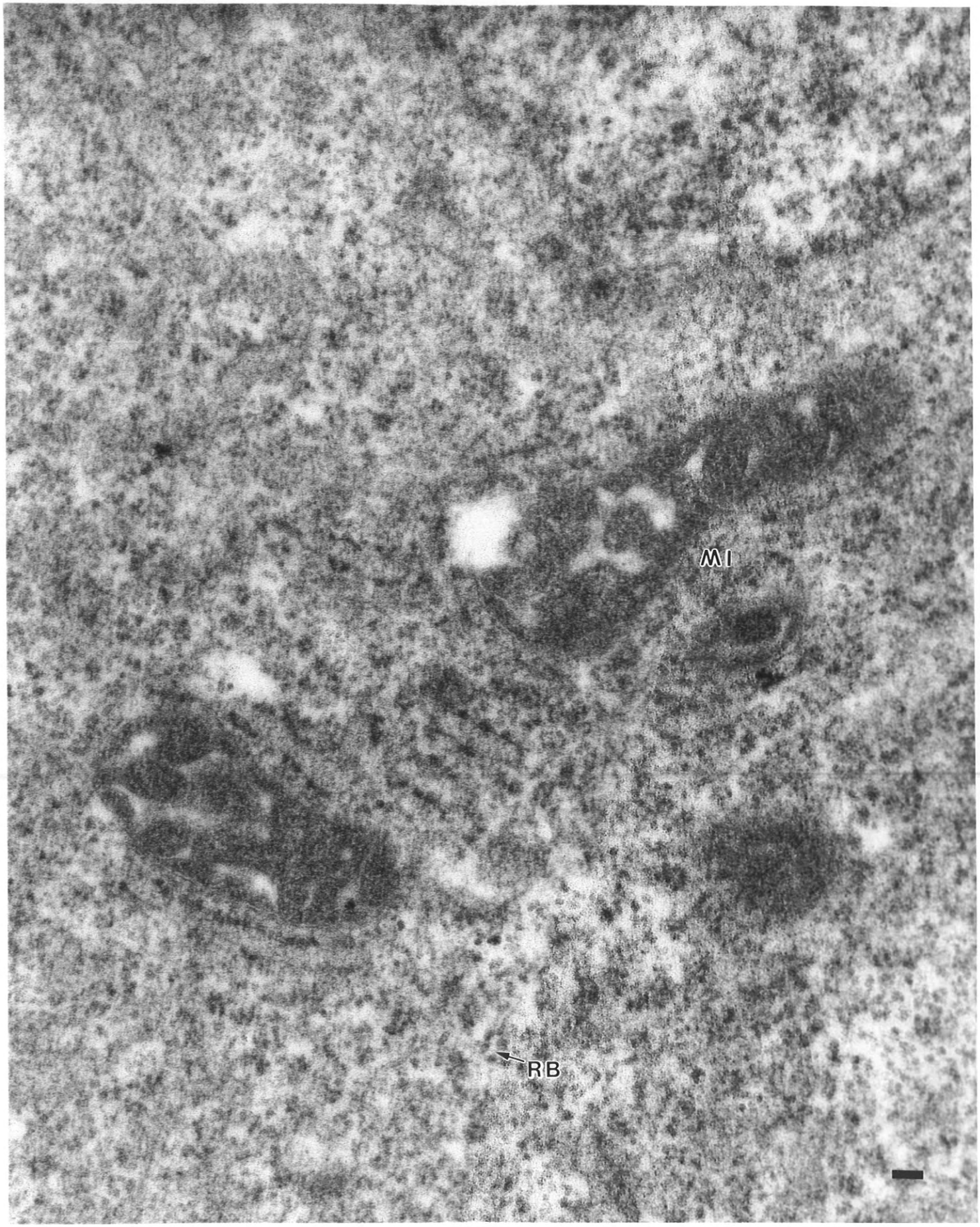
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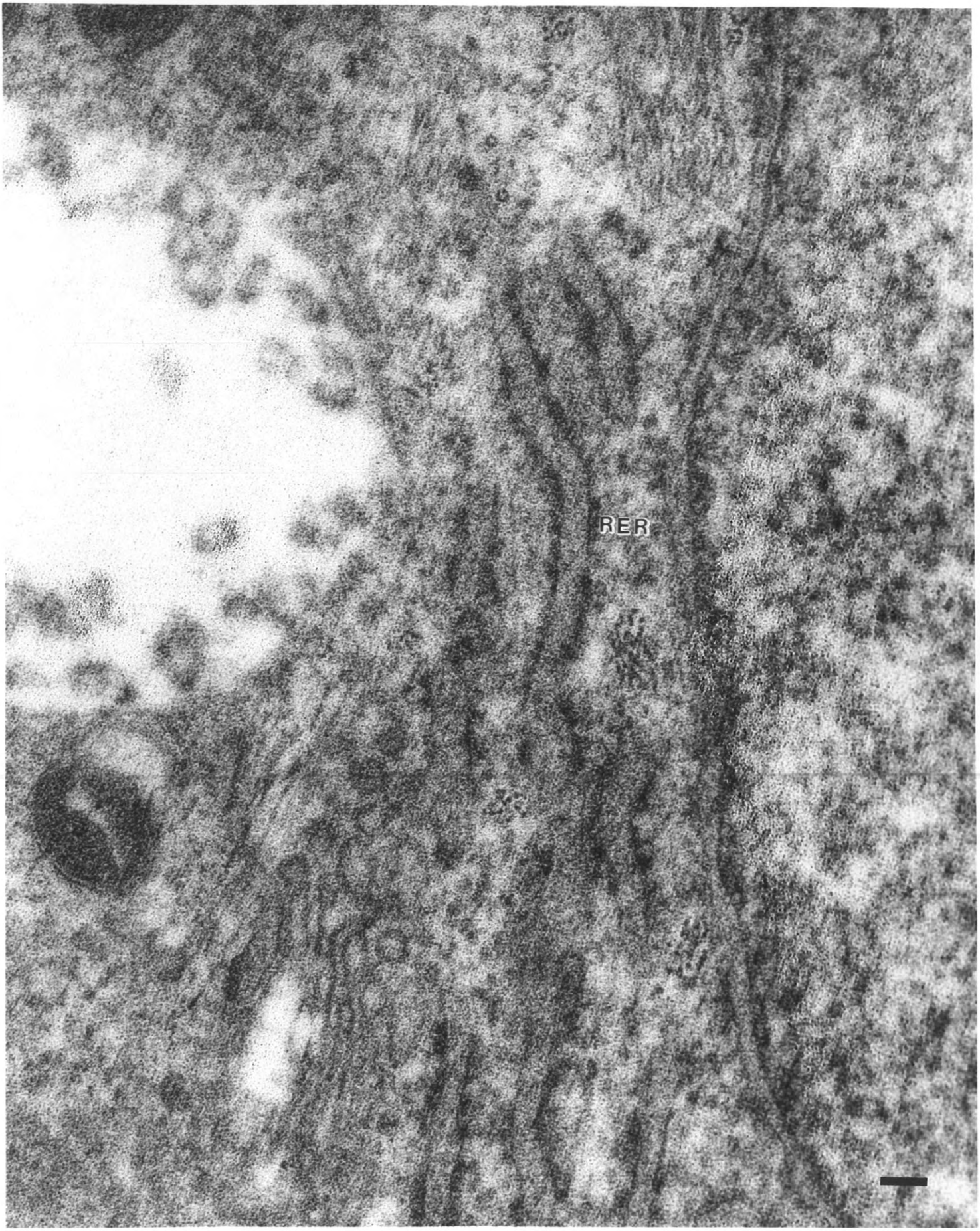
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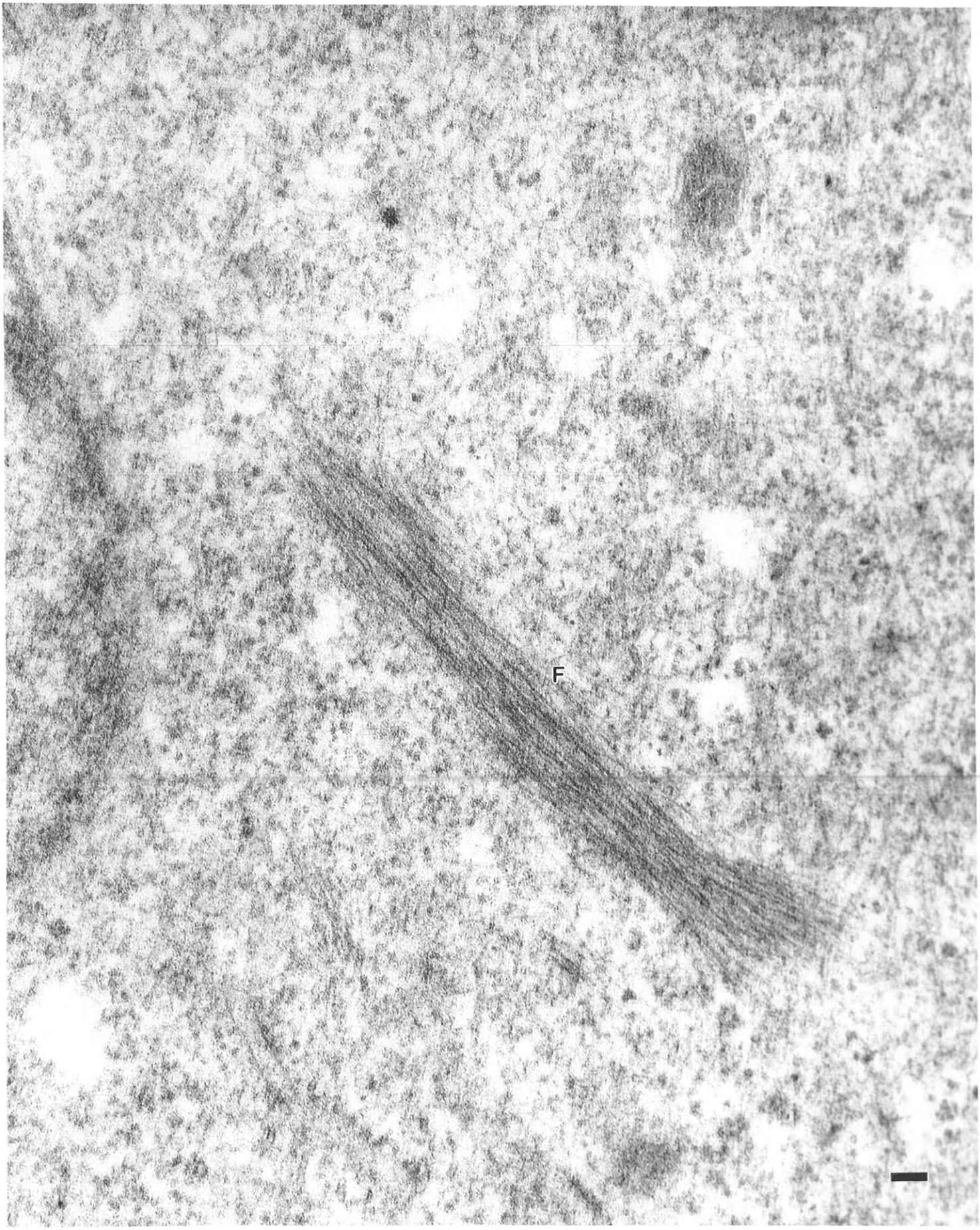
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E-FR-1-09-000860.30201

FINAL STUDY REPORT

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

PROTOCOL: 30201.04

TEST ARTICLE IDENTIFICATION	WUXI APPTEC ACCESSION NUMBER
TE04-MCB-1 #7856	09-000860

SPONSOR: WiCell

PERFORMING LABORATORY: WuXi AppTec, Inc.

WUXI APPTEC ACCESSION NUMBER	RESULTS
09-000860	No evidence for xenotropic, amphotropic, or MCF MuLV retroviral contamination was found in the test article. Following co-cultivation the test article demonstrated a negative response in the PG4 S^+L^- assay.

