



WIC-WA09-MB-001

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## Product Information and Testing - Amended

### Product Information

Product Name	WA09 Cell Bank Produced Under cGMP Conditions
Alias	H9
Lot Number	WIC-WA09-MB-001
Parent Material	WA09-DL-02
Depositor	WiCell
Banked by	Waisman Biomanufacturing
Thaw Recommendation	Thaw 1 vial into 2 wells of a 6 well plate.
Culture Platform	Feeder Independent
	Medium: mTeSR1
	Matrix: Matrigel
Protocol	WiCell Feeder Independent Protocol
Passage Number	p27  These cells were cultured for 26 passages prior to freeze, 4 of them in mTeSR1/Matrigel. One number (+1) is added 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 Viald	13-March-2009
Vial Label	WiCell WA09 Master Cell Bank Lot #: WIC-WA09-MB-001 Viald: 13 March 2009 Store in LN <sub>2</sub>
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.

### Testing Performed by WiCell

Test Description	Test Provider	Test Method	Test Specification	Result
Post-Thaw Viable Cell Recovery	Waisman Center Biomanufacturing Facility	QCP-003	Report result – no specification	89.7% viable recovery
Identity by STR	UW Molecular Diagnostics Laboratory	PowerPlex 1.2 System by Promega	Consistent with known profile	Pass
Sterility - Direct Transfer Method	WuXi Apptec	30744	Negative	Pass
Mycoplasma - FDA PTC method	WuXi Apptec	30055	No contamination detected	Pass
Flow Cytometry for ESC Marker Expression	UW Flow Cytometry Laboratory	SOP-CH-101 SOP-CH-102 SOP-CH-103 SOP-CH-105	Report result– no specification	See report
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



## Product Information and Testing - Amended

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
HLA profile <sup>1</sup>	UW Molecular Diagnostics Laboratory	AlleleSEQR Kits by Abbott	Consistent with known profile	Pass
Bacteriastasis & Fungistasis <sup>1</sup>	WuXi Apptec	30736	Pass	Pass
HIV 1&2 by PCR <sup>1</sup>	Covance	Not available	Negative	Pass
HTLV 1&2 by PCR <sup>1</sup>	BioReliance	105013	Negative	Pass
HTLV 2 by PCR <sup>1</sup>	Covance	Not available	Negative	Pass
HBV by PCR <sup>1</sup>	Covance	Not available	Negative	Pass
HCV by PCR <sup>1</sup>	Covance	Not available	Negative	Pass
CMV by PCR <sup>1</sup>	BioReliance	105012	Negative	Pass
EBV by PCR <sup>1</sup>	Covance	Not available	Negative	Pass
HHV-6 by PCR <sup>1</sup>	BioReliance	105020	Negative	Pass
HHV-7 by PCR <sup>1</sup>	Covance	Not available	Negative	Pass
HHV-8 by PCR <sup>1</sup>	Covance	Not available	Negative	Pass
HP B19 by PCR <sup>1</sup>	Covance	Not available	Negative	Pass
Comparative Genome Hybridization <sup>1</sup>	WiCell Research Institute	SOP-CH-308 SOP-CH-309 SOP-CH-310	Report - no specification	See report
Gene Expression Profile <sup>1</sup>	UW Gene Expression Center	SOP-CH-321 SOP-CH-322 SOP-CH-333 SOP-CH-311	Report - no specification	See report
ABO and rH typing <sup>1</sup>	American Red Cross	ABO/rH System	Report Blood type	A+

<sup>1</sup> Test was performed on WA09-MCB-01.

Amendment(s):

Reason for Amendment	Date
CoA updated to include copyright information.	See Signature
CoA updated for format changes and addition of product information.	09-JUL-2013
CoA updated for clarification of test specifications and test method. Technical service text removed.	05-OCT-2010
CoA updated for format changes, clarification of test specifications, test method, addition of test provider, culture platform, and electronic signature.	11-JUN-2010
Original CoA	01-JUL-2009



## ***Product Information and Testing - Amended***

Date of Lot Release	Quality Assurance Approval
01-July-2009	<div>1/3/2014</div> <div>X AMC</div> <div>AMC</div> <div>Quality Assurance</div> <div>Signed by: Carteron, Anjelica</div>

## Short Tandem Repeat Analysis\*

Sample Report: 4623-STR

UW HLA#: 60889

Sample Date: 05/08/09

Received Date: 05/08/09

Requestor: WiCell Research Institute

Test Date: 05/11/09

File Name: 090512

Report Date: 05/13/09

Sample Name: (label on tube) 4623-STR

Description: DNA Extracted by WiCell  
248.36 ug/mL; 260/280 = 1.87

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

**Comments:** Based on the DNA 4623-STR dated 05/08/09 and received on 05/08/09 from WI Cell, this sample (UW HLA# 60889) matches exactly the STR profile of the human stem cell line H9 comprising 12 allelic polymorphisms across the 8 STR loci analyzed. No STR polymorphisms other than those corresponding to the human H9 stem cell line were detected and the concentration of DNA required to achieve an acceptable STR genotype (signal/noise) was equivalent to that required for the standard procedure (~1 ng/amplification reaction) from human genomic DNA. These results suggest that the 4623-STR DNA sample submitted corresponds to the H9 stem cell line and it was not contaminated with any other human stem cells or a significant amount of mouse feeder layer cells. Sensitivity limits for detection of STR polymorphisms unique to either this or other human stem cell lines is ~5%. In order to accommodate a request from WiCell Research Institute for a fast turnaround time on this sample, these results were communicated via phone to WiCell Cytogenetics laboratory on Wednesday, May 13, 2009 at 1405.

HLA/Molecular Diagnostics Laboratory

HLA/Molecular Diagnostics Laboratory

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

Test Facility:

This report is confidential. No part may be used for advertising or public announcement without written permission. Results apply only to the sample(s) tested.



Report Number

807991

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Waisman Clinical Biomanufacturing

May 14, 2009

P.O. #

## STERILITY TEST REPORT

**Sample Information:** hESC, WA09 MCB, , Lot WIC-WA09-MB-001

**Date Received:** April 28, 2009

**Date in Test:** April 29, 2009

**Date Completed:** May 13, 2009

**Test Information:** Test Codes: 30744, 30744A  
Immersion, USP / 21 CFR 610.12  
Procedure #: BS210WSM.204

TEST PARAMETERS	PRODUCT	
Approximate Volume Tested	1.0 mL	1.0 mL
Number Tested	2	2
Type of Media	SCD	FTM
Media Volume	200 mL	200 mL
Incubation Period	14 Days	14 Days
Incubation Temperature	20 °C to 25 °C	30 °C to 35 °C
RESULTS	2 NEGATIVE	2 NEGATIVE

QA Reviewer

Date

Technical Reviewer

Date

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Testing conducted in accordance with current Good Manufacturing Practices.



LLB  
5/15/09



**FINAL STUDY REPORT**

**STUDY TITLE:** MYCOPLASMA DETECTION:  
"Points to Consider"

**PROTOCOL NUMBER:** 30055G

**TEST ARTICLE IDENTIFICATION:** WA09 Master Cell Bank  
Lot # WIC-WA09-MB-001

**SPONSOR:** Waisman Clinical Biomanufacturing Facility

**PERFORMING LABORATORY:** WuXi AppTec, Inc.

**STUDY NUMBER:** 121178

**RESULT SUMMARY:** Considered **negative** for mycoplasma contamination

*Reference PO #*



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**F01-QCP-028-11222**  
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### QUALITY ASSURANCE UNIT SUMMARY

STUDY: Mycoplasma Detection: "Points to Consider"

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.

<u>Critical Phase</u>	<u>Date</u>	<u>Study Director</u>	<u>Management</u>
Reading	06/05/09	06/18/09	06/29/09
Final Report	06/26/09	06/26/09	06/29/09

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

Quality Assurance Auditor: \_\_\_\_\_

Date: 6/29/09

### 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: 6-29-09

#### Professional Personnel Involved:

Vice President of St. Paul Operations  
Manager, Mycoplasma Testing Laboratory  
Study Director  
Client Relations Manager

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**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.

**2.0 SPONSOR:** Waisman Clinical Biomanufacturing Facility

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

**4.0 SCHEDULING**

**DATE SAMPLE RECEIVED:** 05/19/09  
**STUDY INITIATION DATE:** 05/21/09  
**STUDY COMPLETION DATE:** 06/29/09

**5.0 TEST ARTICLE IDENTIFICATION:** Waisman Clinical Biomanufacturing Facility  
WA09 Master Cell Bank  
Lot # WIC-WA09-MB-001

**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 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.

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## 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 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. 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).

## 10.0 TEST MATERIAL PREPARATION

### 10.1 Test Article Identification:

Test Article Name:	WA09 Master Cell Bank
Lot/Batch #:	WIC-WA09-MB-001
General Description:	hESC
Number of Aliquots used:	1 x 15 mL
Stability (Expiration):	Not Applicable
Storage Conditions:	Ultracold (< -60°C)
Safety Precautions:	BSL-2

### 10.2 Test Sample Preparation

One (1) tube of 15 mL was thawed in a water bath at  $37 \pm 2^\circ\text{C}$  and 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  $36 \pm 1^\circ\text{C}$  / 5 - 10%  $\text{CO}_2$  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  $36 \pm 1^\circ\text{C}$  / 5 - 10%  $\text{CO}_2$ . After three days of incubation, the coverslips were fixed, stained, and then read using an epifluorescent microscope.

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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<sup>2</sup> 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 Controls and Reference Materials

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

#### 10.3.2 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.

#### 10.3.3 Control Preparation

##### a. Negative Controls

- a.1 1.0 mL of sterile SP-4 broth was inoculated onto each of two (2) coverslips containing 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 two (2) SP-4 agar plates to serve as the negative control in the direct assay. 10.0 mL of SP-4 broth was inoculated into a 75 cm<sup>2</sup> flask containing 50 mL of SP-4 broth to serve as the negative control in the direct assay.

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**b. Positive Controls**

- b.1** *M. hyorhinis*, *M. orale*, and *M. pneumoniae* were diluted to less than 100 CFU per inoculum in sterile SP-4 broth. 1.0 mL of *M. hyorhinis* and *M. orale* at less than 100 CFU/mL was inoculated onto each of two (2) coverslips containing Vero cells. These coverslips served as the positive controls in the indirect assay.
- b.2** The coverslips were incubated in incubator E950 for 1-2 hours at  $36 \pm 1$  °C / 5 - 10% CO<sub>2</sub> and then 2.0 mL of EMEM, 8% Fetal Bovine Serum (FBS) was added to each coverslip. The coverslips were returned to incubator E950 at  $36 \pm 1$  °C / 5 - 10% CO<sub>2</sub>. After three days of incubation, the cell cultures 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 was inoculated onto each of two (2) SP-4 agar plates. 10.0 mL of *M. orale* and *M. pneumoniae* at less than 10 CFU/mL ( $\leq 100$  CFU/inoculum) were each inoculated into a 75 cm<sup>2</sup> flask containing 50 mL of sterile SP-4 broth.
- b.4** The agar plates were incubated anaerobically at  $36 \pm 1$  °C for 14 days. The broth cultures were incubated aerobically at  $36 \pm 1$  °C for a minimum of 14 days and were read each working day for 14 days. 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  $36 \pm 1$  °C. The subculture plates were observed microscopically for the presence of mycoplasma colonies 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.

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### 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 COVERSIP REQUIRED FOR THE EVALUATION)
Negative Control	-
<i>M. hyorhinis</i>	+
<i>M. orale</i> ( $\leq 100$ CFU)	+

#### 13.2 Direct Assay

##### DETECTION OF MYCOPLASMA CONTAMINATION BY DIRECT ASSAY

	NEGATIVE CONTROL	<i>M.</i> <i>PNEUMONIAE</i>	<i>M. ORALE</i>
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	-	+	+

### 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 cell debris. The source of DNA is determined by the staining pattern. The indicator cell nuclei fluoresce brightly and are generally 10-20  $\mu\text{m}$  in diameter. Mycoplasma fluorescence is less intense, is extra-nuclear and typically appears as small round bodies approximately 0.3  $\mu\text{m}$  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. These changes must be confirmed by agar plate subculture or DNA-staining since changes in the appearance of the broth culture 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  $\mu\text{m}$ , and can be readily observed unstained using a light microscope.

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#### 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)	-	+/-	+	+/-	-
<b>THEN: OVERALL FINAL RESULT</b>	-	+	+	+	-

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

#### 14.4 Positive Results

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

#### 14.5 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.

### 15.0 RESULTS

#### Indirect Assay and Direct Assay Results

	INDIRECT	DIRECT		OVERALL
		DAY 0 AGAR PLATES	BROTH FLASKS AND ASSOCIATED SUBCULTURE PLATES	
Test Article: WA09 Master Cell Bank Lot # WIC-WA09-MB-001	Negative	Negative	Negative	Negative
Negative Control	Negative	Negative	Negative	Negative
<i>M. hyorhinis</i>	Positive			Positive
<i>M. orale</i>	Positive	Positive	Positive	Positive
<i>M. pneumoniae</i>		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, WA09 Master Cell Bank, Lot # WIC-WA09-MB-001, is considered negative for the presence of mycoplasma contamination.

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**17.0 DEVIATIONS:** None.

**18.0 AMENDMENTS:** None.

**19.0 RECORD RETENTION**

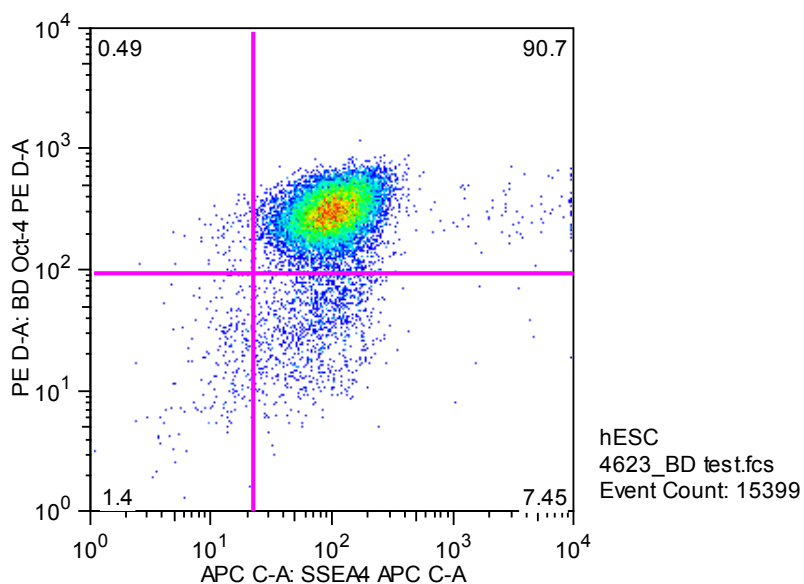
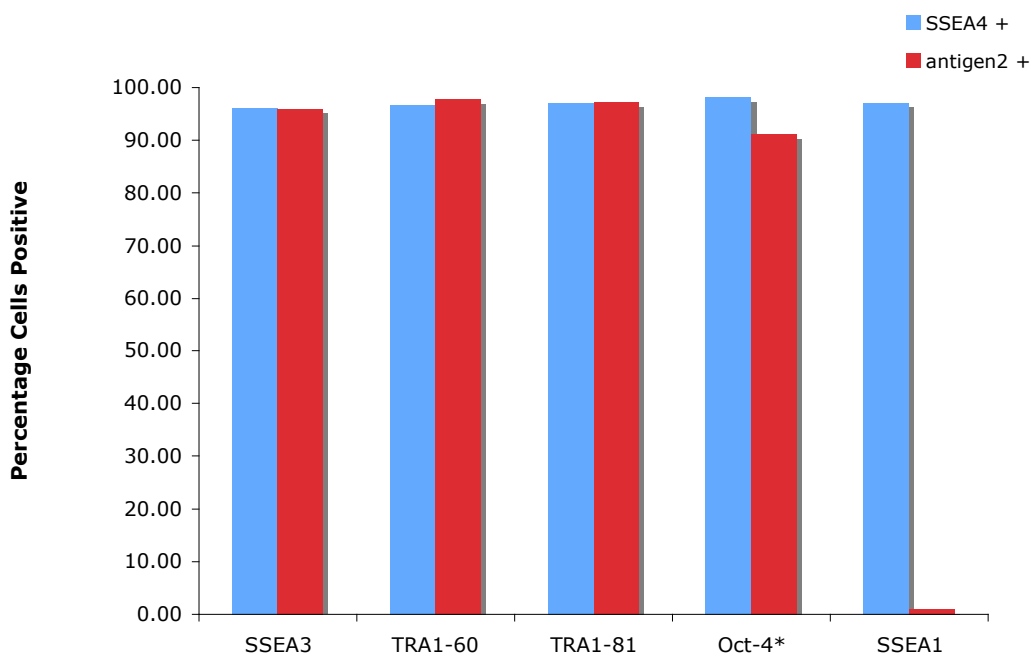
An exact copy of the original final report and all raw data pertinent to this study will be stored at WuXi AppTec, Inc., 2540 Executive Drive, St. Paul, MN 55120. It is the responsibility of the Sponsor to retain a sample of the test article.

**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 Mycoplasmaology, 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 Mycoplasmaology, Vol. II (New York: Academic Press).
- 20.3** 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 Mycoplasmaology, Vol. II (New York: Academic Press).
- 20.4** 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 Mycoplasmaology, Vol. II (New York: Academic Press).
- 20.5** 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).
- 20.6** 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."