QUALITY ASSURANCE UNIT SUMMARY

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

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

Phase Inspected	Date
BR# 30201.04 Step 4.2.10 Initiate the co-cultivation by adding 5 mL of diluted test article cells ($5x10^5$ cells) to each flask prepared for the test article.	May 6, 2009

Quality Assurance — Date: 29 Jun 09

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: 30 JUN 09

Personnel involved in study:

1.0 PURPOSE

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

2.0 SPONSOR: WiCell

3.0 TEST FACILITY: WuXi AppTec, Inc.

4.0 SCHEDULING

DATE SAMPLES RECEIVED: May 5, 2009
STUDY INITIATION DATE: May 6, 2009
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: TE04-MCB-1 #7856

7.0 TEST SYSTEM DESCRIPTION

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

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

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

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

8.0 EXPERIMENTAL DESIGN

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

8.1 Co-Cultivation with *Mus dunni* Cells

8.1.1 *Mus dunni* cells alone served as the negative control and were run in parallel with the test article for 14 days. Three aliquots (3 x 2.0ml) of the conditioned medium were reserved as a time zero (T_0) time point for testing in the PG4 S^+L^- assay

8.1.2 Three aliquots (3 x 2.0ml) of the test article supernatant were saved for testing in the PG4 S^+L^- assay as a time zero (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 (A-MuLV) stocks inoculated with 100 FFU.

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

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

8.1.7 Cell culture supernatants were collected from the negative control, test article, and positive control cultures on day 14, post passage 2. 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 amplification 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 amplification 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 (A-MuLV) was inoculated onto PG4 S^+L^- cells, utilizing a few dilutions of the virus (1:1,000 and 1:10,000).

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

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

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

9.0 TEST ARTICLE PREPARATION

On May 5, 2009, WuXi AppTec, Inc. received 1 flask containing live "hES Cells," at ambient temperature and designated for use in this assay. Upon receipt of the test article cells, the virology laboratory removed the excess supernatant from the flask and saved in a 50ml conical tube; and then replaced the non-vented cap on the flask with a vented cap before storing the flask and supernatant at 37±2°C/5±2% CO_2 until the assay was initiated the following day.

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 to confirm the replication of these viruses.

10.2 Controls for PG4 S^+L^- Assay

A known positive amphotropic murine leukemia virus (A- MuLV) was run along with the test samples in each assay as positive controls.

11.0 NEGATIVE CONTROLS

11.1 Co-Cultivation Controls

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

11.2 Controls for PG4 S^+L^- Assay

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

12.0 ASSAY VALIDITY

12.1 Validity Criteria for Co-Cultivation

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

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

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

13.0 TEST EVALUATION

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

14.0 RESULTS

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

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

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

	Culture Inoculum	Time	FFU/ml
Co-Cultivation Samples	**Accession # 09-000860[1] (diluted 1:2)**	**T_0**	**ND**
	Accession # 09-000860 (diluted 1:2)	**PP2**	**ND**
	Negative control[2] (diluted 1:2)	T_0	ND
	Negative control (diluted 1:2)	PP2	ND
	Positive control (A-MuLV)[3] (diluted 1:10)	PP2	TNTC
	Positive control (A-MuLV)[3] (diluted 1:100)	PP2	TNTC
	Positive control (A-MuLV)[3] (diluted 1:1000)	PP2	TNTC
PG4 S^+L^- - Assay Controls	Negative control (EMEM)	NA	ND
	High positive control (A-MuLV) (diluted 1:1000)	NA	TNTC
	Low positive control (A-MuLV) (diluted 1:10000)	NA	1.73×10^5

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

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

16.0 STATISTICAL DATA ANALYSIS

Statistical analysis of the data was not required.

17.0 DEVIATIONS / AMENDMENTS

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

No amendments to the protocol were generated.

18.0 RECORD RETENTION

The testing facility will retain all records involving the study for ten (10) years including, but not limited to: the signed testing protocol with all amendments, any written communication concerning the conduct of the study, test substance accountability record, raw data worksheets, and an official copy of the final study report.

19.0 REFERENCES

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

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

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

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

Final Report

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

Study Number: AC27WH.105010.BSV

Test Article ID: TE04-MCB-1 NSCB Sample # 7856

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

Ten (10.0) µl of DNA extract isolated from the test article 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.

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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: The test article was received by BioReliance on 04/28/2009. 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: 04/29/2009

Lab Initiation: 04/30/2009

Lab Completion: 05/04/2009

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:	Genomic DNA from MRC5 spiked with 100 copies of pCRII+HIV-1, a plasmid containing the complete genome of HIVZ6 with an interruption in the protease coding region Source: BioReliance
	HIV-2:	Genomic DNA from MRC5 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 pCRII+HIV-1
	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 10.0 µl of test article DNA extract 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 the Kodak Gel Logic 100 Imaging System (SOP OPBT0943).

RESULTS

Ten (10.0) µl of test article DNA extract 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 pCRII+HIV-1 (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 tested negative for the presence of HIV-1/2 proviral DNA.

DEVIATIONS

No known procedural deviations from the study protocol or pertinent assay SOPs occurred during the conduct of the study.

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.

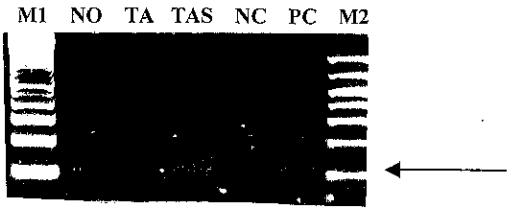
_____ 21 May 09

Ph.D. Date

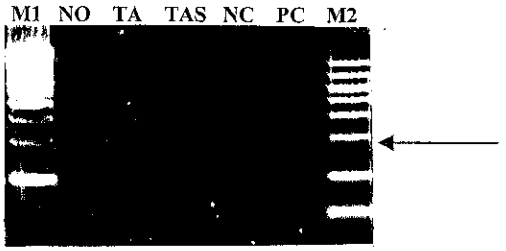
Study Director

FIGURE 1

a. HIV-1



b. HIV-2



Detection of HIV-1 (**a.**) or HIV-2 (**b.**) proviral sequences in the test article 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 (**a.**) pCRII+HIV-1 or (**b.**) pMAHIV2
NC: Negative control (MRC5 genomic DNA)
PC: Positive control (MRC5 genomic DNA spiked with 100 copies of (**a.**) pCRII+HIV-1 or (**b.**) pMAHIV2)
M2: Biomarker low DNA size marker

Arrows indicate specific amplification products.

BioReliance®

Quality Assurance Statement

Study Information

Number: AC27WH.105010.BSV

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

Compliance

Procedures, documentation, equipment and other records were examined in order to assure this study was performed in accordance with the regulation(s) listed below and conducted according to the protocol and relevant Standard Operating Procedures. Verification of the study protocol was performed and documented by Quality Assurance.

US FDA Good Laboratory Practice Regulations (21CFR 58)
UK GLP Regulations
Japanese GLP Standard
OECD Principles of Good Laboratory Practice

Inspections

Quality Assurance performed the inspections(s) below for this study.

Insp. Dates (From/To)		Phase Inspected	To Study Director	To Management
26-May-2009	26-May-2009	Final Reporting	26-May-2009	26-May-2009
31-Mar-2009	31-Mar-2009	Admin. Of Test Substance	22-Apr-2009	22-Apr-2009 *
31-Mar-2009	31-Mar-2009	Manipulation of Test System	22-Apr-2009	22-Apr-2009 *
31-Mar-2009	31-Mar-2009	Observation of Test System	22-Apr-2009	22-Apr-2009 *
31-Mar-2009	31-Mar-2009	Test System Preparation	22-Apr-2009	22-Apr-2009 *

* Process-based Inspection

The Final Report for this study describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

E-signature

Quality Assurance: 26-May-2009 7:23 pm GMT

Reason for signature: QA Approval

Printed by:

Printed on:26-May-09

Final Report

POLYMERASE CHAIN REACTION ASSAY FOR THE DETECTION OF HUMAN T-CELL LYMPHOTROPIC VIRUS TYPES I AND II (HTLV-I/II) IN BIOLOGICAL SAMPLES

Study Number: AC27WH.105013.BSV

Test Article ID: TE04-MCB-1 NSCB Sample # 7856

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

Ten (10.0) µl of DNA extract isolated from the test article 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.

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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:	The test article was received by BioReliance on 04/28/2009. 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:	04/29/2009
Lab Initiation:	04/30/2009
Lab Completion:	05/05/2009
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:	Genomic DNA from MRC5 spiked with 100 copies of pH750, a plasmid containing a 752 bp fragment from the HTLV-I tax/rex gene Source: BioReliance
	HTLV-II:	Genomic DNA from MRC5 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 10µl of test article DNA extract 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 the Kodak Gel Logic 100 Imaging System (SOP OPBT0943).

DEVIATIONS

No known procedural deviations from the study protocol or pertinent assay SOPs occurred during the conduct of the study.

RESULTS

Ten (10.0) µl of test article DNA extract 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-1 and PC-2) produced a 158 bp band. The test article spiked with 100 copies of either pH750 (TAS-1) or pMAHTII (TAS-2) 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 tested negative for the presence of HTLV-I/II proviral DNA.

APPROVAL

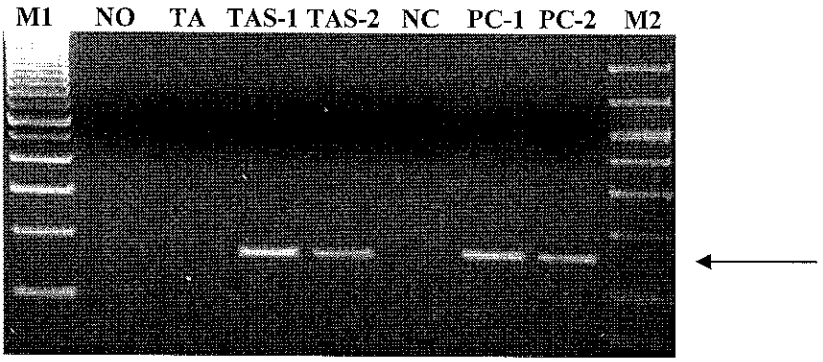
I accept responsibility for the conduct of this study which was performed in compliance with the U.S. FDA Good Laboratory Practice regulations (21 CFR 58), the UK GLP Regulations, the Japanese GLP Standard, and the Organization for Economic Cooperation and Development (OECD) Principles of Good Laboratory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

_____ 21 May 09

Ph.D. Date

Study Director

FIGURE 1



Detection of HTLV-I/II proviral sequences in the test article 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 pH750
TAS-2:	Test article spiked with 100 copies of pMAHTII
NC:	Negative control (MRC5 genomic DNA)
PC-1:	Positive control for HTLV-I (Genomic DNA from MRC5 spiked with 100 copies pH750)
PC-2:	Positive control for HTLV-II (Genomic DNA from MRC5 spiked with 100 copies pMAHTII)
M2:	Biomarker low DNA size marker

The arrow indicates specific amplification products.

BioReliance®

Quality Assurance Statement

Study Information

Number: AC27WH.105013.BSV

Protocol Title: POLYMERASE CHAIN REACTION ASSAY FOR THE DETECTION OF HUMAN T-CELL LYMPHOTROPIC VIRUS TYPES I AND II (HTLV-I/II) IN BIOLOGICAL SAMPLES

Compliance

Procedures, documentation, equipment and other records were examined in order to assure this study was performed in accordance with the regulation(s) listed below and conducted according to the protocol and relevant Standard Operating Procedures. Verification of the study protocol was performed and documented by Quality Assurance.

US FDA Good Laboratory Practice Regulations (21CFR 58)
UK GLP Regulations
Japanese GLP Standard
OECD Principles of Good Laboratory Practice

Inspections

Quality Assurance performed the inspections(s) below for this study.

Insp. Dates (From/To)		Phase Inspected	To Study Director	To Management
26-May-2009	26-May-2009	Final Reporting	26-May-2009	26-May-2009
31-Mar-2009	31-Mar-2009	Admin. Of Test Substance	22-Apr-2009	22-Apr-2009 *
31-Mar-2009	31-Mar-2009	Manipulation of Test System	22-Apr-2009	22-Apr-2009 *
31-Mar-2009	31-Mar-2009	Observation of Test System	22-Apr-2009	22-Apr-2009 *
31-Mar-2009	31-Mar-2009	Test System Preparation	22-Apr-2009	22-Apr-2009 *

* Process-based Inspection

The Final Report for this study describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

E-signature

Quality Assurance: 26-May-2009 7:23 pm GMT

Reason for signature: QA Approval

Final Report

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

Study Number: AC27WH.105042.BSV

Test Article ID: TE04-MCB-1 NSCB Sample # 7856

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

Ten (10.0) µl of DNA extract isolated from the test article 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:	The test article was received by BioReliance on 04/28/2009. 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:	04/29/2009
Lab Initiation:	04/30/2009
Lab Completion:	05/05/2009
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 MRC5 human fetal lung fibroblast line Source: BioReliance
Positive Control:	Genomic DNA from MRC5 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 10.0 ul of test article DNA extract 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 the Kodak Gel Logic 100 Imaging System (SOP OPBT0943).