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<u>antigen2:</u>	<u>SSEA4 -</u> <u>antigen2 +</u>	<u>SSEA4 +</u> <u>antigen2 +</u>	<u>SSEA4 +</u> <u>antigen2 -</u>	<u>SSEA4 -</u> <u>antigen2 -</u>	<u>ALL</u> <u>SSEA4 +</u>	<u>ALL</u> <u>antigen2 +</u>
SSEA3	1.53	94.50	1.62	2.33	96.12	96.03
TRA1-60	3.22	94.60	2.07	0.15	96.67	97.82
TRA1-81	2.65	94.60	2.52	0.26	97.12	97.25
Oct-4*	0.49	90.70	7.45	1.40	98.15	91.19
SSEA1	0.00	1.05	96.10	2.81	97.15	1.05



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

**Report Date:** September 14, 2009

### Case Details:

**Cell Line:** WA09 (WISC1277)

**Passage #:** 30

**Date Completed:** 9/14/2009

**Cell Line Gender:** Female

**Investigator:**

**Specimen:** hESC on Matrigel

**Date of Sample:** 9/11/2009

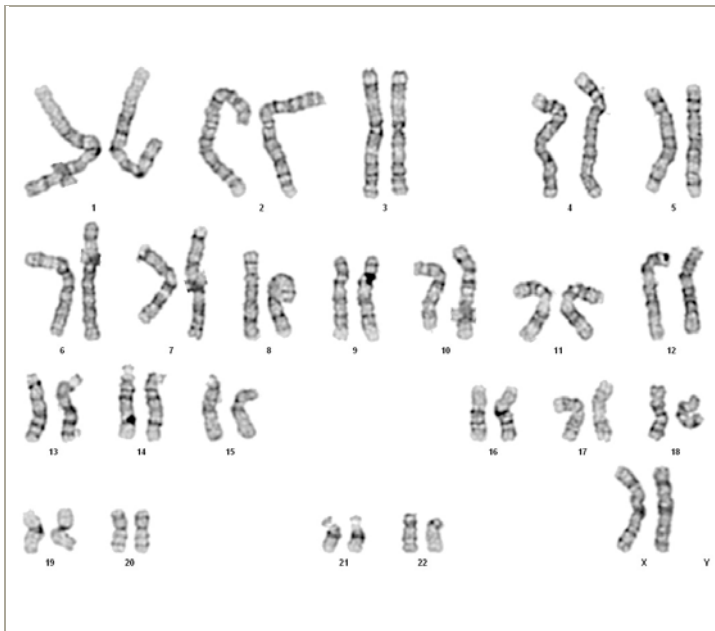
**Tests, Reason for:** Release testing for master bank and pre-freeze research bank

**Results:** 46,XX

Completed by \_\_\_\_\_, CLSp(CG), on 9/14/2009

Reviewed and interpreted by \_\_\_\_\_, PhD, FACMG, on 9/14/2009

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



**Cell:** S01-04

**Slide:** B-20

**Slide Type:** Karyotyping

**# of Cells Counted:** 20

**# of Cells Karyotyped:** 4

**# of Cells Analyzed:** 9

**Band Level:** 450-550

**Results Transmitted by Fax / Email / Post**

**Sent By:** \_\_\_\_\_

**QC Review By:** \_\_\_\_\_

**Date:** \_\_\_\_\_

**Sent To:** \_\_\_\_\_

**Results Recorded:** \_\_\_\_\_

# Final Report

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

**Study Number:** AC32BH.032901.BSV

**Test Article ID:** WIC-WA09-MB-001 #9104

**Sponsor:** WiCell Research Institute

**Authorized Representative:**

### **CONCLUSION**

**Bovine viruses were not detected when the test article, WIC-WA09-MB-001 #9104, was examined for the presence of nine specific bovine viruses using Immunofluorescence Assay (IFA). In addition, hemadsorption and Cytopathic Effect (CPE) were not observed in the test article inoculated cultures.**

**STUDY INFORMATION**

**Test Article:** WIC-WA09-MB-001 #9104 was received by BioReliance on 15-Sep-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:** 21-Sep-2009

**Lab Initiation:** 22-Sep-2009

**Lab Completion:** 14-Oct-2009

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

**Study Director:**

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

BioReliance

**Positive Controls:**

Bovine Viral Diarrhea Virus (BVDV), ATCC VR-534  
Source: American Type Culture Collection (ATCC)  
Manassas, VA

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

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

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

Bovine Respiratory Syncytial Virus (BRSV),  
ATCC VR-1339  
Source: ATCC

Reovirus Type 3 (REO3), 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:**

BT cells: High Glucose Dulbecco's Modified Eagle's  
Medium + 110 mg/L Sodium Pyruvate + 15% Foal Serum,  
4mM L-Glutamine, 0.1% Amphotericin B, and 0.1%  
Gentamicin

Vero cells: Dulbecco's Modified Eagle's Medium/Ham's F-  
12 (1:1 mix) + 15% irradiated FBS, 2mM L-Glutamine,  
0.1% Amphotericin B, and 0.1% Gentamicin

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 REO3, PI3 and Rabies

Erythrocytes:  
Chick 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. After adsorption for  $90 \pm 9$  minutes at  $36 \pm 2^\circ\text{C}$ , 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 75 cm<sup>2</sup> flasks and 6-well plates.

Prior to the second subculture, negative control cells from each indicator line were subcultured to 25 cm<sup>2</sup> flasks and 6-well plates for the positive control inoculation. At the time of the second subculture, a flask of Vero cells were inoculated with REO3 and flasks of BT cells were inoculated with BVDV, BAV5, BPV, BTV, IBR, PI3, and BRSV at 100-300 FAID<sub>50</sub>. The cells were fixed for immunofluorescent staining when the monolayers exhibited  $\geq 10\%$  CPE, or after a minimum of 21 days in culture and slides were stored at  $\leq -60^{\circ}\text{C}$ . 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 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, REO3, 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  $36 \pm 2^{\circ}\text{C}$ . 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 Chick Erythrocytes were inoculated onto the plates and incubated at  $2 - 8^{\circ}\text{C}$  and at  $20 - 25^{\circ}\text{C}$  and 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, WIC-WA09-MB-001 #9104. 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

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.

22 Oct 09  
Date

Study Director

TABLE 1

**Observations for Cytopathic Effects in Cultures of BT and Vero Cells  
Inoculated with WIC-WA09-MB-001 #9104**

Sample	Results
Negative control <sup>a</sup>	-
Test article <sup>a</sup>	-
Negative control <sup>b</sup>	-
Test article <sup>b</sup>	-
<b>Positive Control Cultures:</b>	
BAV5 <sup>a</sup>	+
BPV <sup>a</sup>	+
BRSV <sup>a</sup>	+
BTV <sup>a</sup>	+
BVDV <sup>a</sup>	+
REO3 <sup>b</sup>	+
IBR <sup>a</sup>	+
PI3 <sup>a</sup>	+

<sup>a</sup> Inoculated onto BT cells

<sup>b</sup> Inoculated onto Vero cells

- CPE not observed

+ CPE observed

TABLE 2

Observations for CPE using Cytological Staining on BT and Vero Cultures  
Inoculated with WIC-WA09-MB-001 #9104

Cytological Staining Results	
<b>Day 21 BT Cells</b>	
Negative Control	-
Test Article	-
Positive Control BVDV <sup>a</sup>	+
<b>Day 21 Vero Cells</b>	
Negative Control	-
Test Article	-
Positive Control PI3 <sup>b</sup>	+

<sup>a</sup> Positive control tested on day 17

<sup>b</sup> Positive control tested on day 21

- CPE not observed

+ CPE observed

TABLE 3

**Observations for Hemadsorption in BT and Vero Cultures  
Inoculated with WIC-WA09-MB-001 #9104**

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

<sup>a</sup> Erythrocytes used in these assays: C = Chick, G = Guinea Pig

<sup>b</sup> Positive control tested on day 21

- Hemadsorption not observed

+ Hemadsorption observed

TABLE 4

**Immunofluorescent Staining Results for BT and Vero Cultures  
Inoculated with WIC-WA09-MB-001 #9104**

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

<sup>a</sup> Tested in BT indicator cells<sup>b</sup> Tested in Vero indicator cells<sup>c</sup> Tested on Rabies infected Vero positive control slide

- Immunofluorescence not observed

+ Immunofluorescence observed

<sup>d</sup> Data reflects results of positive control slides that were prepared on day 17

NA = Not Applicable

## Study Information

**Number:** AC32BH.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
21-Oct-2009	21-Oct-2009	Data and Final Reporting	21-Oct-2009	21-Oct-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:**

22-Oct-2009 7:28 pm GMT

Reason for signature: QA Approval

QA Reviewed	
EJM 21 OCT 09	
Init.	Date

## PROTOCOL AMENDMENT NUMBER 1

BIORELIANCE STUDY NO: AC32BH.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: 3.2.3.1 Negative Control Media

AMENDMENT: BT Medium: Dulbecco's Modified Eagle's Medium + 15% foal serum (source to be recorded) supplemented with 110 mg/L sodium pyruvate, 4mM L-Glutamine, 0.1% Amphotericin B, and 0.1% Gentamicin.

REASON FOR AMENDMENT: To reflect changes made to optimize the media composition for this 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:

STUDY DIRECTOR [Signature] 21 Sep 09  
DATE

SPONSOR REPRESENTATIVE [Signature] 13 Nov 09  
DATE

# Final Report

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

**Study Number:** AC32BH.033901.BSV

**Test Article ID:** WIC-WA09-MB-001 #9104

**Sponsor:** WiCell Research Institute

**Authorized Representative:**

### CONCLUSION

Porcine viruses, BVDV, Reovirus and Rabies were not detected when the test article WIC-WA09-MB-001 #9104 was examined for the presence of porcine viruses using Immunofluorescence Assay (IFA). In addition, Hemadsorption and Cytopathic effect (CPE) were not observed in the test article inoculated cultures.

## STUDY INFORMATION

**Test Article:** WIC-WA09-MB-001 #9104 was received by BioReliance on 15-Sep-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:** 21-Sep-2009

**Lab Initiation:** 22-Sep-2009

**Lab Completion:** 14-Oct-2009

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

### Study Director:

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

BioReliance.

**Positive Controls:**

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

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

Porcine Adenovirus (PAV)  
Source: NVSL

Bovine Parainfluenza Virus Type 3 (PI3)  
(Shipping fever virus), ATCC VR-281  
Source: ATCC

Rabies Virus (positive control slide)  
Source: NVSL

**Negative Control:**

Eagle's Minimum Essential Medium + 10% Fetal Bovine  
serum, 2mM 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:  
Chick 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  $90 \pm 9$  minutes at  $36 \pm 2^{\circ}\text{C}$ , 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<sup>2</sup> flasks and 6-well plates.

One day prior to the second subculture, negative control PT-1 cells were subcultured to 25 cm<sup>2</sup> 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  $\geq 10\%$  CPE or after a minimum of day 21 in culture. 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, REO3 and Rabies by immunofluorescent staining according to SOP BPBT0829. FITC-conjugated antibodies were incubated with the fixed cells for approximately 60 minutes at  $36 \pm 2^{\circ}\text{C}$ . 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 Chick Erythrocytes were inoculated onto the plates and incubated at 2 -8°C and at 20 - 25°C and 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 WIC-WA09-MB-001 #9104. 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.

290009

Date

Study Director

TABLE 1

**Observations for Cytopathic Effects in Cultures of PT-1 Cells  
Inoculated with WIC-WA09-MB-001 #9104**

Sample	Results
Negative control	-
Test article	-
<b>Positive Control Cultures</b>	
PAV	+
PPV	+
TGE	+

- CPE not observed

+ CPE observed

TABLE 2

**Observations for Hemadsorption in Monolayers of PT-1 Cells  
Inoculated With WIC-WA09-MB-001 #9104**

	Hemadsorption Results			
	2-8°C		20-25°C	
	C <sup>a</sup>	G <sup>a</sup>	C	G
<b>Day 21 PT-1 Cells</b>				
Negative Control	-	-	-	-
Test Article	-	-	-	-
Positive Control PI3 <sup>b</sup>	+	+	+	+

<sup>a</sup> Erythrocytes used in these assays: C = Chick, G = Guinea Pig

<sup>b</sup> Positive control tested on day 21

- Hemadsorption not observed

+ Hemadsorption observed

TABLE 3

Immunofluorescent Staining Results for PT-1 cells  
Inoculated With WIC-WA09-MB-001 #9104

	PBS	$\alpha$ PAV	$\alpha$ PPV	$\alpha$ TGE	$\alpha$ BVDV	$\alpha$ REO3	$\alpha$ Rabies
<b>Slides Prepared Day 21</b>							
Test Article	-	-	-	-	-	-	-
Negative Control	-	-	-	-	-	-	-
	PBS	$\alpha$ PAV	$\alpha$ PPV	$\alpha$ TGE	$\alpha$ BVDV <sup>b</sup>	$\alpha$ REO3 <sup>b</sup>	$\alpha$ Rabies <sup>a</sup>
Positive Control	-	+	+	+	+	+	+

- = Immunofluorescence not observed

+ = Immunofluorescence observed

<sup>a</sup> Tested on Rabies infected Vero positive control slide

<sup>b</sup> Slides from corresponding bovine study.

## Study Information

**Number:** AC32BH.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
27-Oct-2009	27-Oct-2009	Data and Final Reporting	27-Oct-2009	27-Oct-2009
03-Sep-2009	03-Sep-2009	Admin. Of Test Substance	08-Sep-2009	08-Sep-2009 *
11-Sep-2009	11-Sep-2009	Manipulation of Test System	15-Sep-2009	15-Sep-2009 *
11-Sep-2009	11-Sep-2009	Observation of Test System	15-Sep-2009	15-Sep-2009 *
03-Sep-2009	03-Sep-2009	Test System Preparation	08-Sep-2009	08-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:**

27-Oct-2009 6:38 pm GMT

Reason for signature: QA Approval

PROTOCOL AMENDMENT NUMBER 1

QA Reviewed	
BSM 270009	
Init.	Date

BIORELIANCE STUDY NO: AC32BH.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:

STUDY DIRECTOR

21 Sep 09  
DATE

\_\_\_\_\_  
SPONSOR REPRESENTATIVE

13 NOV 09  
DATE

## **Final Report**

### **MOUSE ANTIBODY PRODUCTION (MAP) TEST**

**Study Number:** AC32BH.004000.BSV

**Test Article ID:** WIC-WA09-MB-001 #9104

**Sponsor:** WiCell Research Institute

**Authorized Representative:**

#### **CONCLUSION**

**Based on the data obtained in the assays performed, the test article, WIC-WA09-MB-001 #9104, has been shown to be free of all of the seventeen murine viruses for which it was examined.**

**STUDY INFORMATION**

**Test Article Receipt:** WIC-WA09-MB-001 #9104 was received at BioReliance on 09/15/2009 (original) and 10/15/2009 (repeat). 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

**Pathologist:** D.V.M., M.S., Ph.D., D.A.C.V.P.

**Tissue Processing Laboratory:** BioReliance

**Study Initiation:** 09/21/2009

**Lab Initiation:** 10/01/2009

**Lab Completion:** 11/11/2009

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

**Study Director:**

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

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

The LCM challenge portion of the assay was performed using a resubmitted sample of the test article, and utilizing the same procedures as the original assay.

### 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 labeled 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.

<u>Virus</u>	<u>Assay</u>
Ectromelia	ELISA <sup>1</sup> or IFA <sup>3</sup>
GDVII	ELISA or IFA
Lactate Dehydrogenase Virus (LDV)	NAD Reduction <sup>2</sup>
Lymphocytic Choriomeningitis	ELISA or IFA and LCM virus challenge
Hantaan Virus	ELISA or IFA
Mouse Minute Virus (MMV)	ELISA, IFA, or HI <sup>4</sup>
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

<sup>1</sup> Enzyme Linked Immunosorbent Assay (OPDL0806)

<sup>2</sup> Testing performed using BioReliance SOP OPVM7009

<sup>3</sup> Indirect Fluorescent Antibody Test (OPDL0810)

<sup>4</sup> Hemagglutination Inhibition (OPDL0621)

**CRITERIA FOR A VALID TEST****Serology Assays**

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

**LDV Assay**

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

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

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

**LCM Virus Assay**

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

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

## EVALUATION OF TEST RESULTS

### Serology Assays

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

### LDV Assay

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

### LCM Virus Assay

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

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

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

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

## RESULTS

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

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

The original LCM challenge portion of the assay was invalidated because one of the three test article 1:10 animals (#2780) died on day 6 post-injection, before the LCM challenge was performed (BRIQS #46680). Animal (#2780) was sent for necropsy. The cause of death of this animal was a brain lesion. (See Pathology Report found in the Appendix.) The brain lesion may have been caused by injection related trauma from the i.c. injection.

Due to the death of one of the test article 1:10 animals, the LCM portion of the assay was performed on a resubmitted sample of the test article, utilizing the same procedures as the original assay.

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

In addition to BRIQS # 46680 mentioned previously, a deviation from a supporting SOP occurred when the Health Status Evaluation and Animal Use Log for the animals used on this study was not completed at assay initiation as required by SOP OPBT0261 (BRIQS #49370).

## 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.

November 18, 2009  
Date

Study Director

Table 1

Group No.	No. of Mice	Test Material	Route of Injection <sup>a</sup>	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 <sub>50</sub> of LCM	i.c.	0.03 ml	No sooner than 14	Animals were observed for death.
III	2	EMEM <sup>b</sup>	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 <sub>50</sub> of LCM	i.c.	0.03 ml	No sooner than 14	Animals were observed for death.

<sup>a</sup> p.o. = per os; i.n. = intranasal; i.p. = intraperitoneal; i.c. = intracerebral

<sup>b</sup> 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 WIC-WA09-MB-001 #9104**

Serum from Animals injected with	PVM <sup>2</sup>	REO3 <sup>2</sup>	SENDAL <sup>2</sup>	GDVII <sup>2</sup>	HANTAAN <sup>2</sup>	POLYOMA <sup>2</sup>	MMV <sup>2</sup>	MPV <sup>2</sup>	ADENO <sup>2</sup>	MHV <sup>2</sup>	LCM <sup>2</sup>	ECTROMELIA <sup>2</sup>	EDIM <sup>2</sup>	MCMV <sup>3</sup>	K <sup>1</sup>	MTV <sup>3</sup>
Test Article	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Negative Control	Neg	Neg	Neg	Neg	I <sup>4</sup>	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Serology Positive Control	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS

<sup>1</sup> Serum antibody was measured by Hemagglutination Inhibition. Neg= Negative, POS = Positive

<sup>2</sup> These titers are measured by ELISA. Neg= Negative, POS = Positive

<sup>3</sup> Serum antibody measured by Indirect Fluorescent Antibody. Neg= Negative, POS = Positive

<sup>4</sup> 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 WIC-WA09-MB-001 #9104

Plasma from Animals Injected with	LDH Titer <sup>a</sup>
Test Article (1:10) (Group II)	289
	200
	417
Negative Control (Group III)	237
	487
LDV Control	1354
	1107

<sup>a</sup> Plasma titers less than 600 IU/L are negative.

APPENDIX

## PATHOLOGY REPORT

QA Reviewed	
BSM 11 Nov 09	
Init.	Date

**Study Number:** AC32BH.004000.BSV

**CAHS Number:** 10816-2780

**Source:** In Vivo Biologics

**Species:** Mouse

**Animal Number:** 2780

**Body Weight (grams):** 25

**Cage Number:** 2

**Sex:** Male

**Test Material:** Test Article 1:10

**Age:** Adult

**Received:** 07-Oct-2009

**Necropsied:** 07-Oct-2009

**Pathology Completed:** 10-Nov-2009

### Gross Observations:

Eye, right, protruding from socket, hard, dark in color. Lumen of GI tract significantly autolyzed, black in color.

### Microscopic Examination:

Heart, lung, liver, kidney, spleen, ileum, cecum, colon, trachea, brain with middle ears, and nasal cavity with eyes, thymus:

Brain, Inflammation, acute, necrotizing and hemorrhagic with foreign material

Thymus, Necrosis, minimal

Spleen, necrosis, minimal

Other organs particularly the gastrointestinal tract is mild to moderately autolyzed however, they are considered to be within normal limits.

### Comments:

There is large lytic area in the brain that contains amphophilic foreign material. There is hemorrhage and infiltration of neutrophils around the lytic area. The cause of death in this animal is the brain lesion. The necrotic changes in the spleen and thymus are considered to be stress induced.

11/11/09  
\_\_\_\_\_  
Date

Director of Veterinary Services

## Study Information

**Number:** AC32BH.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
16-Nov-2009	16-Nov-2009	Data and Final Reporting	16-Nov-2009	16-Nov-2009
03-Sep-2009	03-Sep-2009	Admin. Of Test Substance	08-Sep-2009	08-Sep-2009 *
11-Sep-2009	11-Sep-2009	Manipulation of Test System	15-Sep-2009	15-Sep-2009 *
11-Sep-2009	11-Sep-2009	Observation of Test System	15-Sep-2009	15-Sep-2009 *
03-Sep-2009	03-Sep-2009	Test System Preparation	08-Sep-2009	08-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:**

18-Nov-2009 8:56 pm GMT

Reason for signature: QA Approval



## 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
WIC-WA09-MB-001 #9104	09-001950

**SPONSOR:** WiCell

**PERFORMING LABORATORY:** WuXi AppTec, Inc.

WUXI APPTec ACCESSION NUMBER	RESULTS
09-001950	No evidence of viral contamination was detected.