DEVIATIONS

No known procedural deviations from the study protocol or pertinent assay SOPs occurred during the conduct of the study.

RESULTS

Ten (10.0) µl of test article DNA extract 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 tested negative for the presence of HBV DNA.

APPROVAL

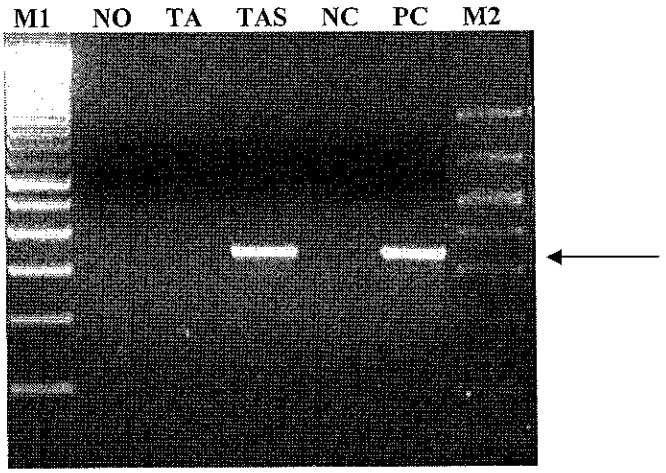
I accept responsibility for the conduct of this study which was performed in compliance with the U.S. FDA Good Laboratory Practice regulations (21 CFR 58), the UK GLP Regulations, the Japanese GLP Standard, and the Organization for Economic Cooperation and Development (OECD) Principles of Good Laboratory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

_____ 21 May 09

Ph.D. Date

Study Director

FIGURE 1



Detection of HBV specific sequences in the test article 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 (Genomic DNA from MRC5)
PC: Positive control (Genomic DNA from MRC5 spiked with 100 copies HBV185)
M2: Biomarker low DNA size marker

Arrow indicates the specific amplification product.

BioReliance®

Quality Assurance Statement

Study Information

Number: AC27WH.105042.BSV

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

Compliance

Procedures, documentation, equipment and other records were examined in order to assure this study was performed in accordance with the regulation(s) listed below and conducted according to the protocol and relevant Standard Operating Procedures. Verification of the study protocol was performed and documented by Quality Assurance.

US FDA Good Laboratory Practice Regulations (21CFR 58)
UK GLP Regulations
Japanese GLP Standard
OECD Principles of Good Laboratory Practice

Inspections

Quality Assurance performed the inspections(s) below for this study.

Insp. Dates (From/To)		Phase Inspected	To Study Director	To Management
26-May-2009	26-May-2009	Final Reporting	26-May-2009	26-May-2009
31-Mar-2009	31-Mar-2009	Admin. Of Test Substance	22-Apr-2009	22-Apr-2009 *
31-Mar-2009	31-Mar-2009	Manipulation of Test System	22-Apr-2009	22-Apr-2009 *
31-Mar-2009	31-Mar-2009	Observation of Test System	22-Apr-2009	22-Apr-2009 *
31-Mar-2009	31-Mar-2009	Test System Preparation	22-Apr-2009	22-Apr-2009 *

* Process-based Inspection

The Final Report for this study describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

E-signature

Quality Assurance: 26-May-2009 7:23 pm GMT

Reason for signature: QA Approval

Final Report

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

Study Number: AC27WH.105025.BSV

Test Article ID: TE04-MCB-1 NSCB Sample # 7856

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

RNA isolated from the test article was analyzed for the presence of Hepatitis C virus (HCV) RNA by the reverse-transcriptase polymerase chain reaction (RT-PCR) technique. The assay can detect 100 copies of HCV target sequence in the presence of 0.5 µg of HCV-negative total RNA.

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

BioReliance®

STUDY INFORMATION

Test Article: The test article was received by BioReliance on 04/28/2009. 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: 04/29/2009

Lab Initiation: 05/05/2009

Lab Completion: 05/06/2009

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 256 bp amplicon. In the presence of p19-100D RNA sequences, these primers produce a 143 bp amplicon. The amplification products are analyzed by agarose gel electrophoresis in the presence of ethidium bromide. The assay is performed according to SOP's OPBT0956, OPBT0967, and OPBT0899. The following controls are included in the assay:

Negative Control:	RNA (0.5 µg) from MRC-5 cells, negative for HCV RNA Source: BioReliance
Positive Control:	RNA (0.5 µg) from MRC-5 cells spiked with 100 copies of p19-100D RNA transcript Source: BioReliance
No RNA Control:	Distilled water, [RNase- and DNase-free] or 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 RNA transcript, 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 oligonucleotide primers HCV-F3 and HCVn-R1, complementary to sequences in 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 the Kodak Gel Logic 100 Imaging System (SOP OPBT0943).

RESULTS

Test article RNA (0.5 µg) 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 143 or 256 bp. The positive control (PC) produced a 143 bp band. The test article spiked with 100 copies of p19-100D (TAS) produced a 143 bp band, demonstrating that the test article did not inhibit the PCR reaction. The test article (TA) produced no bands at 143 or 256 bp.

These results provide evidence that the test article tested negative for the presence of HCV RNA.

DEVIATIONS

No known procedural deviations from the study protocol or pertinent assay SOPs occurred during the conduct of the study.

APPROVAL

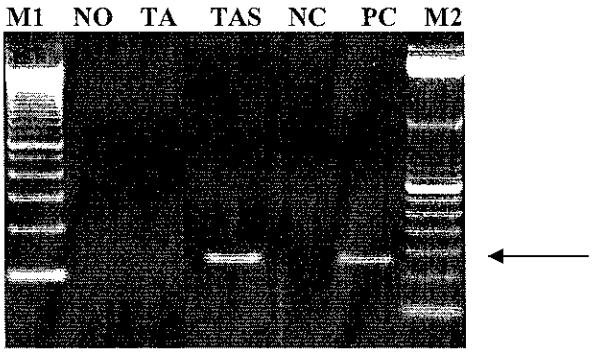
I accept responsibility for the conduct of this study which was performed in compliance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the UK GLP Regulations, the Japanese GLP Standard, and the Organization for Economic Cooperation and Development (OECD) Principles of Good Laboratory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

_____ 21 May 09

Ph.D. Date

Study Director

FIGURE 1



Detection of HCV RNA in the test article utilizing agarose gel electrophoresis in the presence of ethidium bromide

1) **M1:** 100 bp DNA ladder
2) **NO:** No RNA control
3) **TA:** Test Article
4) **TAS:** Test article spiked with 100 copies p19-100D RNA transcript
5) **NC:** Negative control (RNA (0.5 µg) from MRC-5 cells)
6) **PC:** Positive control (MRC-5 spiked with 100 copies p19-100D RNA transcript)
7) **M2:** 50 bp DNA ladder

Arrow indicates the specific amplification product of the positive control.

BioReliance®

Quality Assurance Statement

Study Information

Number: AC27WH.105025.BSV
Protocol Title: RT-PCR ASSAY FOR THE DETECTION OF HEPATITIS C VIRUS (HCV)

Compliance

Procedures, documentation, equipment and other records were examined in order to assure this study was performed in accordance with the regulation(s) listed below and conducted according to the protocol and relevant Standard Operating Procedures. Verification of the study protocol was performed and documented by Quality Assurance.

US FDA Good Laboratory Practice Regulations (21CFR 58)
UK GLP Regulations
Japanese GLP Standard
OECD Principles of Good Laboratory Practice

Inspections

Quality Assurance performed the inspections(s) below for this study.

Insp. Dates (From/To)		Phase Inspected	To Study Director	To Management
26-May-2009	26-May-2009	Final Reporting	26-May-2009	26-May-2009
31-Mar-2009	31-Mar-2009	Admin. Of Test Substance	22-Apr-2009	22-Apr-2009 *
31-Mar-2009	31-Mar-2009	Manipulation of Test System	22-Apr-2009	22-Apr-2009 *
31-Mar-2009	31-Mar-2009	Observation of Test System	22-Apr-2009	22-Apr-2009 *
31-Mar-2009	31-Mar-2009	Test System Preparation	22-Apr-2009	22-Apr-2009 *

* Process-based Inspection

The Final Report for this study describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

E-signature

Quality Assurance: 26-May-2009 7:23 pm GMT

Reason for signature: QA Approval

Printed by:

Printed on:26-May-09

Final Report

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

Study Number: AC27WH.105012.BSV

Test Article ID: TE04-MCB-1 NSCB Sample # 7856

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

Ten (10.0) µl of DNA extract isolated from the test article 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: The test article was received by BioReliance on 04/28/2009. 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: 04/29/2009

Lab Initiation: 04/30/2009

Lab Completion: 05/01/2009

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:	Genomic DNA from MRC5 spiked with 100 copies of pCMVpol, a plasmid containing a 552 bp fragment from the CMV polymerase gene Source: BioReliance
No DNA Control:	Nuclease-free water Source: USB or other commercial source
Spiked Control:	Test article extract spiked with 100 copies of pCMVpol, to verify the absence of PCR inhibitors in the test article DNA (amplification suitability control)

METHODS

Sample Preparation

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

DNA Amplification

PCR amplification was performed on 10.0 µl of test article DNA extract 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 the Kodak Gel Logic 100 Imaging System (SOP OPBT0943).

DEVIATIONS

No known procedural deviations from the study protocol or pertinent assay SOPs occurred during the conduct of the study.

RESULTS

Ten (10.0) µl of test article DNA extract 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 tested negative for the presence of CMV DNA.

APPROVAL

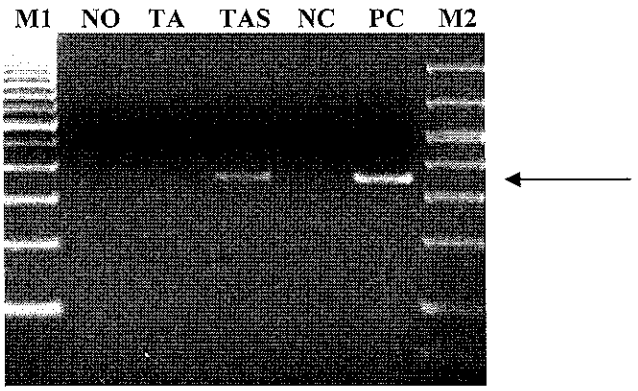
I accept responsibility for the conduct of this study which was performed in compliance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the UK GLP Regulations, the Japanese GLP Standard, and the Organization for Economic Cooperation and Development (OECD) Principles of Good Laboratory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

_____ 21 May 09

Ph.D. Date

Study Director

FIGURE 1



Detection of CMV specific sequences in the test article 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.

BioReliance®

Quality Assurance Statement

Study Information

Number: AC27WH.105012.BSV

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

Compliance

Procedures, documentation, equipment and other records were examined in order to assure this study was performed in accordance with the regulation(s) listed below and conducted according to the protocol and relevant Standard Operating Procedures. Verification of the study protocol was performed and documented by Quality Assurance.

US FDA Good Laboratory Practice Regulations (21CFR 58)
UK GLP Regulations
Japanese GLP Standard
OECD Principles of Good Laboratory Practice

Inspections

Quality Assurance performed the inspections(s) below for this study.

Insp. Dates (From/To)		Phase Inspected	To Study Director	To Management
26-May-2009	26-May-2009	Final Reporting	26-May-2009	26-May-2009
31-Mar-2009	31-Mar-2009	Admin. Of Test Substance	22-Apr-2009	22-Apr-2009 *
31-Mar-2009	31-Mar-2009	Manipulation of Test System	22-Apr-2009	22-Apr-2009 *
31-Mar-2009	31-Mar-2009	Observation of Test System	22-Apr-2009	22-Apr-2009 *
31-Mar-2009	31-Mar-2009	Test System Preparation	22-Apr-2009	22-Apr-2009 *

* Process-based Inspection

The Final Report for this study describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

E-signature

Quality Assurance: 26-May-2009 7:23 pm GMT

Reason for signature: QA Approval

Printed by:

Printed on:26-May-09

Final Report

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

Study Number: AC27WH.105011.BSV

Test Article ID: TE04-MCB-1 NSCB Sample # 7856

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

Ten (10.0) µl of DNA extract isolated from the test article 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 genome 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: The test article was received by BioReliance on 04/28/2009. 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: 04/29/2009

Lab Initiation: 04/30/2009

Lab Completion: 05/06/2009

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 genome, 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:	Genomic DNA from MRC5 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 Molecular Laboratory for testing. DNA was isolated from the test article sample using the Easy DNA™ Kit (Invitrogen) as outlined in the kit procedure and BPBT0920.

DNA Amplification

PCR amplification was performed on 10.0 µl of test article DNA extract using primers TC58 and TC61 specific for the BamW region of the EBV genome, employing conditions optimized to achieve detection of 10 copies of EBV genome. 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 the Kodak Gel Logic 100 Imaging System (SOP OPBT0943).

DEVIATIONS

No known procedural deviations from the study protocol or pertinent assay SOPs occurred during the conduct of the study.

RESULTS

Ten (10.0) µl of test article DNA extract 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 tested negative for the presence of EBV DNA.

APPROVAL

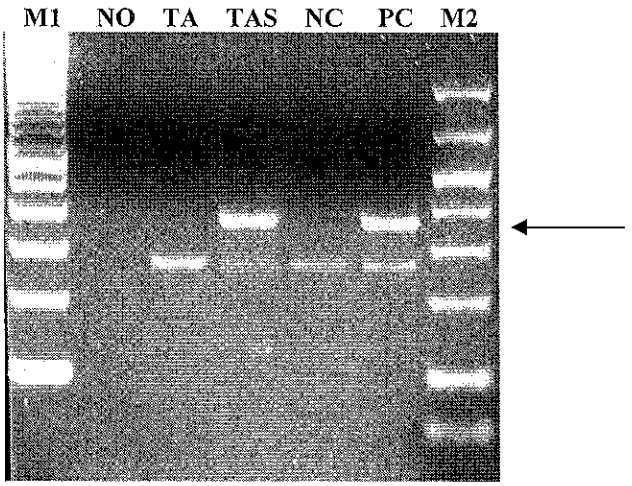
I accept responsibility for the conduct of this study which was performed in compliance with the U.S. FDA Good Laboratory Practice regulations (21 CFR 58), the UK GLP Regulations, the Japanese GLP Standard, and the Organization for Economic Cooperation and Development (OECD) Principles of Good Laboratory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

_____ 21 May 09

Ph.D. Date

Study Director

FIGURE 1



Detection of EBV specific sequences in the test article 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 BamW
NC: Negative control (Genomic DNA from MRC5)
PC: Positive control (Genomic DNA from MRC5 spiked with 100 copies BamW)
M2: Biomarker low DNA size marker

Arrow indicates specific amplification product.

BioReliance®

Quality Assurance Statement

Study Information

Number: AC27WH.105011.BSV

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

Compliance

Procedures, documentation, equipment and other records were examined in order to assure this study was performed in accordance with the regulation(s) listed below and conducted according to the protocol and relevant Standard Operating Procedures. Verification of the study protocol was performed and documented by Quality Assurance.

US FDA Good Laboratory Practice Regulations (21CFR 58)
UK GLP Regulations
Japanese GLP Standard
OECD Principles of Good Laboratory Practice

Inspections

Quality Assurance performed the inspections(s) below for this study.

Insp. Dates (From/To)		Phase Inspected	To Study Director	To Management
26-May-2009	26-May-2009	Final Reporting	26-May-2009	26-May-2009
31-Mar-2009	31-Mar-2009	Admin. Of Test Substance	22-Apr-2009	22-Apr-2009 *
31-Mar-2009	31-Mar-2009	Manipulation of Test System	22-Apr-2009	22-Apr-2009 *
31-Mar-2009	31-Mar-2009	Observation of Test System	22-Apr-2009	22-Apr-2009 *
31-Mar-2009	31-Mar-2009	Test System Preparation	22-Apr-2009	22-Apr-2009 *

* Process-based Inspection

The Final Report for this study describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

E-signature

Quality Assurance: 26-May-2009 7:23 pm GMT

Reason for signature: QA Approval

WuXi AppTec

E-FR-1-09-001101.30863

FINAL STUDY REPORT

STUDY TITLE: **Detection of Human Herpesvirus 6 Variant A (HHV-6 A) and Human Herpesvirus 6 Variant B (HHV-6 B) DNA by Quantitative Polymerase Chain Reaction (qPCR): (GLP)**

TEST PROTOCOL NUMBER: 30863.03

TEST ARTICLE IDENTIFICATION	WUXI APPTEC ACCESSION NUMBER
TE04-MCB-1 NSCB# 2156	09-001101

SPONSOR: WiCell Research Institute

PERFORMING LABORATORY: WuXi AppTec, Inc.

WUXI APPTEC ACCESSION NUMBER	RESULTS
09-001101	**Negative.** HHV-6A/B DNA sequences were not detected in the Sponsor's sample at a 1:10 dilution of the test article DNA. The Limit of Detection of the assay is 10 copies.

QUALITY ASSURANCE UNIT SUMMARY

STUDY: Detection of Human Herpesvirus 6 Variant A (HHV-6 A) and Human Herpesvirus 6 Variant B (HHV-6 B) DNA by Quantitative Polymerase Chain Reaction (qPCR): (GLP)

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
DNA Isolation BR# 36198.07 Step 1.2.5 Add 5 mL of Buffer G2 to completely resuspend the nuclei.	June 11, 2009
qPCR Protocol: Test Article DNA at neat. BR# 30863.03 Step 1.2 See appropriate worksheet for suggested Thermal Cycler Parameters.	June 15, 2009
qPCR Protocol: Test Article DNA at 1:10 dilution. BR# 30863.03 Step 1.2 See appropriate worksheet for suggested Thermal Cycler Parameters.	June 18, 2009

Quality Assurance — Date: 29 Jun 09

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 WuXi AppTec Quality Assurance audited the final report.

Study Director — Date: 22 July 09

Professional Personnel involved in study:

1.0 PURPOSE

The purpose of this assay is to provide a limit test for the detection of human herpesvirus 6 variant A (HHV-6 A) and human herpesvirus 6 variant B (HHV-6 B) DNA in the Sponsor's sample using quantitative Polymerase Chain Reaction (qPCR) technology and to provide data on assay sensitivity within the procedure by including a standard line of 5 levels of HHV-6 A/B DNA.

2.0 SPONSOR: WiCell Research Institute

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

4.0 SCHEDULING

DATE SAMPLES RECEIVED: June 9, 2009
STUDY INITIATION DATE: June10, 2009
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: TE04-MCB-1 NSCB# 2156

7.0 TEST SYSTEM DESCRIPTION

Human herpesvirus 6 (HHV-6) was first isolated from peripheral blood lymphocytes of individuals with lymphoproliferative disease.[1] It is the causative agent of exanthem subitum (Roseola Subitum) and can persist or remain latent in asymptomatic adults.[2] Infection by HHV-6 is widespread, and in healthy populations between 52 to 80% had evidence of seroconversion, many of which were infected in early infancy[3]. The virus shows a tropism for CD4+ T-cells, B-cells, and glial cells and is genetically distinct from other human herpesviruses.[4]

Examination of a large number of HHV-6 isolate markers has demonstrated that HHV-6 isolates consistently fall into two variants (A and B). There has been observed up to 25% difference between these two variants at the nucleotide level and less than 5% difference within a variant group. There is currently no serological test to distinguish the variants.

Cocultivation of susceptible indicator cells (e.g., cord blood mononuclear cells) with test article is often used to detect human herpesvirus. Several cell types should be used to cover all known herpesviruses. Given the high incidence of HHV-6 (and other herpesviruses) in healthy populations, it is obvious that virus-free cord blood cells are hard to obtain and expensive. Moreover, conventional culture assays often require several weeks before a specific cytopathic effect can be observed.

PCR* is the method of choice for detection of viral DNA in cell substrates because of its great sensitivity and specificity for detecting DNA sequences. The technique may be used to detect as few as three copies of a target DNA in a background of half a million cells.[5-6] By defining the borders of a selected region of the HHV-6 A and HHV-6 B sequence with a pair of oligonucleotide primers, it is possible to amplify the target sequence with a thermostable DNA polymerase by a factor of 10 billion in a few hours.

Quantitative PCR offers a unique combination of sensitivity and quantification that had been previously difficult to achieve with classic endpoint PCR. The Roche LightCycler™ is a particularly well-suited tool with which to perform these types of study. It offers extremely rapid cycling times, with PCR runs being completed in 15-20 minutes, and a unique single chamber design, ensuring complete well-to-well uniformity. Sequence-specific hybridization probes are designed to allow detection and analysis of PCR products on the LightCycler without the need for any post-PCR sample manipulation.[7]

The HHV-6 variant A and HHV-6 variant B genomes are linear, double-stranded DNA of about 160 kbp in length. The PCR primers used in this study are derived from the U65-U66 genes of HHV-6 A, and their PCR product is a 196 bp DNA fragment.[8] The amplified sequence corresponds to the coding stand of the nucleotide region 101581-101776 of HHV-6 A U1102 strain genome (159321 bp) and is also identical to the sequence of the coding strand 102832-103018 of HHV-6 B Z29 strain genome (162114 bp). The probes are oligonucleotides that can hybridize specifically to an internal sequence of the 196-bp fragment of HHV-6 A and HHV-6 B. PCR amplifies both extrachromosomal and integrated viral DNA. Detection of the specific amplicon using LightCycler PCR with an FITC-labeled donor probe and an LC640-labeled acceptor probe provides a quantitative, real-time, sequence-specific analysis of amplified target sequences. The assay may be used to detect as few as 10 - 100 copies of HHV-6 A and HHV-6 B viral DNA. The assay can be completed within a single day.

8.0 EXPERIMENTAL DESIGN

8.1 The test article was maintained according to the Sponsor's instructions. Total DNA was isolated from the test article by use of a commercial kit. The purified DNA was resuspended in a final volume of 1X TE buffer that resulted in the concentration of 0.1 µg/µL.

8.2 The DNA template was amplified by PCR in a single reaction mix using specific temperature cycling parameters. A master mix of reagents containing the appropriate primers, fluorescent probes, dNTPs, DEPC-teated water, $MgCl_2$, LightCycler reaction buffer, and LightCycler PCR enzyme mix was prepared and dispensed to every reaction in the assay.

8.3 Five aliquots of test article DNA at 1:10 dilution were dispensed into capillaries. Three aliquots were processed with no additional DNA supplements. The other aliquots were spiked with 100 and 1000 copies of HHV-6 A/B DNA.

* The PCR process is covered by U.S. patents owned by Hoffmann La-Roche Inc.

8.4 Three aliquots of reagent control were dispensed into capillaries. One aliquot of reagent control containing all the components except a DNA template was used as a negative control. Two aliquots of reagent control were spiked with 100 and 1000 copies of HHV-6 A/B DNA and were used as positive controls.

8.5 Four aliquots of H9 DNA were dispensed into capillaries. Two aliquots of H9 DNA processed with no additional DNA supplements were used as negative controls. Two aliquots of H9 DNA were spiked with 100 and 1000 copies of HHV-6 A/B DNA and were used as positive controls.

8.6 HHV-6 A/B DNA corresponding to 10^1 to10^5 copies were prepared in buffer in order to provide a standard line on which linear regression analysis was performed.

9.0 TEST ARTICLE PREPARATION

On June 9, 2009, WuXi AppTec, Inc. received 1 pellet containing $1x10^7$ cells of "hES Cells", frozen on dry ice and designated for use in this assay. The test article was stored at ≤ -60°C until the assay was initiated.

10.0 POSITIVE CONTROLS

Plasmid DNA containing the target HHV-6 A/B viral sequences was spiked into the test article, reagent control, and control DNA as positive controls.

11.0 NEGATIVE CONTROLS

Purified DNA from the human H9 cell line was used as a negative control.

A reaction tube containing all of the components except a DNA template served as a reagent control.

12.0 ASSAY VALIDITY

The test is considered valid if the following criteria were met:

12.1 The 1000-copy dilution of HHV-6 A/B DNA spiked H9 DNA must show a fluorescence signal above background.

12.2 The 100-copy HHV-6 A/B DNA standard must show a fluorescence signal above background.

12.3 Fluorescence signals ≥ the Limit of Detection (LOD) must not be detected in the reagent control.

12.4 Fluorescence signals ≥ the Limit of Detection (LOD) must not be detected in the unspiked H9 DNA samples.

12.5 The R^2 value of the standard curve must be ≥ 0.80.

13.0 TEST EVALUATION

All results will be judged by analysis of the quantitative fluorescence figures (F2/F1 vs. Cycles).