Accession Number: 09-001950  
Final Report Number: 37000.04

WiCell  
Page: 2 of 8

### QUALITY ASSURANCE UNIT SUMMARY

STUDY: Custom In Vitro Assays for Adventitious Viral Contaminants

The Quality Assurance unit monitored the conduct and reporting of this laboratory study. This study has been performed under US FDA Good Laboratory Practice regulations (21 CFR Part 58), EU Good Laboratory Practice regulations (EMA GMP, Rules and Guidance for Pharmaceutical Manufacturers and Distributors, Annex 13), applicable ICH Q7 standards, and/or applicable Good Manufacturing Practices and in accordance with standard operating procedures and a test protocol. The Quality Assurance Unit maintains a copy of the test protocol and standard operating procedures and has inspected this study (as applicable) 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. Add test article to plate.

September 22, 2009

BR 37101.02

Step 4.2.5

Remove medium from one 6-well NIH/3T3 plate. Add test article to plate.

September 22, 2009

Quality Assurance

10-26-09  
Date

### GOOD LABORATORY PRACTICES STATEMENT

The study referenced in this report was conducted in accordance with US FDA Good Laboratory Practices for Nonclinical Laboratory Studies as found in Title 21 Code of Federal Regulations Part 58, EU Good Laboratory Practice regulations (EMA GMP, Rules and Guidance for Pharmaceutical Manufacturers and Distributors, Annex 13), and applicable ICH Q7 standards. The study was inspected during at least one phase, and WuXi AppTec Quality Assurance audited the final report.

Study Director

0

26 OCT 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

3.0 TEST FACILITY: WuXi AppTec, Inc.

## 4.0 SCHEDULING

DATE SAMPLES RECEIVED: September 15, 2009  
STUDY INITIATION DATE: September 22, 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: WIC-WA09-MB-001 #9104

## 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.<sup>1</sup> 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.<sup>2,3</sup> The choice of cell lines used in this assay is dictated by the 1993 Points To Consider directive from the FDA.<sup>4</sup>

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.<sup>5</sup>

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<sup>1</sup>, the presence of these viruses may be detected by their ability to adsorb erythrocytes to the surface of infected cells.<sup>2</sup> 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 MRC-5, VERO, and NIH/3T3 indicator cell monolayers were inoculated with Eagle's Minimum Essential Medium (EMEM) and served as the negative controls.
- 8.1.2 MRC-5, VERO, and NIH/3T3 indicator cell monolayers were inoculated with 0.2 mL per well of disrupted, clarified test article lysate. Six wells were inoculated per cell line.
- 8.1.3 Indicator cell monolayers were inoculated with viruses as appropriate for each cell line chosen to serve as the positive controls. MRC-5 cells were inoculated with encephalomyocarditis virus (EMC), VERO cells were inoculated with adenovirus type 5 (Ad 5), and NIH/3T3 cells were inoculated with herpes simplex type 1 (HSV-1).
- 8.1.4 Cultures were incubated at  $37 \pm 2^{\circ}\text{C}$  in a humidified atmosphere of  $5 \pm 2\%$   $\text{CO}_2$ . 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.<sup>4,5</sup>
- 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 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.<sup>3</sup>
- 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  $2 - 8^{\circ}\text{C}$  or  $15 - 30^{\circ}\text{C}$  for 30 to 45 minutes, washed and examined macroscopically and microscopically for adsorption of erythrocytes to the monolayers.

## 9.0 TEST ARTICLE PREPARATION

On September 15, 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  $\leq -60^{\circ}\text{C}$  until the assay was initiated.

On the day of inoculation (September 22, 2009), the test article was thawed using a  $37\pm 2^{\circ}\text{C}$  waterbath and was subjected to one additional freeze/thaw cycle using a dry ice/ethanol bath and a  $37\pm 2^{\circ}\text{C}$  waterbath. The test article was clarified by low-speed centrifugation and the supernatant 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 Virus Type 1 (HSV-1)

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

## 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 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 +4 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 # 09-001950	0	0	0
EMEM (Negative Control)	0	0	0
Encephalomyocarditis Virus (Positive Control)	+4	NA	NA
Adenovirus type 5 (Positive Control)	NA	+4	NA
Herpes Simplex Virus (Positive Control)	NA	NA	+4

#### Legend:

- 0 No viral cytopathic changes observed during the 28-day test period.
- ~ +1 Up to 25% of the cells in culture show viral cytopathic changes.
- +1 25-50% of the cells in culture show viral cytopathic changes.
- +2 50-75% of the cells in culture show viral cytopathic changes.
- +3 75-90% of the cells in culture show viral cytopathic changes.
- +4 90-100% of the cells in culture show viral cytopathic changes.
- NA Not applicable

**TABLE 2: Hemadsorption Activity of Indicator Cell Lines**

Culture Inoculum	Cell Line	Day of Test	2 - 8°C			15 – 30°C		
			C	GP	H	C	GP	H
Accession #09-001950	MRC-5	28	-	-	-	-	-	-
	VERO	28	-	-	-	-	-	-
	NIH/3T3	28	-	-	-	-	-	-
EMEM (Negative Control)	MRC-5	28	-	-	-	-	-	-
	VERO	28	-	-	-	-	-	-
	NIH/3T3	28	-	-	-	-	-	-
Positive Control <sup>1</sup> (1:2)	VERO	28	+	+	+	+	+	+
Positive Control <sup>1</sup> (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
- +
- <sup>1</sup> Positive reaction indicating presence of viral agent
- 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.

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## 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
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## **Final Report**

### **TEST FOR THE PRESENCE OF INAPPARENT VIRUSES**

**Study Number:** AC32BH.005002.BSV

**Test Article ID:** WIC-WA09-MB-001 #9104

**Sponsor:** WiCell Research Institute

**Authorized Representative:**

#### **CONCLUSION**

**No evidence of contamination with adventitious viral agents was observed due to the test article, WIC-WA09-MB-001 #9104.**

**STUDY INFORMATION**

**Test Article Receipt:** WIC-WA09-MB-001 #9104 was received by BioReliance on 09/15/2009 (original) and 10/21/2009 (repeat). 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:** 09/28/2009

**Lab Initiation Date:** 10/02/2009

**Lab Completion Date:** 11/23/2009

**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:** Hank's Balanced Salt Solution (HBSS)  
Lot No.: 019K2383  
Source: Sigma  
St. Louis, Missouri

**Test System:**

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

Source: Harlan Sprague Dawley  
Frederick, Maryland

Suckling litters (Blind Passage):HSD:ICR, four adult females, each with ten three 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  
Kingston, New York

**Hens' Eggs**

**Original Assay:** Embryonated Hens' Eggs (allantoic route) Primary: Twenty, nine days old

Source: Sunrise (BE Eggs)  
York Springs, Pennsylvania

Embryonated Hens' Eggs (allantoic route) Blind Passage: Twenty, nine days old

Source: Sunrise (BE Eggs)  
York Springs, Pennsylvania

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

Source: Sunrise (BE Eggs)  
York Springs, Pennsylvania

Embryonated Hens' Eggs (yolk sac route): Blind Passage: Twenty, seven days old

Source: Sunrise (BE Eggs)  
York Springs, Pennsylvania

**Repeat Assay:** Embryonated Hens' Eggs (yolk sac route) Primary:  
Twenty, seven days old

Source: Sunrise (BE Eggs)

York Springs, Pennsylvania

Embryonated Hens' Eggs (yolk sac route): Blind

Passage: Twenty, 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

A repeat of the yolk sac portion of the assay was performed on a resubmitted sample of the test article, utilizing the same procedures as the original assay.

### 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 gastrointestinal tract 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  $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$  and  $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$  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  $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$  and  $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ .

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  $36^{\circ}\text{C} \pm 1^{\circ}\text{C}$ . The second plate was incubated anaerobically at  $36^{\circ}\text{C} \pm 1^{\circ}\text{C}$ . The plates were examined for growth after a minimum of 24 hours.

### Animal Husbandry

All animals were fed the following diet *ad libitum*:

Guinea pigs - Teklad Certified Guinea Pig Chow.

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

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

Bedding - corncob, Harlan Teklad. Cages were changed as necessary, usually twice per week.

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

### CRITERIA FOR A VALID TEST

The test will be considered valid if ninety percent of the control adult mice, eighty percent of the control suckling mice, eighty percent of the control embryonated hen's eggs, and seventy-five

percent of the control guinea pigs survive the observation period, show no lesions at the site of injection or signs of viral infection.

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

## **EVALUATION OF TEST RESULTS**

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

## **RESULTS**

### **Mice and Guinea Pigs**

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

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

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**

In the original assay, ten seven day old embryonated hens' eggs were injected by the yolk sac route with the test article or the negative control article. These eggs were examined for viability at 24 and 48 hours and 10 days post-injection. All of the test article injected eggs and nine of the ten negative control article injected eggs appeared viable at 24 hours post-injection. Seven of the test article injected eggs and nine of the negative control article injected eggs appeared viable at 48 hours post-injection. At examination on day 10, seven of the test article injected eggs and nine of the negative control article injected eggs contained viable embryos. Three of the test article injected eggs and one of the negative control article injected eggs contained non-viable embryos. No growth was observed on blood agar plates streaked with fluid from the non-viable eggs. The cause of death of these embryos may have resulted from injection related trauma, as the embryos appeared non viable at 24 and/or 48 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, all of the test article subpassage eggs and nine of the ten negative control article subpassage eggs contained viable embryos. One of the negative control article subpassage eggs contained a non-viable embryo. No growth was observed on blood agar plates streaked with fluid from the non-viable egg. The cause of death of this embryo could not be determined. (See Criteria for a Valid Test/Evaluation of Test Results.)

Due to the survival of less than 80% of the test article injected eggs (BRIQS #46951), a repeat of the yolk sac portion of the assay was performed on a resubmitted sample of the test article, utilizing the same procedures as the original assay.

In the repeat assay, ten seven day old embryonated hens' eggs were injected by the yolk sac route with the test article or the negative control article. These eggs were examined for viability at 24 and 48 hours and 10 days post-injection. Nine of the ten test article injected eggs and nine of the ten negative control article injected eggs appeared viable at 24 hours post-injection. Eight of the test article injected eggs and nine of the negative control article injected eggs appeared viable at 48 hours post-injection. At examination on day 10, eight of the test article injected eggs and nine of the negative control article injected eggs contained viable embryos. Two of the test article

injected eggs and one of the negative control article injected eggs contained non-viable embryos. No growth was observed on blood agar plates streaked with fluid from the non-viable eggs. The cause of death of these embryos may have resulted from injection related trauma, as the embryos appeared non-viable at 24 and/or 48 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. Nine of the ten test article subpassage eggs and nine of the ten 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 this embryo most likely resulted from an injection related trauma, as the embryos appeared non-viable at 24 hours post-injection. (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

In addition to BRIQS #46951 mentioned previously, a deviation from section 7.4.4.1 of the protocol occurred when the observations for the negative control article injected guinea pigs were performed but not recorded on days 17-20 and day 24 post-injection. As determined by the study director this deviation did not affect the results of this assay, as the guinea pigs appeared normal at the completion of the assay (BRIQS #49753).

## 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.

24 Nov 09  
Date

Study Director

**TABLE 1****Suckling Mice**

Group No.	Number of Animals	Sex	Route(s) of Injection <sup>a</sup>	Volume of Test Material	Test Material	Treatment After Injection
SM 1	10	various <sup>b</sup>	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 gastrointestinal) 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						

<sup>a</sup> i.p. = Intraperitoneal injection; p.o. = Per os injection (by mouth); i.c. = Intracranial injection.

<sup>b</sup> 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 <sup>a</sup>	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				

<sup>a</sup> 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 <sup>a</sup>	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					

<sup>a</sup> i.p. = Intraperitoneal injection; i.c. = Intracranial injection.

**TABLE 2**

**Survival Summary  
for WIC-WA09-MB-001 #9104**

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

<sup>a</sup> Number of surviving animals after 28 days/Number of animals injected.

<sup>b</sup> 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 WIC-WA09-MB-001 #9104**

**Guinea Pigs**

<b>Test Material</b>	<b>Animal Number</b>	<b>Clinical Signs</b>	<b>Day of Onset (Post-Injection)<sup>a</sup></b>	<b>Day of Death/Sacrifice (Post-Injection)<sup>a</sup></b>
<b>Test Article</b>	4131	Normal		
	4132	Normal		
	4133	Normal		
	4134	Normal		
	4135	Normal		
	4136	Normal		
<b>Negative Control</b>	4137	Normal		
	4138	Normal		
	4139	Normal		
	4140	Normal		

<sup>a</sup> These columns will only be used if clinical signs, moribund condition, or deaths occur.

**TABLE 3 (Continued)**

**Summary of Daily Observations  
for WIC-WA09-MB-001 #9104**

**Adult Mice**

<b>Test Material</b>	<b>Animal Number</b>	<b>Clinical Signs</b>	<b>Day of Onset (Post-Injection)<sup>a</sup></b>	<b>Day of Death/Sacrifice (Post-Injection)<sup>a</sup></b>
<b>Test Article</b>	4101	Normal		
	4102	Normal		
	4103	Normal		
	4104	Normal		
	4105	Normal		
	4106	Normal		
	4107	Normal		
	4108	Normal		
	4109	Normal		
	4110	Normal		
<b>Negative Control</b>	4111	Normal		
	4112	Normal		
	4113	Normal		
	4114	Normal		
	4115	Normal		
	4116	Normal		
	4117	Normal		
	4118	Normal		
	4119	Normal		
	4120	Normal		

<sup>a</sup> These columns will only be used if clinical signs, moribund condition, or deaths occur.

**TABLE 3 (Continued)**

**Summary of Daily Observations  
for WIC-WA09-MB-001 #9104**

**Suckling Mice**

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

<sup>a</sup> Ten suckling mice injected per cage.

<sup>b</sup> Surviving suckling mice from the primary injection were sacrificed on day 14 for preparation of blind passage tissue homogenate.

<sup>c</sup> These columns will only be used if clinical signs, moribund condition, or deaths occur.

**TABLE 4**

**Embryonated Hens' Eggs**  
**Allantoic Route Survival Summary and Hemagglutination Results**  
**for WIC-WA09-MB-001 #9104**

Test Material	Primary Injection							
	24 Hour Viability	Viability <sup>a</sup> Harvest (Day 3)	Hemagglutination Results <sup>b</sup>					
			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 <sup>a</sup> Harvest (Day 3)	Hemagglutination Results <sup>b</sup>					
			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

<sup>a</sup> Number of viable eggs/number examined.

<sup>b</sup> 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 WIC-WA09-MB-001 #9104**

**Embryonated Hens' Eggs - Yolk Sac Route – Original Assay**

<b>Primary Injection</b>				
<b>Test Material</b>	<b>Number of Eggs Injected</b>	<b>Viability Observations<sup>a</sup></b>		
		<b>24 Hours</b>	<b>48 Hours</b>	<b>Harvest Day 10</b>
<b>Test Article</b>	10	10/10	7/10	7/10
<b>Negative Control Article</b>	10	9/10	9/10	9/10

<sup>a</sup> Number of viable eggs/number examined.

<b>Blind Passage</b>				
<b>Test Material</b>	<b>Number of Eggs Injected</b>	<b>Viability Observations<sup>a</sup></b>		
		<b>24 Hours</b>	<b>48 Hours</b>	<b>Harvest Day 9</b>
<b>Test Article Homogenate</b>	10	10/10	10/10	10/10
<b>Negative Control Article Homogenate</b>	10	10/10	10/10	9/10

<sup>a</sup> Number of viable eggs/number examined.

**TABLE 4 (Continued)**

**Summary of Observations  
for WIC-WA09-MB-001 #9104**

**Embryonated Hens' Eggs - Yolk Sac Route – Repeat Assay**

<b>Primary Injection</b>				
<b>Test Material</b>	<b>Number of Eggs Injected</b>	<b>Viability Observations<sup>a</sup></b>		
		<b>24 Hours</b>	<b>48 Hours</b>	<b>Harvest Day 10</b>
<b>Test Article</b>	10	9/10	8/10	8/10
<b>Negative Control Article</b>	10	9/10	9/10	9/10

<sup>a</sup> Number of viable eggs/number examined.

<b>Blind Passage</b>				
<b>Test Material</b>	<b>Number of Eggs Injected</b>	<b>Viability Observations<sup>a</sup></b>		
		<b>24 Hours</b>	<b>48 Hours</b>	<b>Harvest Day 9</b>
<b>Test Article Homogenate</b>	10	9/10	9/10	9/10
<b>Negative Control Article Homogenate</b>	10	9/10	9/10	9/10

<sup>a</sup> Number of viable eggs/number examined.

## Study Information

**Number:** AC32BH.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
24-Nov-2009	24-Nov-2009	Data and Final Reporting	24-Nov-2009	24-Nov-2009
03-Sep-2009	03-Sep-2009	Admin. Of Test Substance	08-Sep-2009	08-Sep-2009 *
11-Sep-2009	11-Sep-2009	Manipulation of Test System	15-Sep-2009	15-Sep-2009 *
11-Sep-2009	11-Sep-2009	Observation of Test System	15-Sep-2009	15-Sep-2009 *
03-Sep-2009	03-Sep-2009	Test System Preparation	08-Sep-2009	08-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:**

25-Nov-2009 1:59 pm GMT

Reason for signature: QA Approval

## Event Details Report

### Anomalous Result

Record ID: 46951

Date Opened:	12-Oct-2009	Date Occurred:	12-Oct-2009
Final Priority :	Medium	Record State :	Closed - Done

### General Information

Short Description: AC32BH.005002.BSV--Excessive nonviable eggs from yolk sac route (primary)

Department: In Vivo Testing

Impacted Systems : Assay/ Product

#### Product / Assay

TA/Project ID	Protocol No.	Disposition
AC32BH	005002.BSV	Test - Repeat

**Description :** Three out of ten eggs (yolk sac route) from test article AC32BH were observed nonviable during the harvest viability check(primary). These eggs were viable at 24 hour post injection but were observed non viable at 48 hours post injection.

One egg from the negative control group was non-viable at 24 and 48 hour post injection.

Three untreated eggs from same stock were opened up and were observed non-viable from a total of seventeen untreated eggs on the same day of the harvest viability check.

Section 11.1 of protocol P005002.BSV.V05 requires 80% of the test eggs must survive the observation period in order to evaluate the test article ("The test cells, or other test material, will be considered not contaminated if eighty percent of the test animals remain healthy and survive the observation period, and if all the animals used in the test fail to show lesions of any kind at the injection site and fail to show evidence of any viral infection. Statistical evaluation is not required.").

**Immediate / Containment Action :** Study director was notified, sent samples of nonviable eggs to bacteriology for testing, and stored and froze the remaining nonviable eggs contents in <-70 freezer.

### Operations Assessment/Investigation

Valid Assay / Process Criteria Met? Yes

**Preliminary Investigation :** The three eggs found non-viable at harvest of the primary injection were non-viable at 48 hours post-injection. This is indicative of a test system issue. It is likely that the eggs while viable were not hardy.. This conclusion is further supported by the fact that three untreated eggs being held on the same egg rack contained non-viable embryos. The blind passage will be performed, but a repeat assay is necessary.

**Impact Assessment :** A repeat assay is necessary because fewer than 80% of the test article injected eggs were viable. The primary injection of the assay is considered invalid as it cannot be evaluated. The blind passage was performed on the viable eggs and the results were satisfactory. There was no evidence of adventitious viral contamination of the test article. A repeat assay was performed. Both the primary injection and the blind passage had satisfactory results. There was no evidence of adventitious contamination of the test article.