A Limit of Detection (LOD) is determined by examining the data obtained from the standard line and determining the lowest standard detected above background.

A negative result, as judged by the fluorescence signal below the LOD in all of the replicates of the PCR amplified unspiked test article DNA, will indicate that the HHV-6 A/B DNA sequences specific for the primers used are not present in detectable quantities in the Sponsor's sample. The result will be reported as, "Negative. HHV-6 A/B DNA sequences were not detected in the Sponsor's sample. The LOD of the assay is *X* copies."

A positive result will be judged by the presence of a specific hybridization fluorescence signal equal to or greater than the LOD in at least two of the three replicates of the PCR-amplified unspiked test article. The results will be given a mean score and the result will be expressed as, "Positive: *X* copies of HHV-6 A/B DNA sequences per (0.5 μg DNA or other sample amount tested or) per mass or volume extracted were detected in the Sponsor's sample. The LOD of the assay is *X* copies."

If a specific signal is detected in one unspiked test article replicate equal to or greater than the LOD but not the other two, the result will be reported as "Putative Positive: requires verification."

If fluorescence signals are not detected above background in the test article spiked with 100 and 1000 copies of HHV-6 A/B DNA, the results will be reported as, "Test article interferes with the PCR reaction." The Sponsor will be contacted, and a retest of a 1:10 and/or 1:100 diluted test article DNA will be initiated upon Sponsor approval.

14.0 RESULTS

The initial test was valid. However the test article DNA at neat interfered with the PCR reaction. On June 17, 2009 the client was notified of the test article interference and subsequently the client approved of an additional test using 1:10 dilution of the test article DNA. The test article DNA at 1:10 dilution results was negative. The result and DNA calculation for 1:10 dilution was used in this result section.

The test was valid. The 1000 copies of HHV-6 A/B DNA spiked H9 DNA and 100 copies HHV-6 A/B DNA standard showed fluorescence signals above background. Fluorescence signals greater than the Limit of Detection (LOD) were not detected in the reagent control and unspiked H9 DNA samples. The R^2 value of the standard curve was 1.00.

The test article gave a negative result for HHV-6 A/B. The HHV-6 A/B specific probes did not hybridize to the PCR-amplified test article DNA giving fluorescence signals greater than the Limit of Detection, indicating that HHV-6 A/B DNA sequences specific for the primers and probe used were not present in detectable quantities in the Sponsor's sample. The Limit of Detection of the assay was 10 copies.

HHV-6 A/B specific sequences were detected in 100 and 1000 copies of purified HHV-6 A/B DNA spiked into DNA isolated from the test article. HHV-6 A/B specific sequences were detected in 100 and 1000 copies of purified HHV-6 A/B DNA spiked into DNA isolated from H9 control cells. Therefore, the test article and H9 control did not contain material that inhibited the PCR reaction.

DNA was isolated from $1x10^7$ cells of test article. DNA extracted from the test article was re-suspended in 35 µL of 1X TE buffer. 5 µL of DNA was used for the OD scan. The concentration by OD was determined to be 1.132 µg/µL. Therefore the total yield was 35 µL x 1.132 µg/µL = 39.62 µg of DNA.

$1x10^7$ cells of test article generated 39.62 µg of DNA. Since a 1:10 dilution was performed prior to qPCR, 0.05 µg of DNA was therefore obtained from 0.05 µg DNA x $1x10^7$ cells of test article / 39.62 µg of DNA = $1.26x10^4$ cells. Since 0.05 µg of DNA was used per reaction, and the reaction was run in triplicate, an equivalent of $1.26x10^4$ cells x 3 = $3.78x10^4$ cells of original test article was tested.

A printout of the amplification plot is included as Appendix A to this Final Report.

15.0 CONCLUSION

HHV-6 A/B DNA sequences were not detected in the Sponsor's sample at a 1:10 dilution of the test article DNA.

16.0 STATISTICAL DATA ANALYSIS

Linear regression analysis was used to calculate an R^2 value for the standard curve. The copy number of the test article was determined from the standard curve.

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 ten (10) 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. Salahuddin SZ, Ablshi DV, Markham PD, Joseph SF, Sturzenegger S, Kaplan M, Hallligan G, Biberfeld P, Wong-Staal F, Kramarsky B and Gallo RC (1986). "Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders." *Science* 234:596-601

2. Yamanishi K, Okuno T, Shiraki K, Takahashi M, Kondo T, Asano Y and Kurata K (1988). "Identification of human herpesvirus 6 as a causal agent for exanthem subitum." *Lancet* 1:1065-1067

3. Okuno T, Takahashi K, Balachandran K, Shiraki K, Yamanishi K, Takahashi M and Baba K (1989). "Seroepidemiology of human herpesvirus 6 infection in normal children and adults." *Journal of Clinical Microbiology* 27:651-653

4. Lopez C and Honess RW. "Human Herpesvirus-6," in: Fields BN, Knipe DM, Chanock RW, Melnick J, Roizman B, and Shope R, ed. Virology, 2nd edition. Raven Press, New York. 1990. pp. 2055-2062

5. Mullis KB and Faloona FA (1987). "Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction." *Methods in Enzymology* 155:335-350

6. Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis K, and Erlich HA (1988). "Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase." *Science* 239:487-491

7. Wittwer C, Herrmann M, and Moss A (1997). "Continuous fluorescence monitoring of rapid cycle DNA amplification." *BioTechniques* 22(1): 130-138

8. Gautheret-Dejean A, Manichanh C, Thien-Ah-Koon F, Fillet A, Mangeney N, Vidaud M, Dhedin N, Vernant J, and Agut H (2002). "Development of a real-time polymerase chain reaction assay for the diagnosis of human herpesvirus-6 infection and application to bone marrow transplant patients." *J. Virol. Methods* 100:27-35

LightCycler Data Analysis (Lightcycler) - [Quantification: 09-001101 HHV6A+B AYD 18JUN09; F2/F1; CYCLE seg 2]

File Quantification Report Window Help

Analysis
- Fit Points
- (•) Second Derivative Maximum

Baseline Adjustment
- None
- Arithmetic
- (•) Proportional
- Normalized

Analysis Notes

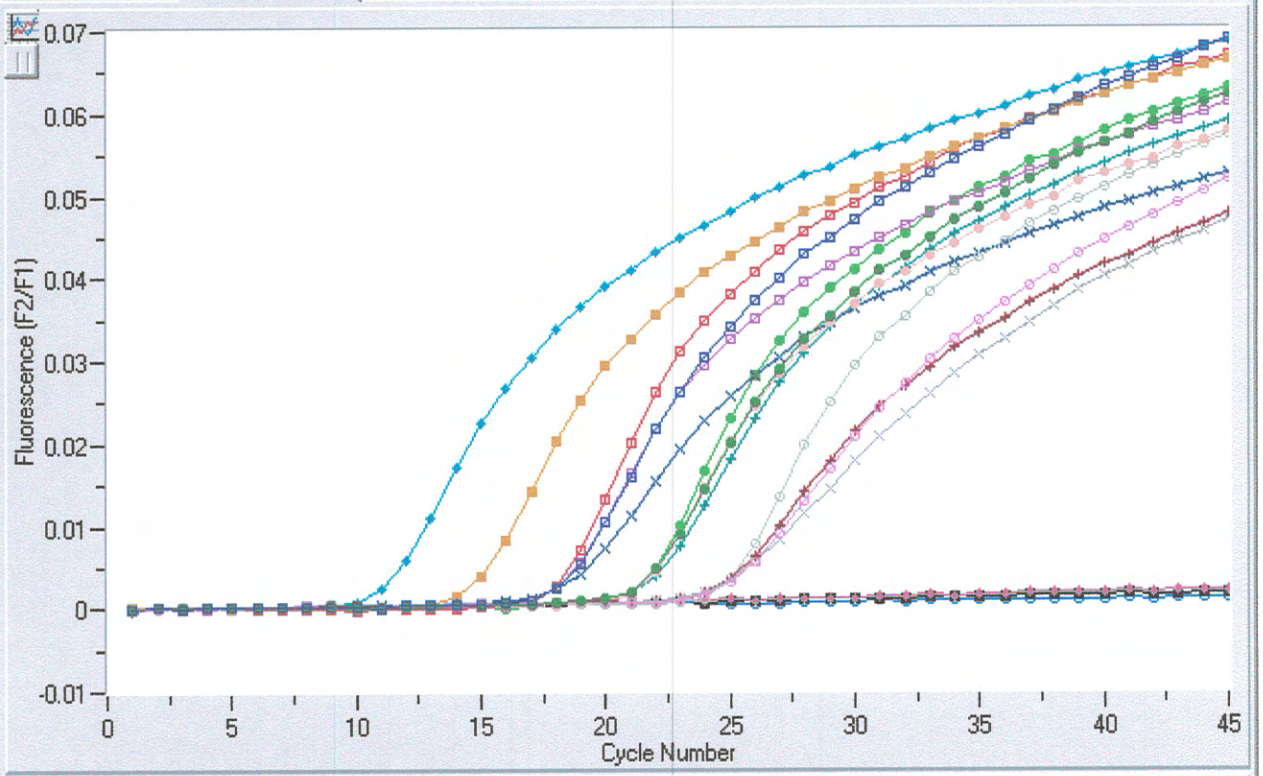
APPENDIX A: HHV6 A&B BR 30863

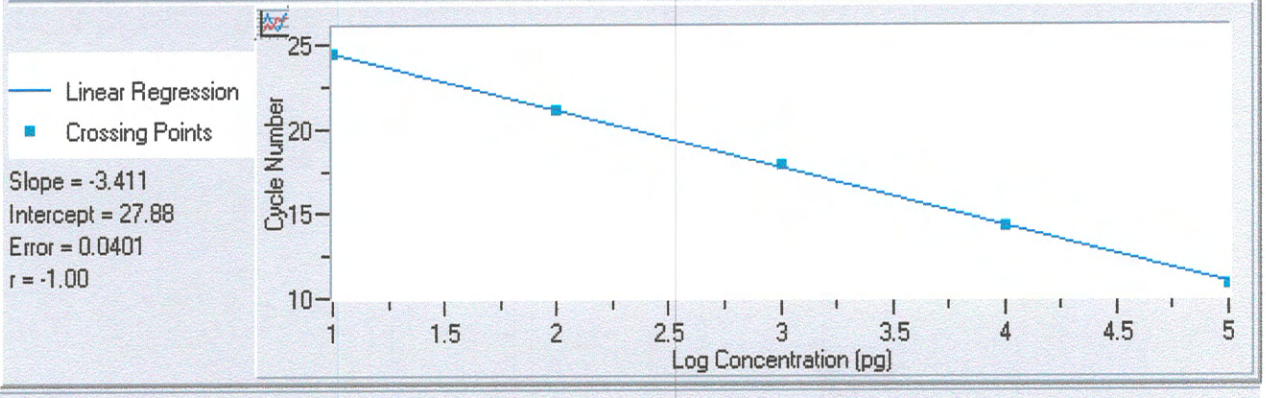
	Position	Name	Standard...	Calculat...	Cro...
	1	RC			
	2	RC 100	1.000E+02	9.946E+01	21.06
	3	RC 1000	1.000E+03	1.012E+03	17.63
	4	H9 NS			
	5	H9 NS			
	6	H9 100	1.000E+02	6.873E+01	21.61
	7	H9 1000	1.000E+03	6.369E+02	18.31
	8	STD 10	1.000E+01	1.055E+01	24.39
	9	STD 100	1.000E+02	1.003E+02	21.05
	10	STD 1000	1.000E+03	8.939E+02	17.81
	11	STD 10000	1.000E+04	1.002E+04	14.23
	12	STD 100000	1.000E+05	1.055E+05	10.74
	13	TA 09-001101 NS 1:10		9.379E+00	24.56
	14	Repli. of TA 09-001101 NS		8.383E+00	24.73
	15	Repli. of TA 09-001101 NS		7.630E+00	24.87
	16	TA 09-001101 100 1:10	1.000E+02	9.081E+01	21.20
	17	TA 09-001101 1000 1:10	1.000E+03	8.029E+02	17.97
	13,14,15	Grouped Means		8.464E+00	24.72
		Grouped S.D.		8.773E-01	0.153

Note: Amplification signal for positions 13, 14, and 15 are below the LOD (STD 10, position 8). TA DNA at 1:10 dilution is negative for HHV6 A/B. SC 25 June 09

AYD 18JUN09

Step 1: Baseline | Step 2: Analysis





Final Report

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

Study Number: AC27WH.105020.BSV

Test Article ID: TE04-MCB-1 NSCB Sample # 7856

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

Ten (10.0) µl of DNA extract isolated from the test article 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.