## Event Details Report

### Anomalous Result

Record ID: 46951

Man	JC was appropriately trained in the procedure.
Method	The protocol was followed.
Machine	The incubator #110237 was functioning appropriately
Material	The correct materials were used.
Measurement	N/A
Environment	There were no facility excursions which impacted the study.
Sample Product	The correct test material was used.

Could this Event have affected other Products/ Services? N/A

Does this Event require Product/ Service Recall? N/A

Is there any evidence that Product/ Service could have been tampered with/ counterfeit? N/A

**Investigation Summary:** A repeat assay is necessary because fewer than 80% of the test article injected eggs were viable. The primary injection of the assay is considered invalid as it cannot be evaluated. The blind passage was performed on the viable eggs and the results were satisfactory. There was no evidence of adventitious viral contamination of the test article. A repeat assay was performed. Both the primary injection and the blind passage had satisfactory results. There was no evidence of adventitious contamination of the test article. The fragility of the test system is considered to be the cause of the embryo death/

**Root Cause Analysis :** The egg fluids were sent for testing for bacterial contamination. The fluids tested negative for bacterial contamination. The cause of the egg deaths could not be definitively determined, but was not due to technical error. It was most likely a result of the fragility of the test system.

### E-signatures

Supervisor Assessment By:	Supervisor Assessment On:	13-Oct-2009 4:32 pm	GMT
Owner Approval By:	Owner Approval On:	24-Nov-2009 7:06 pm	GMT
Final Approval By:	Final Approval On:	24-Nov-2009 7:14 pm	GMT

## Event Details Report

### Unplanned Deviation

Record ID: 49753

Date Opened:	20-Nov-2009	Date Occurred:	19-Oct-2009
Final Priority :	Low	Record State :	Closed - Done

### General Information

**Short Description:** Observations of guinea pigs in study # AC32BH.005002.BSV was performed but inadvertently not recorded.

**Department:** In Vivo Testing

**Impacted Systems :** Assay/ Product

#### Product / Assay

TA/Project ID	Protocol No.	Disposition
AC32BH	005002.BSV	Test - Continue

**Description :** Observations of guinea pigs in study # AC32BH.005002.BSV was performed but inadvertently not recorded for the negative control guinea pigs in cages #7 through cages #10. This deviates from protocol P005002.BSV, section 7.4.4.1 that guinea pigs will be observed every working day of the 28 day test period for clinical signs according to SOP OPBT0217. Observations for the 4 negative control guinea pigs were performed but not recorded on day post injection 17 through day post injection 20 and 24 ( October 19 through October 22 and October 26, 2009 ). Observations in the Test Article portion of the guinea pigs were performed and recorded. Observations continued to be performed and recorded from day post injection 25 through day post injection 28 ( October 27 through October 30th ) and all guinea pigs were normal at study completion.

**Immediate / Containment Action :** Study Director was notified and BRIQS was generated.

### Operations Assessment/Investigation

**Valid Assay / Process Criteria Met?** Yes

**Preliminary Investigation :** This was a technical error by SVA which was a unique event. No preventative or corrective action is necessary.

**Impact Assessment :** Observations were recorded for the TA animals by SVA every working day as required by the protocol. However, for 19,20,21, and 22 October as well as on 26 October the observations were performed on the negative control animals, but they were not recorded. The animals were observed as normal and the observations recorded on 16 October, 23 October and 27 October both before and after the missed observation. The assumption can safely be made that the animals were normal during the period when the observations were not recorded. Additionally, these were negative control animals. This deviation had no impact on the evaluation of the assay results, or the evaluation of the test article as free of adventitious contaminants.

**Root Cause Analysis :** This was an error by the technician SVA, but it has not been a recurring problem and is considered a unique event. To my knowledge, SVA has not had an event raised as a result of missed observation in 2009

## Event Details Report

**Unplanned Deviation****Record ID: 49753****E-signatures****Supervisor Assessment By:****Supervisor Assessment On:**

24-Nov-2009 7:23 pm GMT

**Final Approval By:****Final Approval On:**

24-Nov-2009 8:51 pm GMT



## FINAL STUDY REPORT

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

**TEST PROTOCOL NUMBER:** 30610.07

TEST ARTICLE IDENTIFICATION	WUXI APPTec ACCESSION NUMBER
WIC-WA09-MB-001 #9104	09-001949

**SPONSOR:** WiCell

**PERFORMING LABORATORY:** WuXi AppTec, Inc.

**SUBCONTRACTED TO:** Charles River Laboratories

WUXI APPTec ACCESSION NUMBER	RESULTS
09-001949	Transmission electron microscopic examination of 200 cells revealed no identifiable virus-like particles, nor did it reveal any other identifiable microbial agents.

Accession Number: 09-001949  
Final Report Number: 30610.07

WiCell  
Page: 2 of 7

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**QUALITY ASSURANCE UNIT SUMMARY**

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

The Quality Assurance Unit monitored the conduct and reporting of this laboratory study. This study has been performed under US FDA Good Laboratory Practice regulations (21 CFR Part 58), EU Good Laboratory Practice regulations (EMA GMP, Rules and Guidance for Pharmaceutical Manufacturers and Distributors, Annex 13), and applicable ICH Q7 standards and/or applicable Good Manufacturing Practices and in accordance with standard operating procedures and a 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

November 3, 2009

Quality Assurance

16 Nov 09  
Date

**GOOD LABORATORY PRACTICES STATEMENT**

The study referenced in this report was conducted in accordance with US FDA Good Laboratory Practices for Nonclinical Laboratory Studies as found in Title 21 Code of Federal Regulations Part 58, EU Good Laboratory Practice regulations, (EMA GMP, Rules and Guidance for Pharmaceutical Manufacturers and Distributors, Annex 13), and applicable ICH Q7 standards. The subcontractor inspected the study at least once, and WuXi AppTec Quality Assurance audited the final report.

Study Director

16 Nov 09  
Date

Professional Personnel involved in study:

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**1.0 PURPOSE**

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

**2.0 SPONSOR:** WiCell

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

**SUBCONTRACTOR:** Charles River Laboratories

**4.0 SCHEDULING**

**DATE SAMPLES RECEIVED:** September 15, 2009  
**STUDY INITIATION DATE:** September 15, 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:** WIC-WA09-MB-001 #9104

**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.<sup>1,2,3</sup> 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 fixative 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 September 15, 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 September 28, 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 (J63230). Cells had micovilli (MV: J63228) unevenly distributed on the surface. Nuclei (N: J63230) tended to be rounded to irregular, with chromatin relatively evenly dispersed or clumped along the periphery. Nuclei often had one or more nucleoli (NS: J63230) that were variably located.

The cytoplasm of most cells contained varying numbers of mitochondria (MI: J63233). Ribosomes (RB: J63233) were abundant in the cytoplasm of most cells. Cells were observed to contain autophagic vacuoles (AV: J63233), glycogen (G: J63232) and cleft-like spaces (CS: J63231). Centrioles (CN: J63229), microtubules (T: J63229), and desmosome-like structures (D: J63228) were also seen.

#### 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.

Thirteen percent of the cells were observed to be 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.

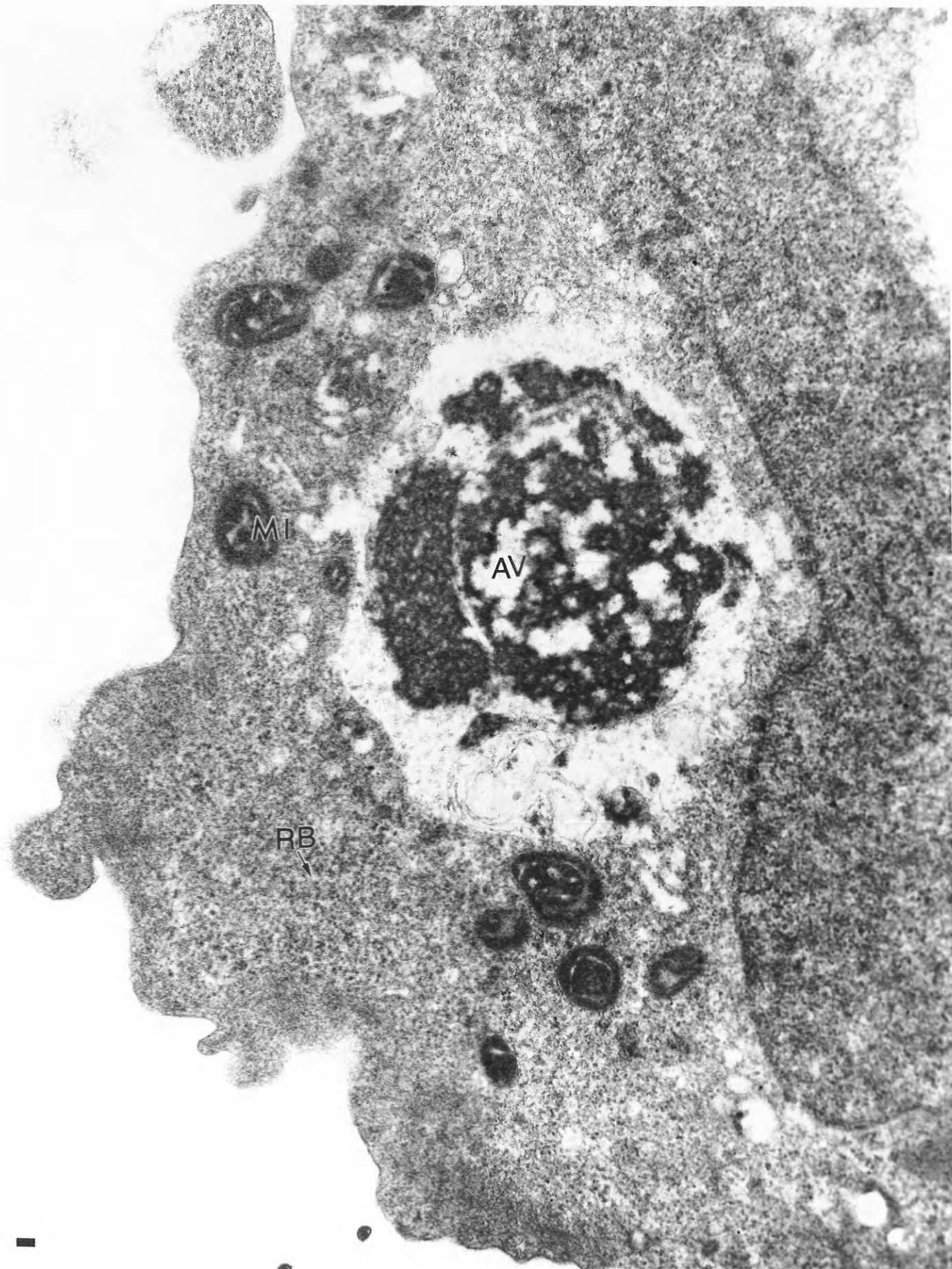
No amendments to the protocol were generated.

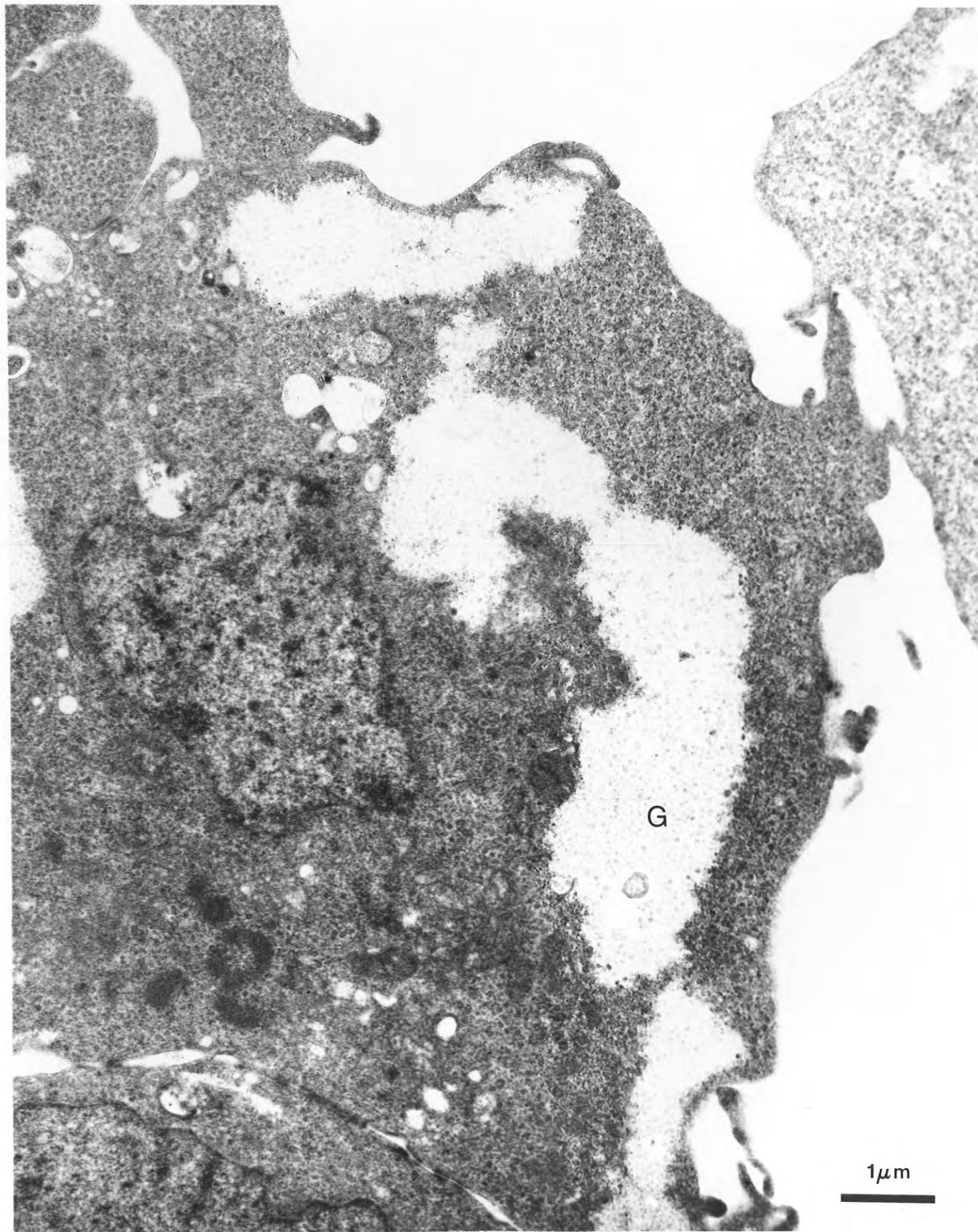
### **17.0 RECORD RETENTION**

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

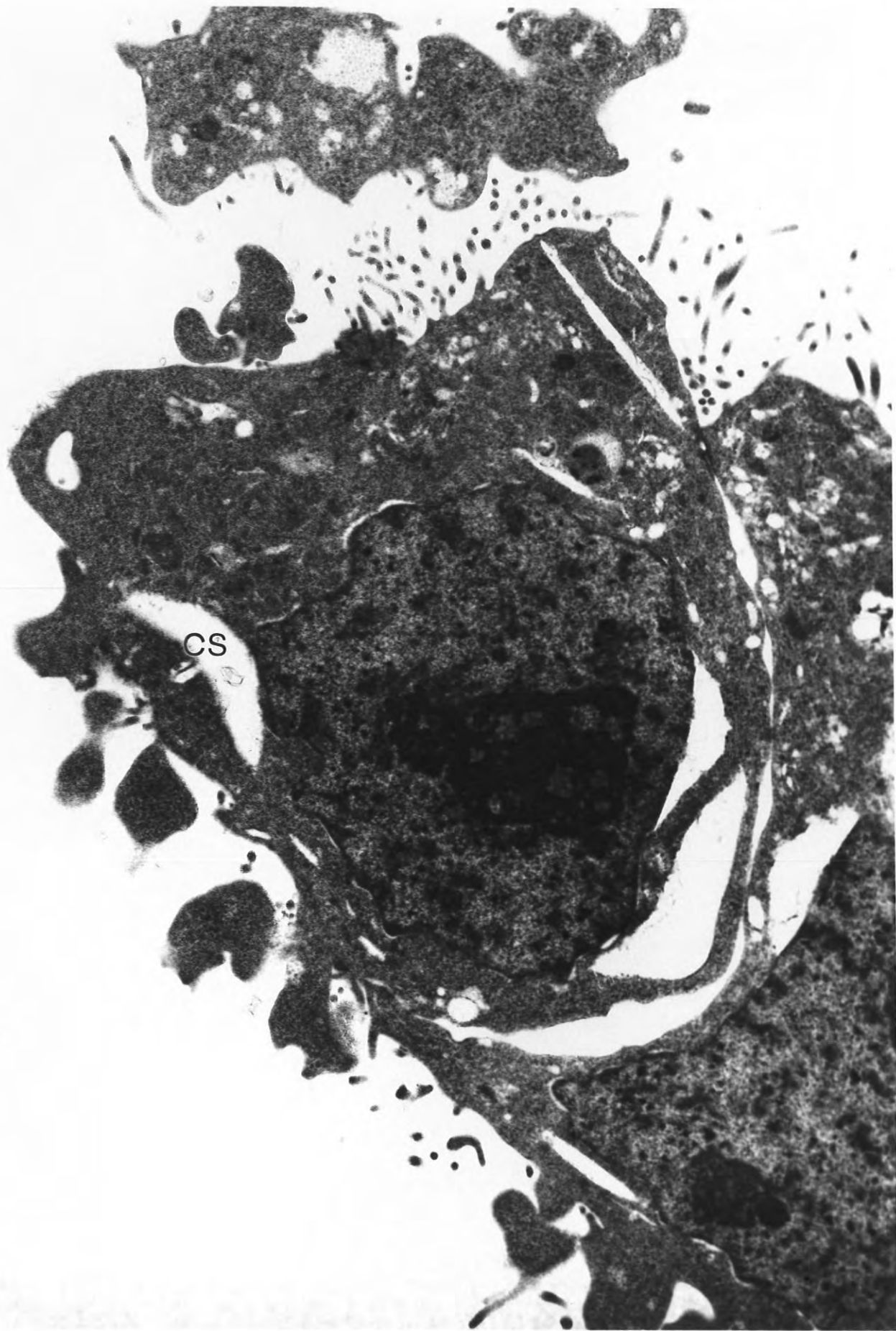
1. Morgan C and Rose HM (1967). "The Application of Thin Sectioning," *Methods in Virology* Vol. 3 (Maramorosch K and Koprowski H, eds.), Academic Press, New York, NY, pp. 576-616
2. Palmer E and Martin M (1988). Retroviridae in "Electron Microscopy in Viral Diagnosis", CRC Press, Boca Raton, FL, pp. 91-103
3. Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals (1993). Office of Biologics Research and Review, Food and Drug Administration
4. Jawetz E, Melnick JL, and Adelberg, EA, eds. (1984). Tumor Viruses in: "Review of Medical Microbiology," 16th Edition, Lange Medical Publications, Drawer L, Los Altos, CA, pp. 495-498