These results provide evidence that the test article tested positive for the presence of HHV-6 variant A viral DNA . The HHV-6 variant B viral DNA was not detected. However, the detection level at 100 copies cannot be claimed for this study as the test article spiked with 100 copies of positive control plasmid pZ29MOD (HHV-6B) did not amplify due to the high level of HHV-6A viral contaminant.

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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: The test article was received by BioReliance on 04/28/2009. 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: 04/29/2009

Lab Initiation: 04/30/2009

Lab Completion: 06/12/2009

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 OPBT0924. In the presence of HHV-6A viral sequences, these primers produce a 328 bp amplification product, while the HHV-6A positive control plasmid (pU1102MOD) generates a 299 bp amplification product. In the presence of HHV-6B viral sequences, the primers produce a 553 bp amplification product, while the HHV-6B positive control plasmid (pZ29MOD) generates a 524 bp amplification product. The following controls are included in the assay:

Negative Control:		Genomic DNA from MRC5 human fetal lung fibroblasts Source: BioReliance
Positive controls:	HHV-6A:	Genomic DNA from MRC5 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:	Genomic DNA from MRC5 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. In initial testing and in subsequent confirmatory 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 10 µl of test article DNA extract 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 the Kodak Gel Logic 100 Imaging System (SOP OPBT0943).

RETESTS

In the first performance of the PCR assay (PCR1), the test article (TA) produced a band at the suspect positive 328 bp (see Figure 1). In accordance to the Retesting Policy of the governing protocol (P105020.BSV.V16, Section 10.0), a retest of the PCR-only using DNA extract from the original extraction, in addition to a complete retest (fresh extraction and PCR) were to be performed.

In both the PCR-only retest (PCR2) and in the complete retest (R1 PCR1), the test article produced a band at the 328 bp, indicating detection of HHV-6 variant A viral DNA. Results are presented in Figures 2 and 3.

INVESTIGATION

The client was informed of the confirmatory testing results, and in efforts to confirm or refute the positive result, an investigation was opened (refer to Event Record # 31626) and DNA sequencing analysis was requested. The PCR products, along with the HHV-6 specific primers and the HHV-6A control plasmid, were sent to an outside, specialized contractor for sequencing analysis. The sequences obtained from the test article and the positive control were analyzed using the BLAST (Basis Local Alignment Search Tool) Search and Sequencher Software.

The positive control HHV-6A plasmid for this assay was designed to contain a 29 bp deletion in the primer-flanking region; the deletion should not be observed in the HHV-6 virus. The sequencing results indicated that the test article contained the HHV-6A virus sequence, since no

deletion of the 29 bp was observed as compared to the sequence of the positive control plasmid for HHV-6A.

Investigation Conclusion

The investigative sequencing confirmed that the test sample is positive for HHV-6A DNA sequences. The investigation is therefore judged successful in confirming the positive results from the PCR assays, and the test article is evaluated as positive for HHV-6A viral DNA.

RESULTS

Test article DNA extract (10 µl) 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 Figures 1 – 3. 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) did produced a band at 524 bp

Due to the presence of HHV-6A viral DNA in the test article, the test article spiked with 100 copies of pU1102MOD (TAS-1) produced a 328 bp band. The test article spiked with 100 copies of pZ29MOD (TAS-2) did not produce a 524 bp band, as a result of the contaminant in the test article. However, as amplification was demonstrated by the HHV-6A DNA present in the test article, the PCR was deemed not inhibitory to HHV-6.

The test article (TA) produced no band at 553 bp, however a band was observed at the 328 bp.

These results provide evidence that the test article tested positive for the presence of HHV-6 variant A viral DNA . The HHV-6 variant B viral DNA was not detected. However, the detection level at 100 copies cannot be claimed for this study as the test article spiked with 100 copies of positive control plasmid pZ29MOD (HHV-6B) did not amplify due to the high level of HHV-6A viral contaminant.

DEVIATIONS

No known procedural deviations from the study protocol or pertinent assay SOPs occurred during the conduct of the study.

APPROVAL

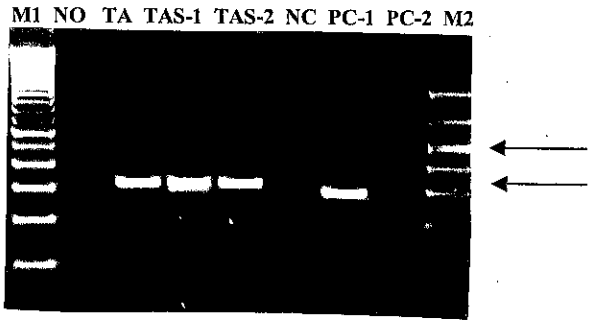
I accept responsibility for the conduct of this study which was performed in compliance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the UK GLP Regulations, the Japanese GLP Standard, and the Organization for Economic Cooperation and Development (OECD) Principles of Good Laboratory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

_____ 12 Jun 09

Ph.D. Date

Study Director

FIGURE 1 (PCR1)

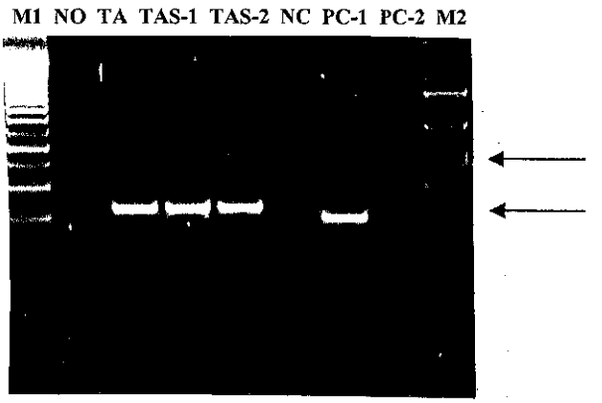


Detection of HHV-6 (variants A and B) viral sequences in the test article utilizing agarose gel electrophoresis in the presence of ethidium bromide

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 (MRC5 genomic DNA spiked with 100 copies of pU1102MOD)
PC-2: Positive control (MRC5 genomic DNA spiked with 100 copies of pZ29MOD)
M2: Biomarker low DNA size marker

Arrows indicate specific amplification products.

FIGURE 2 (PCR2)

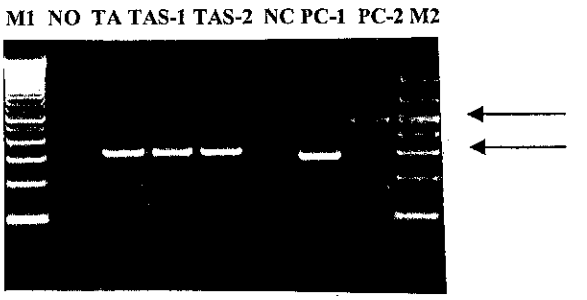


Detection of HHV-6 (variants A and B) viral sequences in the test article utilizing agarose gel electrophoresis in the presence of ethidium bromide

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 (MRC5 genomic DNA spiked with 100 copies of pU1102MOD)
PC-2: Positive control (MRC5 genomic DNA spiked with 100 copies of pZ29MOD)
M2: Biomarker low DNA size marker

Arrows indicate specific amplification products.

FIGURE 3 (R1 PCR1)



Detection of HHV-6 (variants A and B) viral sequences in the test article 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 (MRC5 genomic DNA spiked with 100 copies of pU1102MOD)
PC-2: Positive control (MRC5 genomic DNA spiked with 100 copies of pZ29MOD)
M2: Biomarker low DNA size marker

Arrows indicate specific amplification products.

BioReliance®

Quality Assurance Statement

Study Information

Number: AC27WH.105020.BSV

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

Compliance

Procedures, documentation, equipment and other records were examined in order to assure this study was performed in accordance with the regulation(s) listed below and conducted according to the protocol and relevant Standard Operating Procedures. Verification of the study protocol was performed and documented by Quality Assurance.

US FDA Good Laboratory Practice Regulations (21CFR 58)
UK GLP Regulations
Japanese GLP Standard
OECD Principles of Good Laboratory Practice

Inspections

Quality Assurance performed the inspections(s) below for this study.

Insp. Dates (From/To)		Phase Inspected	To Study Director	To Management
12-Jun-2009	12-Jun-2009	Final Reporting	12-Jun-2009	12-Jun-2009
31-Mar-2009	31-Mar-2009	Admin. Of Test Substance	22-Apr-2009	22-Apr-2009 *
31-Mar-2009	31-Mar-2009	Manipulation of Test System	22-Apr-2009	22-Apr-2009 *
31-Mar-2009	31-Mar-2009	Observation of Test System	22-Apr-2009	22-Apr-2009 *
31-Mar-2009	31-Mar-2009	Test System Preparation	22-Apr-2009	22-Apr-2009 *

* Process-based Inspection

The Final Report for this study describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

E-signature

Quality Assurance: 12-Jun-2009 9:38 pm GMT

Reason for signature: QA Approval

Printed by

Printed on:12-Jun-09

Event Details Report

Positive Result — Record ID: 61626

Date Opened:	11-May-2009	**Date Occurred:**	04-May-2009
Final Priority :	High	**Record State :**	Closed - Done

General Information

Short Description: BioReliance Study Number AC27WH.105020.BSV tested positive for HHV-6 DNA.

Department: Molecular - Molecular Biology

Impacted Systems : Assay/ Product

Product / Assay

TA/Project ID	Protocol No.	Disposition
AC27WH	105020.BSV	Test - Retest

Description : The test article AC27WH tested positive for HHV-6 DNA (assay # 105020.BSV). In initial performance of the PCR assay, the test article (TA) produced a band at the ~299bp, indicating detection of the HHV-6 DNA sequence. The client was notified of the result and indicated that the result obtained was unexpected.
Further investigation is needed to identify the cause of the positive results.

Immediate / Containment Action : In accordance to the retesting policy of the governing protocol, P105020.BSV.V16, section 10.0, the positive result obtained from initial performance of the PCR assay (PCR1) was to be confirmed by retesting the PCR analysis, using extracted DNA obtained from the original extraction (PCR2), in addition to a complete retest (fresh extraction of a backup sample and PCR analysis) (R1 PCR1). The laboratory was thoroughly cleaned prior to the performance of any retesting procedures, using appropriate materials designed to rid surfaces and equipment of any trace DNA. In addition, two different technicians were used to perform the retest analyses, in order to rule out technician error as a possibility of root cause.
Both retest assays (PCR2 and R1 PCR1) produced the same positive result, with the test article (TA) producing a band at the ~299bp.
Further investigation is needed to identify the cause of the positive results.

Operations Assessment/Investigation

Valid Assay / Process Criteria Met? Yes

Preliminary Investigation : All variables (i.e: environment, materials, technicians, etc) were within regulations and compliance. After obtaining the result from PCR1, the rooms were cleaned with appropriate materials to ensure integrity of the environment. All machines were functioning properly during performance of the initial assay and retest analyses. Proper methods were utilized in performing the assays, and documentation was found to be free of error; in addition, three separate technicians were assigned to each of the three analyses, in order to rule out any error by man.

The two positive controls of the assay (PC-I and PC-II) produced the expected amplification at the 299bp and 524bp, respectively. No amplification was observed in the negative control (NC) well or the No DNA control (NO), indicating absence of contamination. With the meeting of these criteria, all three analyses were valid.

Impact Assessment : The impact is scored as medium since this is unexpected positive result from the client. Retest confirmed the positive result. Further investigation is needed to identify the nature of the positive finding.