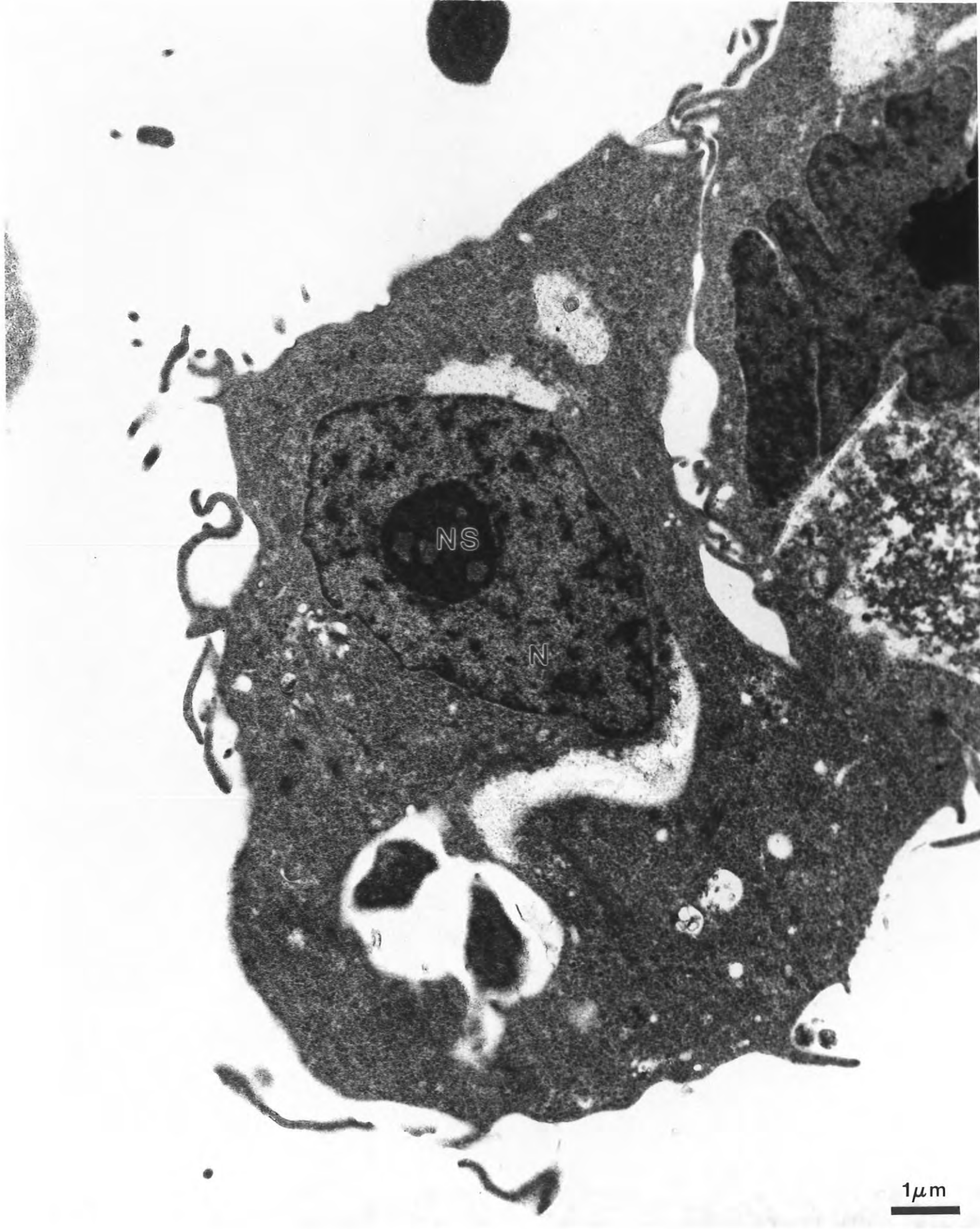


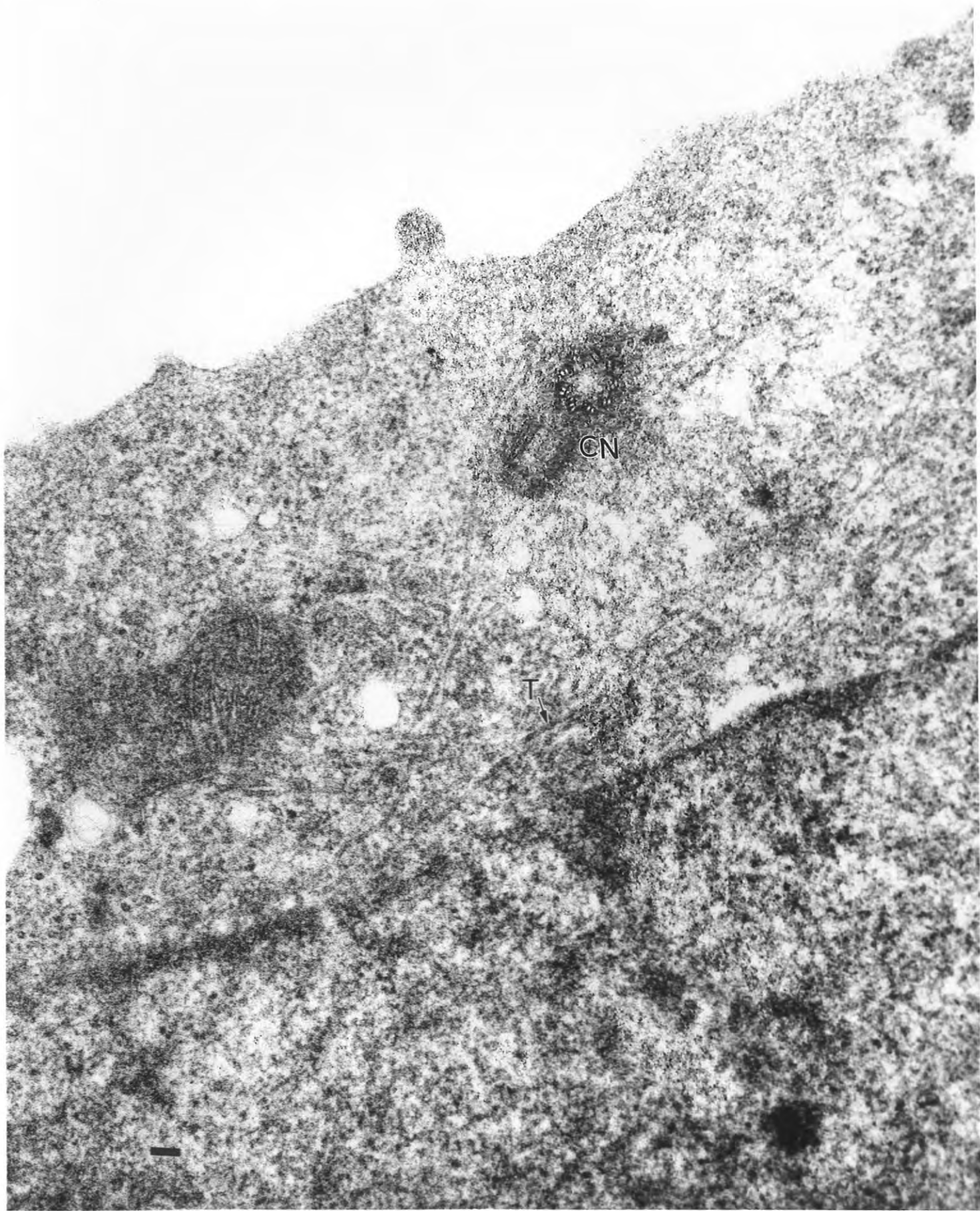


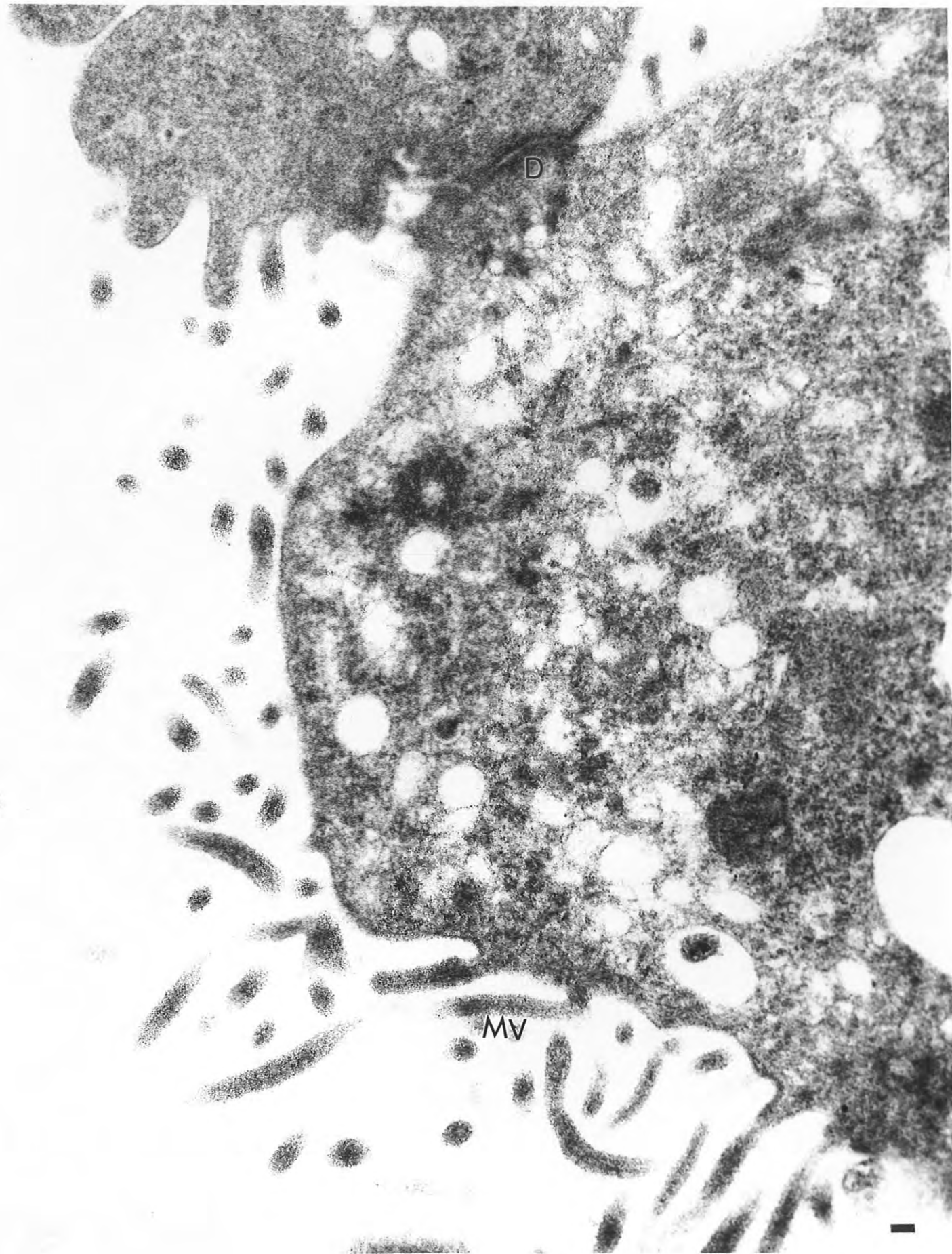
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## FINAL STUDY REPORT

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

**PROTOCOL:** 30201.04

TEST ARTICLE IDENTIFICATION	WUXI APPTec ACCESSION NUMBER
WIC-WA09-MB-001 #9104	09-001948

**SPONSOR:** WiCell Research Institute

**PERFORMING LABORATORY:** WuXi AppTec, Inc.

WUXI APPTec ACCESSION NUMBER	RESULTS
09-001948	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 <sup>+</sup> L <sup>-</sup> assay.

Accession Number: 09-001948  
Final Report Number: 30201.04

WiCell Research Institute  
Page: 2 of 8

### 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 ( $5 \times 10^5$  cells) to each flask prepared for the test article.

September 16, 2009

Quality Assurance

09 Oct 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

12 Oct 09  
Date

Personnel involved in study:

**1.0 PURPOSE**

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

**2.0 SPONSOR:** WiCell Research Institute

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

**4.0 SCHEDULING**

**DATE SAMPLES RECEIVED:** September 15, 2009  
**STUDY INITIATION DATE:** September 15, 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:** WIC-WA09-MB-001 #9104

**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<sup>1</sup>. (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<sup>+</sup>L<sup>-</sup> assay (outlined below, and as described further in protocol 30165). The PG4 S<sup>+</sup>L<sup>-</sup> assay is a very sensitive S<sup>+</sup>L<sup>-</sup> 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 stored according to the Sponsor's instructions. Indicator cell lines were maintained by the Cell Biology Laboratory.

### 8.1 Co-cultivation with *Mus dunni* Cells (30201)

- 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 2.0 mL) of the conditioned medium were reserved as a time zero (T<sub>0</sub>) time point for testing in the PG4 S<sup>+</sup>L<sup>-</sup> assay.
- 8.1.2 Three aliquots (3 x 2.0 mL) of the test article supernatant were saved for testing in the PG4 S<sup>+</sup>L<sup>-</sup> assay as a time zero (T<sub>0</sub>) time point.
- 8.1.3 Equal numbers of the *Mus dunni* cells (5x10<sup>5</sup> cells) and the test article cells (5x10<sup>5</sup> cells) were mixed to initiate two T150 flasks for the co-cultivation.
- 8.1.4 Positive controls were established last, using viral amphotropic murine retrovirus stocks (A-MuLV) inoculated at 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<sub>2</sub> 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 post-passage 2 were collected from the negative control, test article, and positive control cultures on day 14. The supernatants were frozen at -60°C or below until tested.

## 8.2 PG4 S<sup>+</sup>L<sup>-</sup> 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 three dilutions 1:10, 1:100, and 1:1000.
- 8.2.3 The assay positive control (A-MuLV) was inoculated onto PG4 S<sup>+</sup>L<sup>-</sup> 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<sub>2</sub> 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 September 15, 2009, WuXi AppTec, Inc. received one (1) T25 flask containing "hES Cells," at ambient temperature and designated for use in this assay. The Virology department appropriately removed the excess medium from the T25 flask and reserved it, and also re-placed the non-vented cap with a vented cap. The excess medium and T25 flask containing "hES Cells" were placed in an incubator at 37±2°C/5±2% CO<sub>2</sub> until initiation of this assay on September 16, 2009.

## 10.0 POSITIVE CONTROLS

### 10.1 Co-Cultivation Controls (30201)

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<sup>+</sup>L<sup>-</sup> assay on day 5 to confirm the replication of these viruses.

### 10.2 Controls for PG4 S<sup>+</sup>L<sup>-</sup> Assay (30165)

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

## 11.0 NEGATIVE CONTROLS

### 11.1 Co-Cultivation Controls (30201)

*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<sup>+</sup>L<sup>-</sup> Assay (30165)

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 (30201)

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

### 12.2 Validity Criteria for PG4 S<sup>+</sup>L<sup>-</sup> Assay (30165)

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<sup>+</sup>L<sup>-</sup> 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<sup>+</sup>L<sup>-</sup> assay, and the positive control co-cultivation cultures inoculated with A-MuLV demonstrated a positive reaction in the PG4 S<sup>+</sup>L<sup>-</sup> assay. No foci were observed in the negative assay control for the PG4 S<sup>+</sup>L<sup>-</sup> assay, and the positive assay control displayed viral-specific focus formation.

The test article supernatant from T<sub>0</sub> produced a negative PG4 S<sup>+</sup>L<sup>-</sup> result. Following co-cultivation with *Mus dunni* cells, the test article supernatants from post-passage 2 produced a negative PG4 S<sup>+</sup>L<sup>-</sup> result.

**TABLE 1: Observation of PG4 S<sup>+</sup>L<sup>-</sup> - Assay**

	Culture Inoculum	Time	FFU/mL
Co-Cultivation Samples	Accession #09-001948 <sup>1</sup> (diluted 1:2)	T <sub>0</sub>	ND
	Accession #09-001948 (diluted 1:2)	PP2	ND
	Negative control <sup>2</sup> (diluted 1:2)	T <sub>0</sub>	ND
	Negative control (diluted 1:2)	PP2	ND
	Positive control (A-MuLV) <sup>3</sup> (diluted 1:10)	PP2	TNTC
	Positive control (A-MuLV) <sup>3</sup> (diluted 1:100)	PP2	TNTC
	Positive control (A-MuLV) <sup>3</sup> (diluted 1:1000)	PP2	TNTC
PG4 S <sup>+</sup> L <sup>-</sup> - 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	3.13x10 <sup>5</sup>

**Legend:**

T <sub>0</sub>	-	Time 0
PP2	-	Post passage 2
NA	-	Not applicable
ND	-	None detected
TNTC	-	Too numerous to count

- <sup>1</sup> Supernatant collected from initial test article cultures used to prepare cultures for this assay.
- <sup>2</sup> Controls prepared from supernatant taken from fresh *M. dunni* cultures used to prepare cultures for assay.
- <sup>3</sup> Stock virus used to initiate positive control in co-cultivation assay.

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

## 15.0 CONCLUSION

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

## 16.0 STATISTICAL DATA ANALYSIS

Statistical analysis of the data was not required.

## 17.0 DEVIATIONS / AMENDMENTS

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

No amendments to the protocol were generated.

## 18.0 RECORD RETENTION

The testing facility will retain all records involving the study for 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)

H9 MCB.1



University of Wisconsin  
Hospital and Clinics

Histocompatibility/Molecular Diagnostics Laboratory

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

To: WiCell Research Institute

Re: High-resolution HLA results

**Patient**

Name HLA / MR# received	Method / Test date		HLA DNA-based typing*							
			Method: PCR-SSP			Direct Sequencing				PCR-SSP
			A*	B*	C*	DRB1*	DRB3*	DRB4*	DRB5*	DQB1*
WICELL, NSCB# 6185-HLA	DQB SSP		0201/24/2 6/34/90	3503/13	0401/09N	1501				
56749 /	A,B,C Seq	08/01/2007	0301/7/8/ 9/17	4427/12	0704/11	1601				
08/01/2007	DRB Seq	08/01/2007								

\_\_\_\_\_, Manager  
HLA/Molecular Diagnostics Laboratory

\_\_\_\_\_, PhD, Director  
HLA/Molecular Diagnostics Laboratory

\_\_\_\_\_  
Date

\_\_\_\_\_  
Date

Test Facility:

This report is confidential. No part may be used for advertising or public announcement without written permission. Results apply only to the sample(s) tested.

Report Number  
**807987**

Page 1 of 1

Waisman Clinical Biomanufacturing

May 05, 2009

P.O. #

## GENERAL MICROBIOLOGY TEST REPORT

**Sample Information:** hESC, WA09 MCB, Lot WIC-WA09-MB-001

**Date Received:** April 28, 2009

**Date in Test:** April 30, 2009

**Date Completed:** May 04, 2009

**Test Information:** Test Code: 30736  
Sterility Test Validation (Bacteriostasis / Fungistasis)  
Immersion, USP / 21 CFR 610.12  
Procedure #: BS210WSM.204  
Media Volume: 200 mL  
Volume Tested: 1.0 mL

SCD	<i>B. subtilis</i> ATCC 6633	<i>C. albicans</i> ATCC 10231	<i>A. brasiliensis</i> ATCC 16404
Test Sample	Positive	Positive	Positive
Inoculated Control	Positive	Positive	Positive
Inoculum Level (CFU)	28	30	37
RESULTS	PASS	PASS	PASS

FTM	<i>B. subtilis</i> ATCC 6633	<i>P. aeruginosa</i> ATCC 9027	<i>C. sporogenes</i> ATCC 11437
Test Sample	Positive	Positive	Positive
Inoculated Control	Positive	Positive	Positive
Inoculum Level (CFU)	26	31	28
RESULTS	PASS	PASS	PASS

**Conclusion:** The above test parameters do not demonstrate bacteriostatic / fungistatic activity. A sterility test performed using a media volume equal to or greater than that shown is acceptable.

**Note:** Reference Sterility Test Report(s): 807991

QA Reviewer

Date

Technical Reviewer

Date

ATTACH TO  
F01-QCP-028-11221

Testing conducted in accordance with current Good Manufacturing Practices.

LAB  
5/15/09

# Final Report

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

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## **STUDY DIRECTOR AUTHENTICATION AND GLP COMPLIANCE STATEMENT**

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

The study was performed in accordance with the agreed Protocol and with Covance Laboratories Limited Standard Operating Procedures, unless otherwise stated, and the study objectives were achieved. The work and generated data are scientifically acceptable and valid and this Report provides a true and accurate record of the results obtained.

The study was conducted in compliance with\*:

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

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

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

---

Study Director

30 May 2008  
Date

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## QUALITY ASSURANCE STATEMENT

### **H9-MCB.1: Detection of HIV-1, HIV-2, HTLV-2, EBV, HHV-7, HHV-8 and HBV Sequences using the Polymerase Chain Reaction (PCR) Technique.**

### **Detection and Quantification of B19 Sequences using the Quantitative Polymerase Chain Reaction Technique (QPCR). Detection and Quantification of HCV Sequences using the Reverse Transcriptase Quantitative Polymerase Chain Reaction Technique (RT-QPCR)**

This study has been reviewed by the Quality Assurance Unit of Covance Laboratories Ltd. and the report accurately reflects the raw data. The following inspections were conducted and findings reported to the Study Director (SD) and associated management. Critical procedures, which are performed routinely in an operational area, may be audited as part of a "process" inspection programme. This can be in addition to phases scheduled on an individual study basis. Selected process inspections conducted and considered applicable to this study are included below.

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

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

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

Quality Assurance Unit

30 May 2008  
Date

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## RESPONSIBLE PERSONNEL

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

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

Study Director:

Study Supervisor:

---

## STUDY SCHEDULE

The study schedule was as follows:

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

## **ARCHIVE STATEMENT**

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

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

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

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## SUMMARY

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

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

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

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

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

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

## INTRODUCTION

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

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

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

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

## **OBJECTIVE**

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

## **MATERIALS**

### **Protocol Adherence**

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

### **Test Article**

**Identification:** H9-MCB.1.

**Source:** WiCell Research Institute.

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

The exact details as presented on the test article vessel:

COVANCE 1 pellet @ 1x10<sup>6</sup> cells Human Virus Panel MCB.A.H9p27. 22 JAN 07. DF.

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

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

Appearance: Cell Pellet.  
Concentration:  $1 \times 10^6$  cells.  
Cell Line Information: Human Embryonic Stem Cells.  
Storage Temperature:  $-70^{\circ}\text{C}$ .  
Expiry Date: N/A.  
Unused test article to be disposed of by incineration.  
The test article does not contain chemicals which may be hazardous.

This study, for the presence of extraneous agents was conducted to define the purity of the test article. Therefore, information regarding the purity of the test article was not available at study initiation. Stability of the test article was not considered relevant, as the objective of this study was to test for extraneous agents that may be present in the test materials.

#### **PCR and QPCR Test Systems**

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

#### **Controls**

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

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

## PROCEDURES

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

## RESULTS

### Human Immunodeficiency Virus-1 (HIV-1) Results

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

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

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

### **Human Immunodeficiency Virus-2 (HIV-2) Results**

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

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

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

### **Human T Cell Lymphotropic Virus-2 (HTLV-2) Results**

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

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

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

### **Epstein Barr Virus (EBV) Results**

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

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

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

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

### **Human Herpes Virus 7 (HHV-7) Results**

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

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

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

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

### **Human Herpes Virus 8 (HHV-8) Results**

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

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

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

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

### **Hepatitis B Virus (HBV) Results**

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

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

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

### **Human Parvovirus (B19) Results**

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

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

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

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

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

### **Hepatitis C Virus (HCV) Results**

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

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

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

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

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

## CONCLUSIONS

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

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

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

## TABLES

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

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

N = Negative.

P = Positive.

vp = Virus particles.

\* = Non-specific PCR product(s) present.

pg = Picograms.

NA = Not applicable.

<sup>1</sup> = RT-QPCR assay.

<sup>2</sup> = QPCR assay.

<sup>3</sup> = Two positive control dilutions containing the highest levels of positive control were detected. This meets the PCR acceptance criteria.

**Table 2: Results of B19 QPCR**

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

U = Undetermined.

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

**Table 3: Results of HCV RT-QPCR**

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

U = Undetermined.

\* = Omitted from calculations as undetermined.

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

## **APPENDIX**

### **Minor Deviations from the Definitive Protocol**

1. On page 7 of the Definitive Protocol for the RNA extraction it states that the test article will be resuspended in 20µl of DNase, RNase none detected 0.1µm filtered water. However, the test article was resuspended in 30µl of DNase, RNase none detected 0.1µm filtered water in order to ensure that there was sufficient volume for spectrophotometry and RT-PCR analysis. This minor deviation is deemed not to have affected the integrity or outcome of the study.
2. On page 7 of the Definitive Protocol for the RNA extraction it states that the pellets will be washed with 1ml of 75% (v/v) ethanol. However, the pellets were washed with 1ml of 76% (v/v) ethanol in error. This minor deviation is deemed not to have affected the integrity or outcome of the study.
3. On page 14 of the Definitive Protocol for the EBV PCR it states that the spike will be 0.1pg and the positive control dilutions (approximate range) will be 0.01, 0.1 and 1pg. However, this should have read 1 in 10 for the spike and 1 in 100, 1 in 10 and neat EBV for the positive control dilutions (approximate range). This deviation is deemed not to have affected the integrity or outcome of the study.
4. On page 11 of the Definitive Protocol for the B19 QPCR it states 'Three replicates of 1µg of DNA will be assayed directly, with no further treatment and three replicates of 1µg of DNA will be spiked (1000 copies) to assess for inhibition. Where 1µg of DNA is unavailable, neat aliquots of the test article sample will be assayed. The extraction control will also be included in triplicate in QPCR.' The DNA was diluted to a concentration of 0.2µg/µl instead of 0.25µg/µl and therefore 0.8µg of DNA was tested spiked and un-spiked in triplicate. This minor deviation is deemed not to have affected the integrity or outcome of the study.
5. On page 11 of the Definitive Protocol for the B19 QPCR the reaction conditions are incorrect and should have included a cycle of 50°C for 2 minutes. The B19 QPCR run included this cycle and therefore this minor deviation is deemed not to have affected the integrity or outcome of the study.

6. On page 13 of the Definitive Protocol for the HCV probe concentration it states 0.15µM this should read 0.1µM as in the table on page 9 for the HCV master mix. This minor deviation is deemed not to have affected the integrity or outcome of the study.
7. On page 9 of the Definitive Protocol for the HCV RT-QPCR it states 'Three replicates of 1µg of RNA will be assayed directly, with no further treatment and three replicates of 1µg of RNA will be spiked (e.g. 100pg) to assess for inhibition. If 1µg of RNA is unattainable, neat aliquots of the test article will be tested.' However, the DNA was diluted to a concentration of 0.2µg/µl instead of being used neat, therefore, 0.6µg of DNA was tested spiked and un-spiked in triplicate. This minor deviation is deemed not to have affected the integrity or outcome of the study.
8. MRC-5 cell RNA batch 050722 expired on 22/07/07, however, was used on 06/08/07. The RT-QPCR passed all the assay acceptance criteria and the assay was valid. Therefore, this minor deviation is deemed not to have affected the integrity or outcome of the study.
9. On page 7 of the Definitive Protocol the second paragraph of the RNA extraction section states that, 'Each positive control sample will be resuspended in 10.5µl DNase, RNase none detected 0.1µm filtered water'. This is a typographical error and should not have been included.
10. On page 10 of the Definitive Protocol it states: 'In addition, a second "master mix" will be prepared containing all the reagents used with the exception of Reverse Transcriptase (EuroScript RT), which will be replaced with water. The RT negative control will be analysed in triplicate and will control for the presence of contaminating DNA'. This second master mix was not prepared. This second master mix controls for the presence of contaminating DNA, however as the test article samples were shown to be negative using only the master mix containing Reverse Transcriptase (EuroScript RT), viral RNA and contaminating DNA was therefore not present in the test article. This minor deviation is deemed not to have affected the integrity or outcome of the study.
11. On page 5 of the Definitive Protocol, for the Preparation of Test Article for DNA Extraction, it states: 'The appropriate number of cells will be centrifuged at 300 x 'g' for approximately 5 minutes. The supernatant will be removed and discarded and cell pellet used directly'.

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

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

## **ANNEX**

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

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

# Definitive Protocol

Title H9-MCB.1: Detection of HIV-1, HIV-2, HTLV-1, HTLV-2, HCMV, EBV, HHV-7, HHV-8 and HBV Sequences using the Polymerase Chain Reaction (PCR) Technique. Detection and Quantification of HHV-6 and B19 Sequences using the Quantitative Polymerase Chain Reaction Technique (QPCR). Detection and Quantification of HCV Sequences using the Reverse Transcriptase Quantitative Polymerase Chain Reaction Technique (RT-QPCR)

Study Director

Testing Facility

Covance Laboratories Ltd.,

Study Monitors

Sponsor

Waisman Clinical BioManufacturing Facility

Covance Study Number

2823/004

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## INTRODUCTION

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

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

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

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

### **OBJECTIVE**

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

### **TEST ARTICLE**

**Identification:** H9-MCB.1.

**Source:** WiCell Research Institute.

The exact details as presented on the test article vessel are: COVANCE 1 pellet @  $1 \times 10^6$  cells Human Virus Panel MCB.A.H9p27. 22JAN07 DF.

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

Appearance: Cell pellet

Concentration:  $1 \times 10^6$  cells

Vial size: 1.5mL

Cell line information: Human Embryonic Stem Cells

Storage temperature: -70°C

Expiry date: N/A

Unused test article to be disposed of by incineration.

The test article does not contain chemicals which may be hazardous.

This study, for the presence of extraneous agents will be conducted to define the purity of the test article. Therefore, information regarding the purity of the test article is not available at study initiation. Stability of the test article is not considered relevant, as the objective of this study is to test for extraneous agents that may be present in the test materials.

## PCR AND QPCR TEST SYSTEMS

### Controls

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

Blank, sentinel and RNA extraction control:

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

Source: Sigma-Aldrich Company Ltd.

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

Source: Invitrogen Ltd.

## PROCEDURES

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

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

### Preparation of Test Article for DNA Extraction

A maximum of  $5 \times 10^6$  cells can be extracted per column using the DNeasy™ Tissue and Blood Kit. Total cell count (not viable cell count) is required, therefore no trypan blue should be added. The appropriate number of cells will be centrifuged at  $300 \times 'g'$  for approximately 5 minutes. The supernatant will be removed and discarded and cell pellet used directly.

### DNA Extraction

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

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

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

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

#### **Determination of the DNA Concentration of Samples**

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

Ideally, the DNA should have an OD<sub>260</sub>:280nm value of 1.6 to 1.9 for PCR analysis. However, samples in the range of 1.0 to 2.5 will be accepted for PCR analysis. If a sample has a ratio outside the range of 1.6 to 1.9 and is shown to be inhibitory, the Sponsor will be contacted with the results prior to proceeding with further extraction.

### **Preparation of Test Article for RNA Extraction**

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

### **RNA Extraction**

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

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

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

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

#### **Determination of the RNA Concentration of Samples**

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

Ideally, the RNA should have an OD<sub>260</sub>:280nm value of 1.65 to 2.1 for PCR analysis. However, samples in the range of 1.4 to 2.1 will be accepted for PCR analysis. If a sample has a ratio outside the range of 1.65 to 2.1 and is shown to be inhibitory, the Sponsor will be contacted with the results prior to proceeding with further extraction.

#### **PCR Amplification**

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

1 x PCR buffer	50 mM KCl, 10 mM Tris pH 8.3
dNTPs	200 µM each
MgCl <sub>2</sub>	as optimised for each primer pair, 1.5 - 3.5 mM
Primers	0.03 - 0.3 µM each
Amplitaq Gold	
DNA polymerase	1.25 - 2.5 units

Refer to primers section for precise reaction component concentrations.

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

## Reverse Transcriptase (RT)-QPCR Amplification

### HCV RT-QPCR

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

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

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

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

<sup>1</sup> The amount of water added will bring the total volume per reaction to 20µl after the addition of sample.

<sup>2</sup> Reaction buffer contains HotGoldStar, dNTP's, MgCl<sub>2</sub>, ROX™ passive reference and stabilisers (proprietary formulation).

<sup>3</sup> The final concentration is based on a total volume of 66 µl of reaction buffer plus water/sample prepared and then 20µl of this used for each of the three replicates to be tested.

<sup>4</sup> TaqMan® exogenous internal positive control (IPC) reagents.

N/A = Not applicable.

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

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

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

One cycle of:

48°C for 30 minutes

95°C for 10 minutes

Then 40 cycles of:

95°C for 15 seconds

60°C for 1 minute

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

## **QPCR Amplification**

### **B19 QPCR**

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

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

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

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

<sup>1</sup> The amount of water added will bring the total volume per reaction to 20µl after the addition of sample.

<sup>2</sup> Reaction buffer contains HotGoldStar, dNTP's, MgCl<sub>2</sub>, ROX™ passive reference and stabilisers (proprietary formulation).

<sup>3</sup> The final concentration is based on a total volume of 66 µl of reaction buffer plus water/sample prepared and then 20µl of this used for each of the three replicates to be tested.

<sup>4</sup> TaqMan® exogenous internal positive control (IPC) reagents.

N/A = Not applicable.

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

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

One cycle of:

95°C for 10 minutes

Then 40 cycles of:

95°C for 15 seconds

60°C for 1 minute

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

### HHV-6 QPCR

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

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

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

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

<sup>2</sup> The amount of water added will bring the total volume per reaction to 25µl after the addition of sample.

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

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

One cycle of:

95°C for 10 minutes

Then 40 cycles of:

95°C for 15 seconds

60°C for 1 minute

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

## PCR Primers

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

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

## RT-QPCR and QPCR Primers and Probe

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

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

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

### PCR Controls

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

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

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

### B19 QPCR Controls

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

### **HHV-6 QPCR Controls**

The positive control will be included and will contain a range of DNA (e.g.  $1 \times 10^7$  -  $1 \times 10^2$  copies/ $\mu$ l), ensuring comparability between QPCR runs. The data generated from this dilution series will be used to construct a standard curve and generate quantitative data. Four blank reactions using water as the template will also be used as negative controls. Two wells will remain open throughout the assay preparation steps until initiation of QPCR. These wells function as sentinel controls by monitoring for airborne contamination. The second set of wells function as assay blank controls to monitor for reagent contamination. A negative control DNA sample will be included in triplicate in the assay to ensure specificity.

### **HCV RT-QPCR Controls**

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

### PCR Thermal Profiles

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

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

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

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

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

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

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

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

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

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

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

#### HCMV gB1/HCMV gB 2 primers

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

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

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

**KS330 233A/KS330 233E primers**

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

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

**Gel Electrophoresis**

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

**Southern Blot Hybridisation**

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

## DATA ANALYSIS

### PCR Assay Acceptance Criteria

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

- 1) No viral specific amplicon is detected in the negative control, blank reaction or sentinel control following each PCR assay.
- 2) No viral specific amplicon is detected in the extraction control(s) following each PCR assay.
- 3) Viral specific amplicon is detected in at least the two positive control dilutions containing the highest levels of positive control, following each PCR.
- 4) Viral specific amplicon is detected in each spiked test article DNA or RNA sample following each PCR assay.

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

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

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

### **PCR Evaluation Criteria**

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

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

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

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

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

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

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

### **QPCR Assay Acceptance Criteria**

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

1. Amplification is detected in the appropriate positive control dilutions by QPCR.
2. No amplification is detected in the negative, extraction, water or sentinel controls by QPCR.
3. Linear regression analysis of the standard curve dilutions produces a slope of less than -2.2 and greater than -4.4 and a correlation coefficient ( $R^2$ ) of greater than 0.9.

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

#### **QPCR Evaluation Criteria**

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

- 1) The samples will be judged positive for the test article if the level of fluorescence (e.g. from FAM) rises >10 standard deviations above background fluorescence, once, and the accumulation of fluorescence is indicative of exponential amplification.
- 2) By comparing the  $C_T$  values generated from the spiked test article samples with those generated from the spiked control material that forms the standard curve. Test article samples will be judged totally inhibitory if the spiked sample produced a  $C_T$  value of 40. Test article samples will be judged partially inhibitory if the spiked sample produced a  $C_T$  value, 3.3 cycles or more, greater than the  $C_T$  predicted from the standard curve.

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

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

#### **RT-QPCR Assay Acceptance Criteria**

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

4. Amplification is detected in the appropriate positive control dilutions by RT-QPCR.
5. No amplification is detected in the negative, RT negative, extraction, water or sentinel controls by RT-QPCR.
6. Linear regression analysis of the standard curve dilutions produces a slope of less than -2.2 and greater than -4.4 and a correlation coefficient ( $R^2$ ) of greater than 0.9.

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

#### **RT-QPCR Evaluation Criteria**

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

1. The samples will be judged positive for the test article if the level of fluorescence (e.g. from FAM) rises >10 standard deviations above background fluorescence, once, and the accumulation of fluorescence is indicative of exponential amplification.
2. By comparing the  $C_T$  values generated from the spiked test article samples with those generated from the spiked control material that forms the standard curve. Test article samples will be judged totally inhibitory if the spiked sample produced a  $C_T$  value of 40. Test article samples will be judged partially inhibitory if the spiked sample produced a  $C_T$  value, 3.3 cycles or more, greater than the  $C_T$  predicted from the standard curve.

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

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

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

## **GLP COMPLIANCE**

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

The study will be performed in compliance with\*:

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

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

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

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

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

## ARCHIVE STATEMENT

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

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

## REFERENCES

1. Saiki R K, Scharf S, Faloona F, Mullis K B, Horn G T, Erlich H A and Arnheim N (1985) Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anaemia. *Science* 230 1350 - 1354.
2. Mullis K B and Faloona F (1987) Specific synthesis of DNA in vitro via a polymerase-catalysed chain reaction. *Methods in Enzymol.* 155 335 - 350.
3. Saiki R K, Gelfand D H, Stoffel S, Scharf S J, Higuchi R, Horn G T, Mullis K G and Erlich H A (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 239 487 - 491.
4. Bootman J S and Kitchin P A (1992) An international collaborative study to assess a set of reference reagents for HIV-1 PCR. *J. Virological Methods* 37 23 - 42.
5. Kwok S and Higuchi R (1989) Avoiding false positives with PCR. *Nature* 339 237 - 238.

## APPENDIX 1

### Study Records

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

Definitive Protocol

Amendment(s)\*

File note(s)\*

Study related correspondence\*

Test article description

Test article receipt and utilisation

Metrology<sup>#</sup>

Records for reagents and stock solutions<sup>#</sup>

Test article cell culture records\*

Work sheets

\* where appropriate

<sup>#</sup> some records held centrally

## **APPENDIX 2**

### **Draft Report**

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

- The objectives stated in the approved Protocol.
- The identity of the test substance as specified by the Sponsor.
- Any unforeseen circumstances which may have affected the quality or integrity of the study.
- Location and storage of all raw data.