Event Details Report

Positive Result — Record ID: 31626

Man	The operators for this assay were properly trained. All data are documented clearly and promptly by the operators in the batch records. No error was found after inspection of the data by the Study Director.
Method	The testing method has been used in this lab for many years and has been performed robustly. The assay is a validated assay and there is no issue with the suitability of the testing method.
Machine	All the equipments are maintained under GLP regulation. Calibrations, routine cleaning and preventive maintenance are up to date. No record of misconduct of the laboratory equipments was found. All the controls worked as expected, indicating the equipments are suitable for the testing.
Material	Testing reagents: All reagents for sample prep and PCR were within the material expiration date. The lot/batch numbers were properly documented in the batch record. The spiked control amplified as expected, indicating that the reagents for extraction met the requirements. The assay positive control amplified as expected, indicating that the reagents for PCR are suitable for the testing.
Measurement	PCR for positive control produced correct size band indicating proper amplification of the assay. PCR for the test article spiked control produced a band indicating no sample matrix inhibition from the test article.
Environment	The Molecular Biology Lab has been following the one-way system for conducting molecular assays. The routine cleaning for the lab bench top and equipments are up to the date. All the negative controls (no DNA control and negative extraction controls) are negative for this assay. The rooms used are: PCR set up room, DNA extraction room, positive control room and amplification room. All rooms are maintained and cleaned as required.
Sample Product	The sample for study was received by BioReliance in integrity and no discrepancy was found in terms of label ID and storage condition. The sample was inspected upon receival of the sample by Molecular Biology Lab as required by the governing SOP for sample integrity, storage condition and label ID. No discrepancy was found and 'Test Article Label Verification' form was properly filled by the operator on the day of initiation.

Could this Event have affected other Products/ Services?	No
Does this Event require Product/ Service Recall?	No
Is there any evidence that Product/ Service could have been tampered with/ counterfeit?	No

Investigation Summary: According to DNA Sequencing of TA and Postive Controls it has been concluded sample is positive for HHV-6. Attached you will find sequence data. Postive Control sequence as expected showed 29 bps deletion and TA article did not have the deletion of HHV-6. This expected and therefore confirms that there was no contamination of Postive Cotnrol with TA. TA sequence matches HHV-6 wild type sequence. Therefore the TA is positive and client has been notified. Client expected negative but through our investigation they were convinced the sample is positive.

Root Cause Analysis : The TA is positive for HHV6-DNA. We have confirmed the positive result by DNA sequencing.

Event Details Report

Positive Result — Record ID: 31626

E-signatures

Supervisor Assessment By:		**Supervisor Assessment On:**	12-May-2009 1:05 pm GMT
Owner Approval By:		**Owner Approval On:**	08-Jun-2009 5:33 pm GMT
Final Approval By:		**Final Approval On:**	09-Jun-2009 1:44 pm GMT

Final Report

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

Study Number: AC27WH.105029.BSV

Test Article ID: TE04-MCB-1 NSCB Sample # 7856

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

Ten (10.0) µl of DNA extract isolated from the test article was analyzed for the presence of Human Herpesvirus 7 (HHV-7) DNA by the polymerase chain reaction (PCR)[1] technique. The assay can detect 100 copies of HHV-7 in the presence of 0.5 µg of genomic DNA.

The results presented herein indicate that the test article tested negative for the presence of HHV-7 DNA.

---

[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: The test article was received by BioReliance on 04/28/2009. 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: 04/29/2009

Lab Initiation: 04/30/2009

Lab Completion: 05/05/2009

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:	Genomic DNA from MRC5 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 10µl of test article DNA extract 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 the Kodak Gel Logic 100 Imaging System (SOP OPBT0943).

DEVIATIONS

No known procedural deviations from the study protocol or pertinent assay SOPs occurred during the conduct of the study.

RESULTS

Ten (10.0) µl of test article DNA extract 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 tested negative for the presence of HHV-7 DNA.

APPROVAL

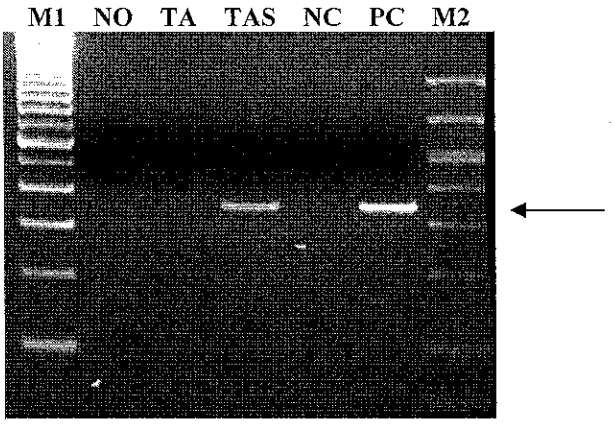
I accept responsibility for the conduct of this study which was performed in compliance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the UK GLP Regulations, the Japanese GLP Standard, and the Organization for Economic Cooperation and Development (OECD) Principles of Good Laboratory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

_____ 21 May 09

Ph.D. Date

Study Director

FIGURE 1



Detection of HHV-7 specific sequences in the test article 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 pHH7
NC: Negative control (MRC5 genomic DNA)
PC: Positive control (MRC5 genomic DNA spiked with 100 copies pHH7)
M2: Biomarker low DNA size marker

Arrow indicates the amplification product.

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Quality Assurance Statement

Study Information

Number: AC27WH.105029.BSV

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

Compliance

Procedures, documentation, equipment and other records were examined in order to assure this study was performed in accordance with the regulation(s) listed below and conducted according to the protocol and relevant Standard Operating Procedures. Verification of the study protocol was performed and documented by Quality Assurance.

US FDA Good Laboratory Practice Regulations (21CFR 58)
UK GLP Regulations
Japanese GLP Standard
OECD Principles of Good Laboratory Practice

Inspections

Quality Assurance performed the inspections(s) below for this study.

Insp. Dates (From/To)		Phase Inspected	To Study Director	To Management
26-May-2009	26-May-2009	Final Reporting	26-May-2009	26-May-2009
31-Mar-2009	31-Mar-2009	Admin. Of Test Substance	22-Apr-2009	22-Apr-2009 *
31-Mar-2009	31-Mar-2009	Manipulation of Test System	22-Apr-2009	22-Apr-2009 *
31-Mar-2009	31-Mar-2009	Observation of Test System	22-Apr-2009	22-Apr-2009 *
31-Mar-2009	31-Mar-2009	Test System Preparation	22-Apr-2009	22-Apr-2009 *

* Process-based Inspection

The Final Report for this study describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

E-signature

Quality Assurance: 26-May-2009 7:23 pm GMT

Reason for signature: QA Approval

Printed by:

Printed on:26-May-09

Final Report

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

Study Number: AC27WH.105056.BSV

Test Article ID: TE04-MCB-1 NSCB Sample # 7856

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

Ten (10.0) µl of DNA extract isolated from the test article 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:	The test article was received by BioReliance on 04/28/2009. 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:	04/29/2009
Lab Initiation:	04/30/2009
Lab Completion:	05/01/2009
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 MRC5 human fetal lung fibroblasts Source: BioReliance
Positive Control:	Genomic DNA from MRC5 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 BPBT0920.

DNA Amplification

PCR amplification was performed on 10 µl of test article DNA extract 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 the Kodak Gel Logic 100 Imaging System (SOP OPBT0943).

RESULTS

Test article DNA extract (10 µl) 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 tested negative for the presence of HHV-8 DNA.

DEVIATIONS

No known procedural deviations from the study protocol or pertinent assay SOPs occurred during the conduct of the study.

APPROVAL

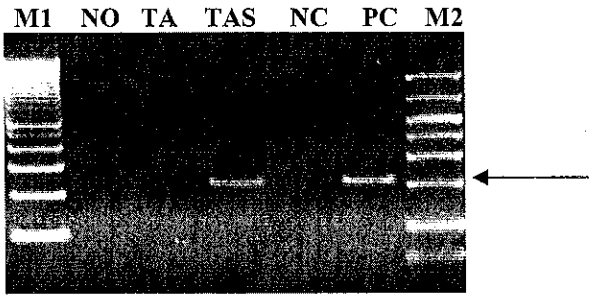
I accept responsibility for the conduct of this study which was performed in compliance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the UK GLP Regulations, the Japanese GLP Standard, and the Organization for Economic Cooperation and Development (OECD) Principles of Good Laboratory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

_____ 21 May 09

Ph.D. Date

Study Director

FIGURE 1



Detection of HHV-8 specific sequences in the test article 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 from MRC5)
PC: Positive control (Genomic DNA from MRC5 spiked with 100 copies pHHV-8)
M2: Biomarker low DNA size marker

Arrow indicates the 225 bp amplification product.

BioReliance®

Quality Assurance Statement

Study Information

Number: AC27WH.105056.BSV
Protocol Title: PCR ASSAY FOR THE DETECTION OF HUMAN HERPESVIRUS TYPE 8 (HHV-8)

Compliance

Procedures, documentation, equipment and other records were examined in order to assure this study was performed in accordance with the regulation(s) listed below and conducted according to the protocol and relevant Standard Operating Procedures. Verification of the study protocol was performed and documented by Quality Assurance.

US FDA Good Laboratory Practice Regulations (21CFR 58)
UK GLP Regulations
Japanese GLP Standard
OECD Principles of Good Laboratory Practice

Inspections

Quality Assurance performed the inspections(s) below for this study.

Insp. Dates (From/To)		Phase Inspected	To Study Director	To Management
26-May-2009	26-May-2009	Final Reporting	26-May-2009	26-May-2009
31-Mar-2009	31-Mar-2009	Admin. Of Test Substance	22-Apr-2009	22-Apr-2009 *
31-Mar-2009	31-Mar-2009	Manipulation of Test System	22-Apr-2009	22-Apr-2009 *
31-Mar-2009	31-Mar-2009	Observation of Test System	22-Apr-2009	22-Apr-2009 *
31-Mar-2009	31-Mar-2009	Test System Preparation	22-Apr-2009	22-Apr-2009 *

* Process-based Inspection

The Final Report for this study describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

E-signature

Quality Assurance: 26-May-2009 7:23 pm GMT

Reason for signature: QA Approval

Printed by:

Printed on:26-May-09

Final Report

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

Study Number: AC27WH.105037.BSV

Test Article ID: TE04-MCB-1 NSCB Sample # 7856

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

Ten (10.0) µl of DNA extract isolated from the test article 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 human parvorvovirus 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 human parvovirus B19 DNA.

---

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: The test article was received by BioReliance on 04/28/2009. 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: 04/29/2009

Lab Initiation: 04/30/2009

Lab Completion: 05/05/2009

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 human parvovirus 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 human parvovirus B19-specific primers. In the presence of human parvovirus 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:	Genomic DNA from MRC5 spiked with 100 copies of pNPS-1, a plasmid containing a 3.6 Kb fragment from the human parvovirus 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 10 µl of test article DNA extract and on the assay controls using primers B19F and B19R specific for the capsid gene of human parvovirus B19, employing conditions optimized to achieve detection of 100 copies of human parvovirus 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 the Kodak Gel Logic 100 Imaging System (SOP OPBT0943).

RESULTS

Test article DNA extract (10 µl) was analyzed for the presence of human parvovirus 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 tested negative for the presence of human parvovirus B19 DNA.

DEVIATIONS

No known procedural deviations from the study protocol or pertinent assay SOPs occurred during the conduct of the study.

APPROVAL

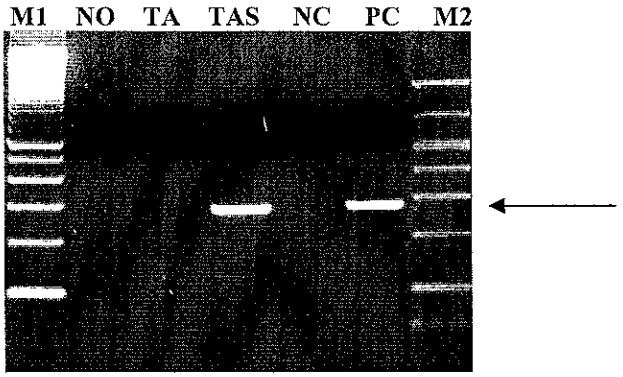
I accept responsibility for the conduct of this study which was performed in compliance with the U.S. FDA Good Laboratory Practice regulations (21 CFR 58), the UK GLP Regulations, the Japanese GLP Standard, and the Organization for Economic Cooperation and Development (OECD) Principles of Good Laboratory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

_____ 26 May 09

Ph.D. Date

Study Director

FIGURE 1



Detection of human parvovirus B19 specific sequences in the test article 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 (MRC5 genomic DNA spiked with 100 copies of pNPS-1
M2: Biomarker low DNA size marker

Arrow indicates the specific amplification product.

BioReliance®

Quality Assurance Statement

Study Information

Number: AC27WH.105037.BSV

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

Compliance

Procedures, documentation, equipment and other records were examined in order to assure this study was performed in accordance with the regulation(s) listed below and conducted according to the protocol and relevant Standard Operating Procedures. Verification of the study protocol was performed and documented by Quality Assurance.