The following items will be presented in the Draft Report:

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

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

### **Final Report**

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

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

## APPENDIX 3

### RESPONSIBLE PERSONNEL AND STUDY SCHEDULE

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

#### Distribution

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

#### Hard Copy

Head of Quality Systems<sup>1</sup>  
Resource Management  
Resource Management

#### Electronic Access

Study Supervisor<sup>1</sup>  
Analytical Staff  
Study Co-ordinator  
Project Manager  
VP of Biotechnology

<sup>1</sup> = Any change documented in study records

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#### PROPOSED DATES

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

**PROTOCOL APPROVAL**

\_\_\_\_\_  
Study Monitor  
Waisman Clinical Biomanufacturing Facility

7/11/07

\_\_\_\_\_  
Quality Assurance  
WiCell Research Institute

7/13/07

\_\_\_\_\_  
Study Director  
Covance Laboratories Ltd

28 June 2007  
Date

\_\_\_\_\_  
Head of Biosafety  
Covance Laboratories Ltd

28 Jun 2007  
Date

# Protocol Amendment

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

This Amendment documents the following:

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

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

### **OBJECTIVE**

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

PROTOCOL APPROVAL

Study Monitor  
Waisman Clinical Biomanufacturing Facility

11/2/07

Quality Assurance  
WiCell Research Institute

11/2/07

Study Director  
Covance Laboratories Ltd

01/20/2007  
Date

Project Manager  
Covance Laboratories Ltd

01/20/2007  
Date

# Final Report

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

**Study Number:** AC08DS.105013.BSV

**Test Article ID:** H9 MCB.1

**Sponsor:** WiCell Research Institute

**Authorized Representative:**

### **CONCLUSION**

**One-half (0.5)  $\mu$ g of DNA (representing approximately  $7.5 \times 10^4$  cells) isolated from test article H9 MCB.1 was analyzed for the presence of human T-cell lymphotropic virus types I and II (HTLV-I/II) proviral DNA by the polymerase chain reaction (PCR)<sup>1</sup> technique. The assay can detect 100 copies of HTLV-I/II proviral DNA in the presence of 0.5  $\mu$ g of genomic DNA.**

**The results presented herein indicate that the test article tested negative for the presence of HTLV-I/II DNA.**

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

**STUDY INFORMATION**

**Test Article:** H9 MCB.1 was received by BioReliance on 09/25/2007. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor. Retention of reserve sample from each batch of test article is the responsibility of the sponsor.

**Testing Facility:** BioReliance

**Schedule:**

<b>Study Initiation:</b>	09/26/2007
<b>Lab Initiation:</b>	10/02/2007
<b>Lab Completion:</b>	10/04/2007
<b>Study Completion:</b>	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:

<b>Negative Control:</b>	Genomic DNA from MRC5 human fetal lung fibroblasts Source: BioReliance
<b>Positive Controls:</b>	<div>HTLV-I: Negative control DNA spiked with 100 copies of pH750, a plasmid containing a 752 bp fragment from the HTLV-I tax/rex gene Source: BioReliance</div> <div>HTLV-II: Negative control DNA spiked with 100 copies of pMAHTII, a plasmid containing a 552 bp fragment from the HTLV-II tax/rex gene Source: BioReliance</div>
<b>No DNA Control:</b>	Nuclease-free water Source: USB or other commercial supplier
<b>Spiked Control:</b>	The spiked controls (amplification suitability controls) verify the absence of PCR inhibitors in the test article DNA. <div>HTLV-I: Test article extract spiked with 100 copies of pH750.</div> <div>HTLV-II: Test article extract spiked with 100 copies of pMAHTII.</div>

## METHODS

### Sample Preparation

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

### DNA Amplification

Each PCR amplification was performed on 0.5 µg of test article DNA and on the assay controls, using primers HT-OS and HT-OA, specific for the tax/rex region of HTLV-I/II, employing conditions optimized to achieve detection of 100 copies of proviral DNA. Aliquots of the amplification products obtained from the test article and from the control samples were analyzed by agarose gel electrophoresis in the presence of ethidium bromide. Amplification products were visualized on a UV transilluminator and photographed using Polaroid 667 film (OPBT0932).

### RESULTS

Test article DNA (0.5 µg), representing approximately  $7.5 \times 10^4$  test article cells, was analyzed for the presence of HTLV-I/II proviral DNA by PCR amplification and agarose gel electrophoresis in the presence of ethidium bromide. The results are shown in Figure 1. The No DNA control (NO) and Negative control (NC) showed no bands at 158 bp. The positive controls (PC-I and PC-II) produced a 158 bp band. The test article spiked with 100 copies of either pH750 (TAS-I) or pMAHTII (TAS-II) produced a 158 bp band, demonstrating that the test article did not inhibit the PCR reaction. The test article (TA) produced no bands at 158 bp.

These results provide evidence that the test article H9 MCB.1 tested negative for the presence of HTLV-I/II proviral DNA.

### APPROVAL

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

\_\_\_\_\_, Ph.D.  
Study Director

23 Oct 07  
\_\_\_\_\_  
Date

FIGURE 1



Detection of HTLV-I/II proviral sequences in test article H9 MCB.1 utilizing agarose gel electrophoresis in the presence of ethidium bromide. The arrow indicates specific amplification products.

M1:	100 bp DNA ladder
NO:	No DNA control
TA:	Test Article
TAS-I:	Test article spiked with 100 copies of pH750
TAS-II:	Test article spiked with 100 copies of pMAHTII
NC:	Negative control (MRC5 genomic DNA)
PC-I:	Positive control for HTLV-I (negative control DNA spiked with 100 copies pH750)
PC-II:	Positive control for HTLV-II (negative control DNA spiked with 100 copies pMAHTII)
M2:	Biomarker low DNA size marker.

# Quality Assurance Statement

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

**Study Number:** AC08DS.105013.BSV

**Study Director:** , Ph.D.

Quality Assurance performed the inspections listed below for this study. Verification of the study protocol was also performed and documented by QA. Procedures, documentation, equipment records, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. FDA Good Manufacturing Practices (21 CFR 210 and 211), the UK GLP Regulations, the Japanese GLP Standard, and the OECD Principles of Good Laboratory Practice and to assure that the study is conducted according to the protocol and relevant Standard Operating Procedures.

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

**	Inspect On	23-Oct-07 - 23-Oct-07 To Study Dir 23-Oct-07 To Mgmt 23-Oct-07
	Phase	Final Report and data audit
*	Inspect On	24-Sep-07 - 25-Sep-07 To Study Dir 24-Sep-07 To Mgmt 24-Sep-07
	Phase	Systems Inspection - Administration of Test Substance to Test System
*	Inspect On	24-Sep-07 - 25-Sep-07 To Study Dir 25-Sep-07 To Mgmt 25-Sep-07
	Phase	Systems Inspection - Manipulation of Test System
*	Inspect On	24-Sep-07 - 25-Sep-07 To Study Dir 25-Sep-07 To Mgmt 25-Sep-07
	Phase	Systems Inspection - Observation of Test System/Data Collection and/or Analysis
*	Inspect On	24-Sep-07 - 25-Sep-07 To Study Dir 25-Sep-07 To Mgmt 25-Sep-07
	Phase	Systems Inspection - Test System Preparation

\*\* Inspection specific for this study

\* Systems Inspection

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

\_\_\_\_\_

23 Oct 07

DATE

QUALITY ASSURANCE

## SUMMARY

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

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

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

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

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

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

## INTRODUCTION

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

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

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

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

## **OBJECTIVE**

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

## **MATERIALS**

### **Protocol Adherence**

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

### **Test Article**

**Identification:** H9-MCB.1.  
**Source:** WiCell Research Institute.

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

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

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

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

Appearance: Cell Pellet.  
Concentration:  $1 \times 10^6$  cells.  
Cell Line Information: Human Embryonic Stem Cells.  
Storage Temperature:  $-70^\circ\text{C}$ .  
Expiry Date: N/A.  
Unused test article to be disposed of by incineration.  
The test article does not contain chemicals which may be hazardous.

This study, for the presence of extraneous agents was conducted to define the purity of the test article. Therefore, information regarding the purity of the test article was not available at study initiation. Stability of the test article was not considered relevant, as the objective of this study was to test for extraneous agents that may be present in the test materials.

### PCR and QPCR Test Systems

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

#### Controls

Positive Controls: DNA extracted from HIV-1.  
DNA extracted from HIV-2.  
DNA extracted from HTLV-2.  
DNA extracted from EBV.  
DNA extracted from HHV-7.  
DNA extracted from HHV-8.

Source: Advanced Biotechnologies Inc.

Positive Control: B19 DNA synthetic oligonucleotide.

Source: Eurogentec.

Positive Control: HBV, full length genome in pEco63, extracted DNA.

Source: American Type Tissue Culture Collection.

Positive Control: Armored RNA<sup>®</sup> HCV (Genotype 1a) in TSM III buffer.

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

## PROCEDURES

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

## RESULTS

### Human Immunodeficiency Virus-1 (HIV-1) Results

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

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

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

#### **Human Immunodeficiency Virus-2 (HIV-2) Results**

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

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

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

#### **Human T Cell Lymphotropic Virus-2 (HTLV-2) Results**

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

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

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

### **Epstein Barr Virus (EBV) Results**

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

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

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

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

### **Human Herpes Virus 7 (HHV-7) Results**

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

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

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

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

### **Human Herpes Virus 8 (HHV-8) Results**

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

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

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

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

### **Hepatitis B Virus (HBV) Results**

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

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

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

### **Human Parvovirus (B19) Results**

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

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

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

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

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

### **Hepatitis C Virus (HCV) Results**

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

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

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

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

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

## CONCLUSIONS

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

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

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

## TABLES

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

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

N = Negative.  
P = Positive.  
vp = Virus particles.

\* = Non-specific PCR product(s) present.  
pg = Picograms.  
NA = Not applicable.

<sup>1</sup> = RT-QPCR assay.

<sup>2</sup> = QPCR assay.

<sup>3</sup> = Two positive control dilutions containing the highest levels of positive control were detected. This meets the PCR acceptance criteria.

**Table 2: Results of B19 QPCR**

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

U = Undetermined.

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

**Table 3: Results of HCV RT-QPCR**

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

U = Undetermined.

\* = Omitted from calculations as undetermined.

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

# Final Report

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

**Study Number:** AC08DS.105012.BSV

**Test Article ID:** H9 MCB.1

**Sponsor:** WiCell Research Institute

**Authorized Representative:**

### CONCLUSION

One-half (0.5) µg of DNA isolated from test article H9 MCB.1 (representing approximately  $7.5 \times 10^4$  cells) was analyzed for the presence of human cytomegalovirus (CMV) DNA by the polymerase chain reaction (PCR)<sup>1</sup> 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.

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

**STUDY INFORMATION**

**Test Article:** H9 MCB.1 was received by BioReliance on 09/25/2007. Determination of the stability, purity, and concentration of the test article is the responsibility of the sponsor. Retention of reserve sample from each batch of test article is the responsibility of the sponsor.

**Testing Facility:** BioReliance

**Schedule:**

**Study Initiation:** 09/26/2007

**Lab Initiation:** 10/02/2007

**Lab Completion:** 10/08/2007

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

**Study Director:** , 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:

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

**METHODS****Sample Preparation**

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

**DNA Amplification**

PCR amplification was performed on 0.5µg of test article DNA using primers CMV100 and CMV150 specific for the polymerase region of CMV, employing conditions optimized to achieve detection of 100 copies of CMV. Aliquots of the amplification products obtained from the test article and from the control samples were analyzed by agarose gel electrophoresis in the presence of ethidium bromide. Amplification products were visualized on a UV transilluminator and photographed using Polaroid 667 film (SOP OPBT0935).

## REPEATS

The first performance of the PCR assay, testing 0.5 µg amount of test article DNA, provided an invalid test. The positive control (PC) failed to produce a 363 bp band (results not presented). The PCR assay was repeated, using the same amount of test article DNA. The repeated assay provided a valid test with a negative result (results are presented in Results section below).

## RESULTS

Test article DNA (0.5 µg), representing approximately  $7.5 \times 10^4$  test article cells, was analyzed for the presence of CMV DNA by PCR amplification and agarose gel electrophoresis in the presence of ethidium bromide. The results are shown in Figure 1. The No DNA control (NO) showed no bands and the Negative control (NC) showed no bands at 363 bp. The positive control (PC) produced a 363 bp band. The test article spiked with 100 copies of pCMVpol (TAS) produced a 363 bp band, demonstrating that the test article did not inhibit the PCR reaction. The test article (TA) produced no bands at 363 bp.

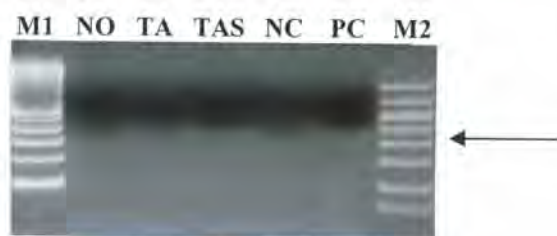
These results provide evidence that the test article H9 MCB.1 tested negative for the presence of CMV DNA.

## APPROVAL

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

\_\_\_\_\_, Ph.D.  
Study Director

23 Oct 07  
\_\_\_\_\_  
Date

**FIGURE 1**

Detection of CMV specific sequences in the test article H9 MCB.1 utilizing agarose gel electrophoresis in the presence of ethidium bromide

M1: 100 bp DNA ladder

NO: No DNA control

TA: Test Article

TAS: Test article spiked with 100 copies pCMVpol

NC: Negative control (MRC5 DNA)

PC: Positive control (MRC5 DNA spiked with 100 copies pCMVpol)

M2: Biomarker low, a DNA size marker

Arrow indicates the specific amplification product.

# Quality Assurance Statement

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

**Study Number:** AC08DS.105012.BSV

**Study Director:** , Ph.D.

Quality Assurance performed the inspections listed below for this study. Verification of the study protocol was also performed and documented by QA. Procedures, documentation, equipment records, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. FDA Good Manufacturing Practices (21 CFR 210 and 211), the UK GLP Regulations, the Japanese GLP Standard, and the OECD Principles of Good Laboratory Practice and to assure that the study is conducted according to the protocol and relevant Standard Operating Procedures.

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

**	Inspect On	23-Oct-07 - 23-Oct-07 To Study Dir 23-Oct-07 To Mgmt 23-Oct-07
	Phase	Final Report and data audit
*	Inspect On	24-Sep-07 - 25-Sep-07 To Study Dir 24-Sep-07 To Mgmt 24-Sep-07
	Phase	Systems Inspection - Administration of Test Substance to Test System
*	Inspect On	24-Sep-07 - 25-Sep-07 To Study Dir 25-Sep-07 To Mgmt 25-Sep-07
	Phase	Systems Inspection - Manipulation of Test System
*	Inspect On	24-Sep-07 - 25-Sep-07 To Study Dir 25-Sep-07 To Mgmt 25-Sep-07
	Phase	Systems Inspection - Observation of Test System/Data Collection and/or Analysis
*	Inspect On	24-Sep-07 - 25-Sep-07 To Study Dir 25-Sep-07 To Mgmt 25-Sep-07
	Phase	Systems Inspection - Test System Preparation

\*\* Inspection specific for this study

\* Systems Inspection

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

\_\_\_\_\_  
, B.A.  
QUALITY ASSURANCE

23 OCT 07  
\_\_\_\_\_  
DATE

# Final Report

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

**Study Number:** AC08DS.105020.BSV

**Test Article ID:** H9 MCB.1

**Sponsor:** WiCell Research Institute

**Authorized Representative:**

### CONCLUSION

One-half (0.5) µg of DNA (representing approximately  $7.5 \times 10^4$  cells) isolated from test article H9 MCB.1 was analyzed for the presence of human herpesvirus 6 (HHV-6) viral DNA by the polymerase chain reaction (PCR)<sup>1</sup> technique. The assay can detect 100 copies of HHV-6 (variants A and B) viral DNA in the presence of 0.5 µg of genomic DNA.

The results presented herein indicate that the test article tested negative for the presence of HHV-6 DNA.

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

**STUDY INFORMATION**

**Test Article:** H9 MCB.1 was received by BioReliance on 09/25/2007. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor. Retention of reserve sample from each batch of test article is the responsibility of the sponsor.

**Testing Facility:** BioReliance

**Schedule:**

**Study Initiation:** 09/26/2007

**Lab Initiation:** 10/02/2007

**Lab Completion:** 10/04/2007

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

**Study Director:** , 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:

<b>Negative Control:</b>		Genomic DNA from MRC5 human fetal lung fibroblasts Source: BioReliance
<b>Positive controls:</b>	HHV-6A:	Negative control DNA spiked with 100 copies of plasmid pU1102MOD. Plasmid pU1102MOD contains a 2.3 Kb region from the HHV-6A (strain U1102) genome. The 2.3 Kb region contains a 29 bp internal deletion to distinguish it from the wild type HHV-6A sequence. Source: BioReliance
	HHV-6B:	Negative control DNA spiked with 100 copies of plasmid pZ29MOD. Plasmid pZ29MOD contains a 2.3 Kb region from the HHV-6B (strain Z29) genome. The 2.3 Kb region contains a 29 bp internal deletion to distinguish it from the wild type HHV-6B sequence. Source: BioReliance
<b>No DNA Control:</b>		Nuclease free water Source: USB or other commercial supplier
<b>Spiked Controls:</b>		The spiked controls (amplification suitability controls) verify the absence of PCR inhibitors in the test article DNA.
	HHV-6A:	Test article spiked with 100 copies of plasmid pU1102MOD
	HHV-6B:	Test article spiked with 100 copies of plasmid pZ29MOD

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

## METHODS

### Sample Preparation

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

### DNA Amplification

PCR amplification was performed on 0.5µg of test article DNA and on the assay controls using primers HHV-6F and HHV-6R, specific for the immediate-early region of HHV-6, employing conditions optimized to achieve detection of 100 copies of viral DNA. Aliquots of the amplification products obtained from the test article and from the control samples were analyzed by agarose gel electrophoresis in the presence of ethidium bromide. Amplification products were visualized on a UV transilluminator and photographed using Polaroid 667 film.

## RESULTS

Test article DNA (0.5µg), representing approximately  $7.5 \times 10^4$  test article cells, was analyzed for the presence of HHV-6 viral DNA by PCR amplification and agarose gel electrophoresis in the presence of ethidium bromide. The results are shown in Figure 1. The following results provide evidence that the assay was valid and free of contamination:

- a. the No DNA control (NO) showed no amplification bands
- b. the Negative control (NC) showed no bands at 553, 524, 328 or 299 bp
- c. the positive control (PC-1) produced a band at 299 bp
- d. the positive control (PC-2) produced a band at 524 bp
- e. the test article showed no bands at 524 or 299 bp.

The test article spiked with 100 copies of pU1102MOD (TAS-1) produced a 299 bp band and the test article spiked with 100 copies of pZ29MOD (TAS-2) produced a 524 bp band, demonstrating that the test article did not inhibit the PCR reaction.

The test article (TA) produced no bands at 553 or 328 bp.

These results provide evidence that the test article H9 MCB.1 tested negative for the presence of HHV-6 (variants A and B) viral DNA.

**APPROVAL**

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

\_\_\_\_\_, Ph.D.  
Study Director

23 Oct 07  
\_\_\_\_\_  
Date

**FIGURE 1**

Detection of HHV-6 (variants A and B) viral sequences in test article H9 MCB.1 utilizing agarose gel electrophoresis in the presence of ethidium bromide

- M1: 100 bp DNA ladder  
NO: No DNA control  
TA: Test Article  
TAS-1: Test article spiked with 100 copies of pU1102MOD  
TAS-2: Test article spiked with 100 copies of pZ29MOD  
NC: Negative control (MRC5 genomic DNA)  
PC-1: Positive control (negative control DNA spiked with 100 copies of pU1102MOD)  
PC-2: Positive control (negative control DNA spiked with 100 copies of pZ29MOD)  
M2: Biomarker low DNA size marker

Arrows indicate specific amplification products.