US FDA Good Laboratory Practice Regulations (21CFR 58)
UK GLP Regulations
Japanese GLP Standard
OECD Principles of Good Laboratory Practice

Inspections

Quality Assurance performed the inspections(s) below for this study.

Insp. Dates (From/To)		Phase Inspected	To Study Director	To Management
26-May-2009	26-May-2009	Final Reporting	26-May-2009	26-May-2009
31-Mar-2009	31-Mar-2009	Admin. Of Test Substance	22-Apr-2009	22-Apr-2009 *
31-Mar-2009	31-Mar-2009	Manipulation of Test System	22-Apr-2009	22-Apr-2009 *
31-Mar-2009	31-Mar-2009	Observation of Test System	22-Apr-2009	22-Apr-2009 *
31-Mar-2009	31-Mar-2009	Test System Preparation	22-Apr-2009	22-Apr-2009 *

* Process-based Inspection

The Final Report for this study describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

E-signature

Quality Assurance: 26-May-2009 7:23 pm GMT

Reason for signature: QA Approval

WiCell Cytogenetics Report: 000236 NSCB2793

Report Date: *1/3/10*

Case Details:

Cell Line: *TE04-MCB-1-p54 (female)*

Reference: *WA01-MCB-3-L.3-p29(2) (male)*

Investigator: *National Stem Cell Bank*

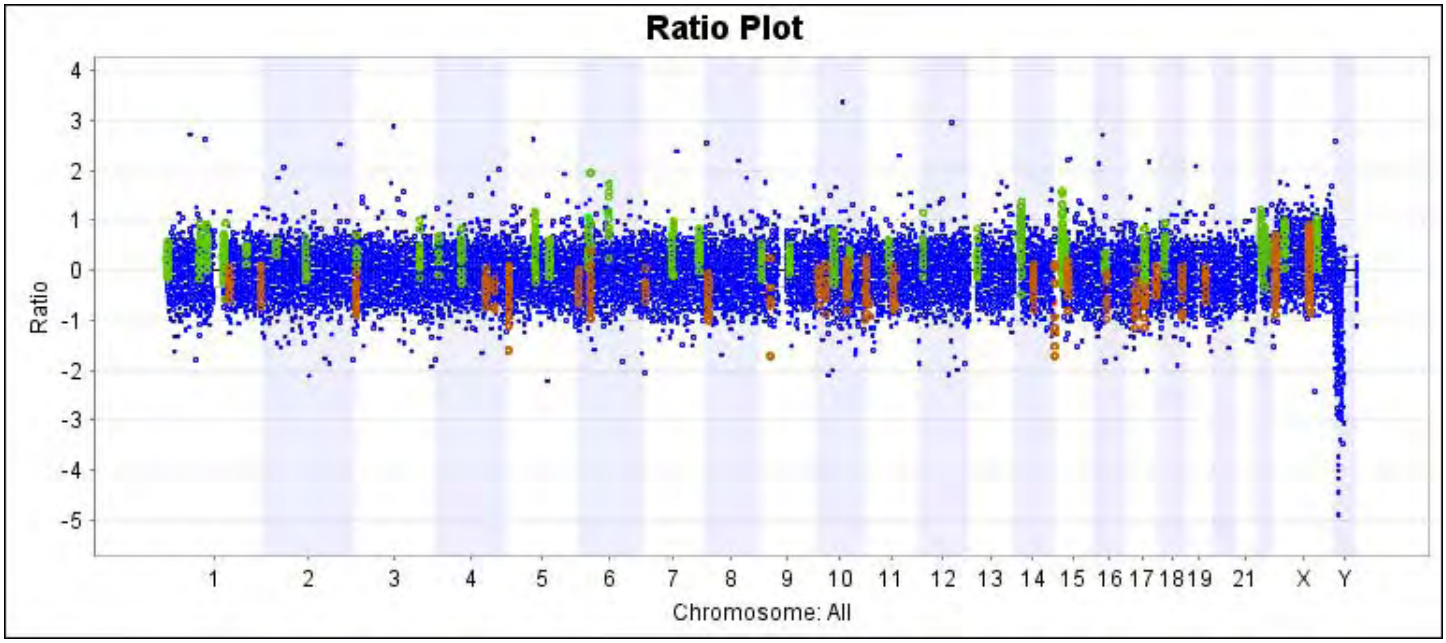
Specimen: *hESC on MEF feeder*

Date of Sample: *5/6/2009*

Reason for Testing: *MCB Testing*

GEO Accession #: *GSM456764*

aCGH Results:
Results are given in the attached Excel spreadsheet labeled "report." There were 81 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]	*2 of 8*
Reference DNA Copy Number Changes[2]	*4 of 8*
Select Differentially Expressed Genes	*0 of 88*

WiCell Cytogenetics Report: 000236 NSCB2793

These results are consistent with karyotype results [46,XX] as reported in 000983-040109 4016-KAR. This karyotype corresponds to NSCB 2793.

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.

Results Completed By: *MS, CG(ASCP)^{CM}*

Reviewed and Interpreted By: *, PhD, FACMG*

***aCGH Specifications**:*

- *Platform: NimbleGen 385K array (HG18 CGH 385K WG Tiling v2)*
- *Relative copy number is determined by competitive differential hybridization of labeled genomic DNA to the 385,000 oligonucleotide whole genome tiling array*
- *Probe length = 50-75mers for v1 and 60mers for v2, spanning non-repetitive regions of the human genome*
- *Median probe spacing = 6270bp for v1 and 7073bp for v2*
- *Analysis software: NimbleScan™ , SignalMap™, OneClickCGH (RBS v1.0)™, OneClickFusion (RBS v1.0)™*
- *Array design, genomic position, genes and chromosome banding are based on HG18.*
- *Analysis is based on examination of unaveraged and/or 60Kbp (10X) averaged data tracks as noted. Settings for data analysis in Infoquant include an average log-ratio threshold of 0.2 and no minimum aberration length.*
- *Raw data is deposited in GEO, accession number shown above.*
- *Reported gains and losses are based on test to reference ratios within OneClickCGH™, size of aberration, 8-9 probes per gene, and coverage of at least one reported gene or overlap with the DGV.*

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

Literature Sources:

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

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

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

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

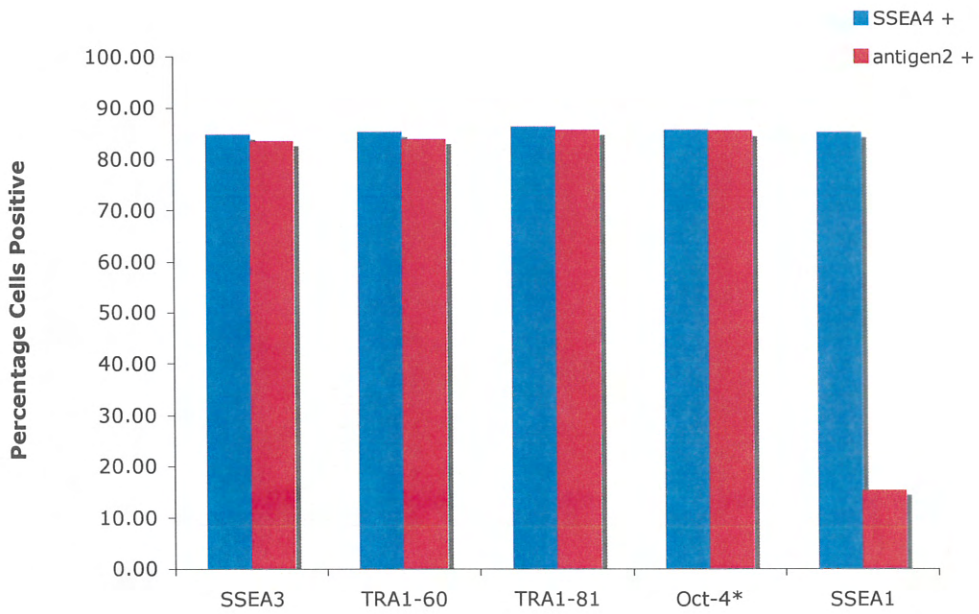
NSCB National Stem Cell Bank

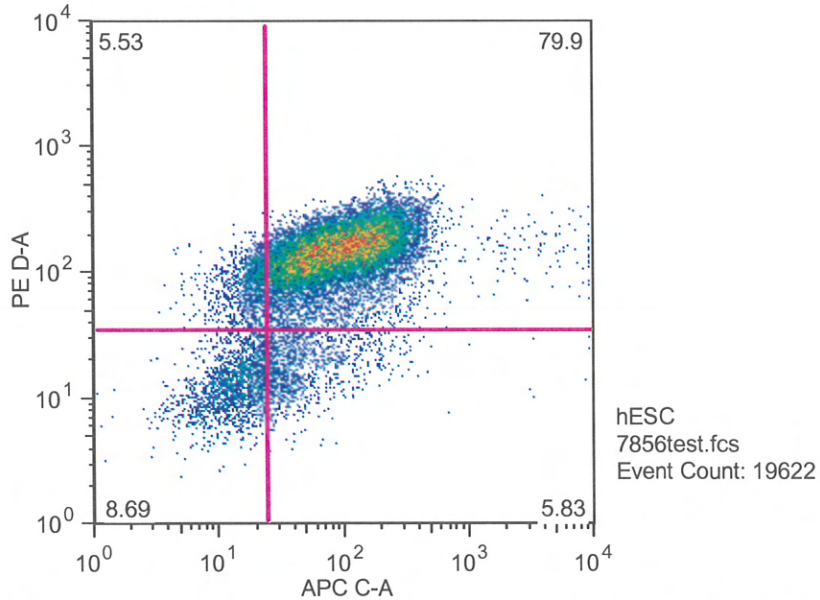
Procedures performed:
SOP-CH-101 SOP-CH-102
SOP-CH-103 SOP-CH-105

Cell Line:
Passage
Sample ID: 7856-FAC

Date of: *(mm/dd/yy)*
acquisition: 04/30/09
file creation: 04/30/09
file submission: 05/05/09

antigen2:	SSEA4 - antigen2 +	SSEA4 + antigen2 +	SSEA4 + antigen2 -	SSEA4 - antigen2 -	ALL SSEA4 +	ALL antigen2 +
SSEA3	2.41	81.10	3.71	12.70	84.81	83.51
TRA1-60	6.66	77.20	8.16	7.98	85.36	83.86
TRA1-81	6.34	79.30	7.03	7.30	86.33	85.64
Oct-4*	5.53	79.90	5.83	8.69	85.73	85.43
SSEA1	4.13	11.10	74.10	10.70	85.20	15.23





*PE-conjugated Oct-3/4 from BD Biosciences was used (cat #560186).

Blood Services
Penn-Jersey Region
Musser Blood Center

American Red Cross

05/11/09

SAMPLE: DNA from Cell Lines:
NSCB#7594 (ML09-0471)
NSCB#7856 (ML09-0472)
NSCB#1962 (ML09-0473)
NSCB#4964 (ML09-0474)
NSCB#8935 (ML09-0475)

Date received: 04/29/09

INSTITUTION: WiCell Research Institute (WICELL) / National Stem Cell Bank

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
NSCB#**7594:**	*ABO* inconclusive; *RHD, RHC, RHe*	**See below. RhD**+, C+, c-, E-, e+
NSCB#**7856:**	*ABO* O^1/O^1; RHD, RHC, RHc, RHE, RHe*	**Group O; RhD**+, C+, c+, E+, e+
NSCB#**1962:**	*ABO*O^1/O^1; RHD, RHC, RHc, RHE, RHe*	**Group O; RhD**+, C+, c+, E+, e+
NSCB#**4964:**	*ABO*O^1/O^1; RHD, RHC, RHe*	**Group O; RhD**+, C+, c-, E-, e+
NSCB#**8935:**	*ABO*O^1/O^1; RHD, RHC, RHc, RHE, RHe*	**Group O; RhD**+, C+, c+, E+, e+

ABO COMMENTS: #7594 - *ABO* testing gave B-specific, faint A, and O^1 banding patterns on initial and repeat testing suggesting a chimera or mixed cell population. Repeat testing on a new sample is suggested.

RH COMMENTS: All samples were negative for the *RHD*-inactivating pseudogene and the D-, *RHD-CE-D* hybrid.

SBB, PhD
Scientific Director

MS
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.