# Quality Assurance Statement

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

**Study Number:** AC08DS.105020.BSV

**Study Director:** , Ph.D.

Quality Assurance performed the inspections listed below for this study. Verification of the study protocol was also performed and documented by QA. Procedures, documentation, equipment records, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. FDA Good Manufacturing Practices (21 CFR 210 and 211), the UK GLP Regulations, the Japanese GLP Standard, and the OECD Principles of Good Laboratory Practice and to assure that the study is conducted according to the protocol and relevant Standard Operating Procedures.

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

**	Inspect On Phase	23-Oct-07 - 23-Oct-07 To Study Dir 23-Oct-07 To Mgmt 23-Oct-07 Final Report and data audit
*	Inspect On Phase	24-Sep-07 - 25-Sep-07 To Study Dir 24-Sep-07 To Mgmt 24-Sep-07 Systems Inspection - Administration of Test Substance to Test System
*	Inspect On Phase	24-Sep-07 - 25-Sep-07 To Study Dir 25-Sep-07 To Mgmt 25-Sep-07 Systems Inspection - Manipulation of Test System
*	Inspect On Phase	24-Sep-07 - 25-Sep-07 To Study Dir 25-Sep-07 To Mgmt 25-Sep-07 Systems Inspection - Observation of Test System/Data Collection and/or Analysis
*	Inspect On Phase	24-Sep-07 - 25-Sep-07 To Study Dir 25-Sep-07 To Mgmt 25-Sep-07 Systems Inspection - Test System Preparation

\*\* Inspection specific for this study  
\* Systems Inspection

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

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B.A.

QUALITY ASSURANCE

23 Oct 07  
DATE

**Report Date:** March 13, 2009

**Case Details:**

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

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

**Investigator:** National Stem Cell Bank

**Specimen:** hES cells on MEFs

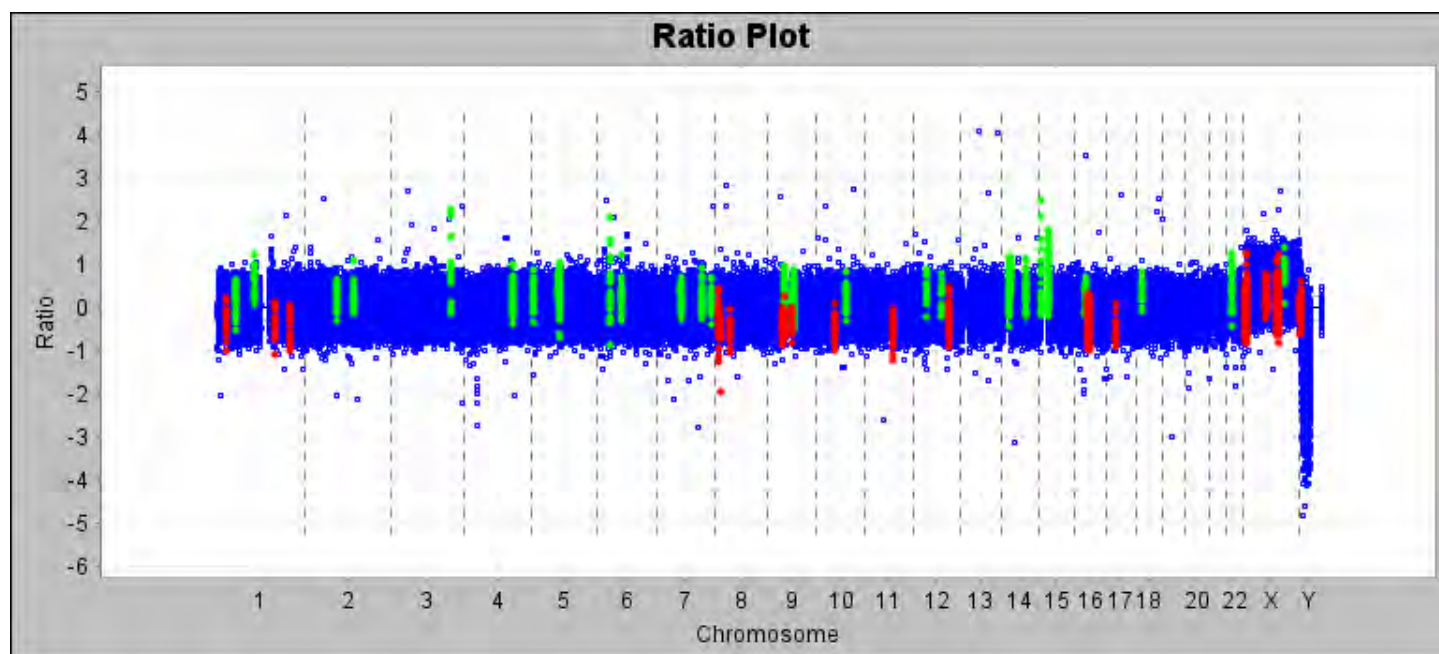
**Date of Sample:** 7/23/2007

**Reason for Testing:** NSCB MCB Testing

**GEO Accession #:** GSM347606

**Results:**

Results are given in the attached excel spreadsheet labeled „report.‘ There were 61 copy number gains and losses identified by modified circular binary segmentation<sup>1</sup>. 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<sup>2, 3</sup> or may be related to differential gene expression that is monitored in the NSCB characterization protocol and the ISCI study<sup>4</sup>. Changes associated with karyotype abnormalities and/or previously reported publications<sup>2, 5</sup> 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 <sup>3</sup>	2 of 2
Published Copy Number Changes <sup>5, 6</sup>	1 of 8
Reference DNA Copy Number Changes <sup>2</sup>	12 of 18
Select Differentially Expressed Genes	0 of 45

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

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

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

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**aCGH Specifications:**

- Platform: NimbleGen 385K array (HG18 CGH 385K WG Tiling v2 X1)
- Relative copy number is determined by competitive differential hybridization of labeled genomic DNA to the 385,000 oligonucleotide whole genome tiling array
- Probe length = 50-75mers spanning non-repetitive regions of the human genome
- Average probe spacing = 6270bp
- Analysis software: NimbleScan™, SignalMap™, OneClickCGH™, OneClickFusion™
- Analysis is based on examination of unaveraged and/or 60Kbp (10X) averaged data tracks as noted.
- Raw data is deposited in GEO, accession number shown above.
- Reported gains and losses are based on test to reference ratios within OneClickCGH™, size of aberration, 8-9 probes per gene, and coverage of at least one reported gene or overlap with the DGV.

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

**Literature Sources:**

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

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

---

**Results Transmitted by Fax / Email / Post**  
**Sent By:** \_\_\_\_\_

**Date:** \_\_\_\_\_  
**Sent To:** \_\_\_\_\_

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

1. Chip design: 2007-03-02\_WiCell\_HG18

2. Sample labeling:

Cy5: 6185-GEP 2ug (Barcode: LR00350);

Cy3: Sonicated H1 gDNA 4.5ug (Barcode: LD00123);

3. QC comments:

Box plots and distribution graphs are within acceptable range.

4. Expression of ES markers:

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

5. Expression of differentiation markers:

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



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Blood Services

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10/25/07

**SAMPLES: DNA from Cell Lines:**

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

Date received: 09/28/07

**INSTITUTION:** WiCell Research Institute

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

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

**DNA MOLECULAR RESULTS:**

	<u>Genotype</u>	<u>Predicted Phenotype</u>
ES03-MCB-1	NSCB 1590: <i>ABO</i> * <i>O</i> <sup>1</sup> / <i>O</i> <sup>1</sup> ; <i>RHD</i> ; <i>RHCE</i> * <i>Ce</i> / <i>Ce</i>	NSCB 1590: Group O; RhD+, C+, c-, E-, e+
H9-MCB-1	NSCB 6185: <i>ABO</i> * <i>A</i> <sup>1</sup> / <i>O</i> <sup>1</sup> ; <i>RHD</i> ; <i>RHCE</i> * <i>cE</i> / <i>ce</i>	NSCB 6185: Group A; RhD+, C-, c+, E+, e+
H1-MCB-1	NSCB 9592: <i>ABO</i> * <i>O</i> <sup>1</sup> / <i>O</i> <sup>1</sup> ; <i>RHD</i> ; <i>RHCE</i> * <i>Ce</i> / <i>Ce</i>	NSCB 9592: Group O; RhD+, C+, c-, E-, e+
HSF1-MCB-1	NSCB 5456: <i>ABO</i> * <i>O</i> <sup>1</sup> / <i>O</i> <sup>1</sup> ; <i>RHD</i> ; <i>RHCE</i> * <i>Ce</i> / <i>ce</i>	NSCB 5456: Group O; RhD+, C+, c+, E-, e+

**COMMENTS:** All samples were negative for the *RHD*-inactivating pseudogene and the *RHD-CE-D* hybrid which cause a D- phenotype and are common in African Black ethnic groups.

Scientific Director

Molecular Biologist

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

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## End of Production Testing Summary Report

End of Production (EOP) testing including karyotype, marker expression, STR, and CGH was performed at p+10. Summary results are presented here for the material described.

Product Name	WA09 Cell Bank Produced Under cGMP Conditions
Alias	H9
Lot Number	WIC-WA09-MB-001
Parent Material	WA09-DL-02
Depositor	WiCell
Banked By	Waisman Biomanufacturing
Culture Platform	Feeder Independent
	Medium: mTeSR1
	Matrix: Matrigel
Passage number at initiation of EOP Study	p27 These cells were cultured for 26 passages prior to freeze, 4 of them in mTeSR1/Matrigel. One number (+1) is added 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 Viald	13-March-2009

Passage	Karyotype by G-banding		Identity by STR		Flow Cytometry for ESC Marker Expression		Comparative Genome Hybridization	
	Test Specification	Result	Test Specification	Result	Test Specification	Result	Test Specification	Result
37	Normal Karyotype	Pass	Consistent with known profile	Pass	Report - no specification	See report	Not completed at this passage	
48	Normal Karyotype	Pass	Not completed at this passage		Not completed at this passage		Not completed at this passage	
58	Normal Karyotype	Pass	Not completed at this passage		Not completed at this passage		Not completed at this passage	
68	Normal Karyotype	Pass	Consistent with known profile	Pass	Report	See report	Report	See report
78	Normal Karyotype	Pass	Not completed at this passage		Not completed at this passage		Not completed at this passage	

Quality Assurance Approval	
	1/10/2014
<input checked="" type="checkbox"/> AMC	
AMC Quality Assurance Signed by: _____	

**Report Date:** October 25, 2009

### Case Details:

**Cell Line:** WIC-WA09-MB-001 (8125)

**Passage #:** 37

**Date Completed:** 10/25/2009

**Cell Line Gender:** Female

**Investigator:**

**Specimen:** hESC on Matrigel

**Date of Sample:** 10/14/2009

**Tests, Reason for:** end of production testing

**Results:** 46,XX

Completed by [REDACTED] MS, CLSp(CG), on 10/15/2009

Reviewed and interpreted by [REDACTED] PhD, FACMG, on 10/25/2009

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

Preliminary results were communicated by [REDACTED] on 10/15/09.



**Cell:** S01-04

**Slide:** B-18

**Slide Type:** Karyotyping

**# of Cells Counted:** 20

**# of Cells Karyotyped:** 4

**# of Cells Analyzed:** 8

**Band Level:** 475-550

**Results Transmitted by Fax / Email / Post**  
**Sent By:** \_\_\_\_\_  
**QC Review By:** \_\_\_\_\_

**Date:** \_\_\_\_\_  
**Sent To:** \_\_\_\_\_  
**Results Recorded:** \_\_\_\_\_

## Short Tandem Repeat Analysis\*

Sample Report: 8125-STR

UW HLA#: 61904

Sample Date: 10/22/09

Received Date: 10/22/09

Requestor: WiCell Research Institute

Test Date: 10/25/09

File Name: 091026

Report Date: 10/28/09

Sample Name: (label on tube) 8125-STR

Description: DNA Extracted by WiCell  
231.26 ug/mL; 260/280 = 1.89

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

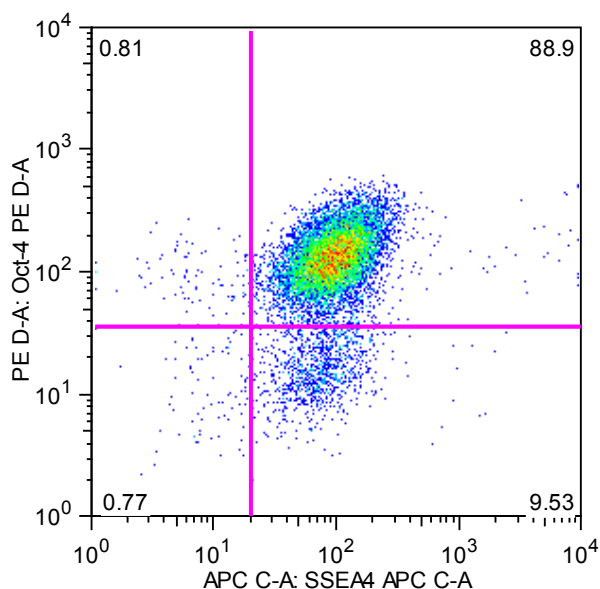
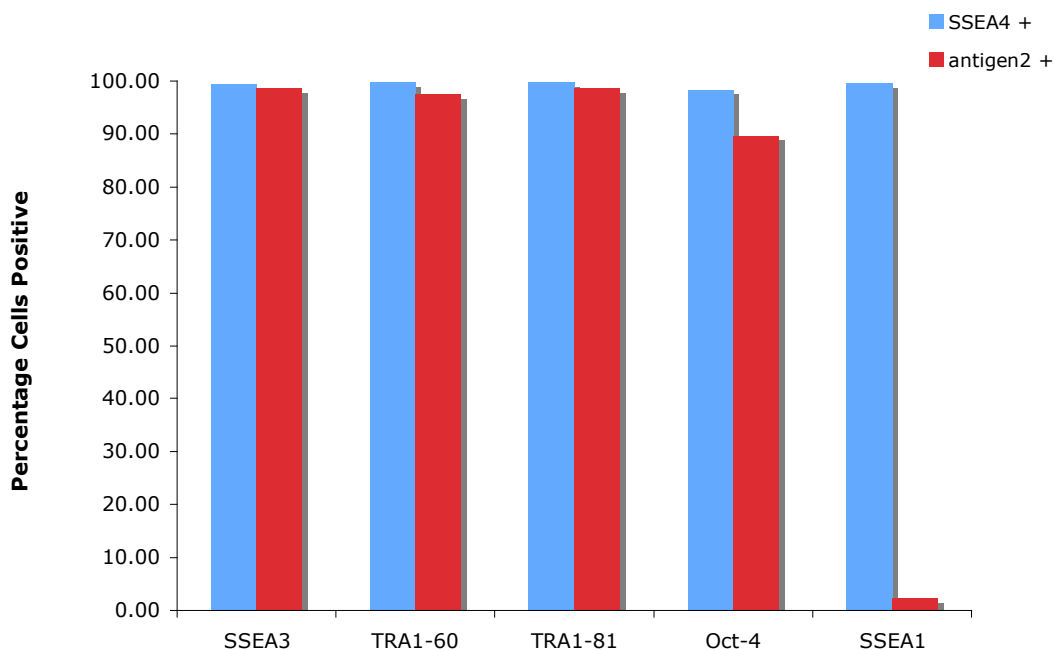
**Comments:** Based on the DNA 8125-STR dated and received on 10/22/09 from WI Cell, this sample (UW HLA# 61904) matches exactly the STR profile of the human stem cell line H9 comprising 12 allelic polymorphisms across the 8 STR loci analyzed. No STR polymorphisms other than those corresponding to the human H9 stem cell line were detected and the concentration of DNA required to achieve an acceptable STR genotype (signal/noise) was equivalent to that required for the standard procedure (~1 ng/amplification reaction) from human genomic DNA. These results suggest that the 8125-STR DNA sample submitted corresponds to the H9 stem cell line and it was not contaminated with any other human stem cells or a significant amount of mouse feeder layer cells. Sensitivity limits for detection of STR polymorphisms unique to either this or other human stem cell lines is ~5%.

HLA/Molecular Diagnostics Laboratory

HLA/Molecular Diagnostics Laboratory

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

antigen2:	SSEA4 - antigen2 +	SSEA4 + antigen2 +	SSEA4 + antigen2 -	SSEA4 - antigen2 -	ALL SSEA4 +	ALL antigen2 +
SSEA3	0.16	98.50	0.94	0.37	99.44	98.66
TRA1-60	0.11	97.40	2.40	0.06	99.80	97.51
TRA1-81	0.22	98.50	1.23	0.09	99.73	98.72
Oct-4	0.81	88.90	9.53	0.77	98.43	89.71
SSEA1	0.00	2.24	97.40	0.41	99.64	2.24



hESC  
8125\_Test.fcs  
Event Count: 11137

**Report Date:** December 07, 2009

### Case Details:

**Cell Line:** WIC-WA09-MB-001(8870)

**Passage #:** 48

**Date Completed:** 12/7/2009

**Cell Line Gender:** Female

**Investigator:**

**Specimen:** hESC on Matrigel

**Date of Sample:** 12/4/2009

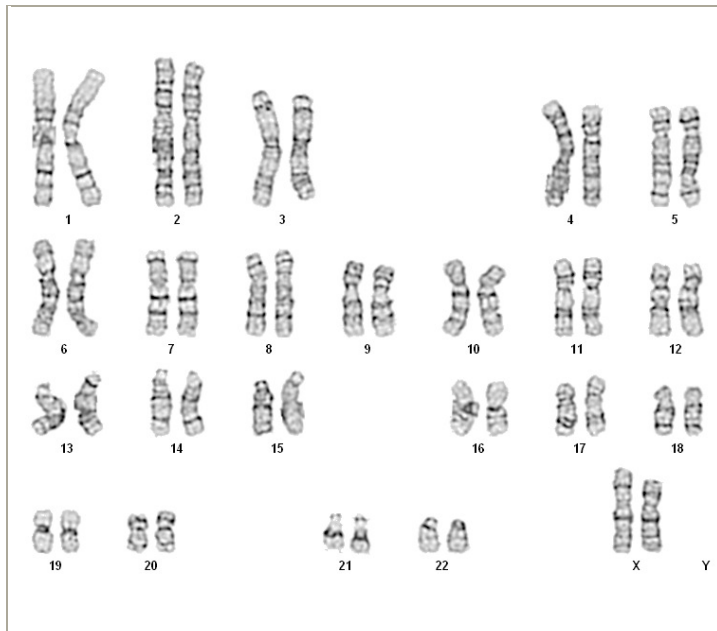
**Tests, Reason for:** GMP genetic stability

**Results:** 46,XX

**Completed by** [REDACTED] *CLSp(CG), on 12/7/2009*

**Reviewed and interpreted by** [REDACTED] *PhD, FACMG, on 12/7/2009*

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



**Cell:** S01-01

**Slide:** A5

**Slide Type:** Karyotyping

**# of Cells Counted:** 20

**# of Cells Karyotyped:** 4

**# of Cells Analyzed:** 8

**Band Level:** 400-450

**Results Transmitted by Fax / Email / Post**

**Sent By:** \_\_\_\_\_

**QC Review By:** \_\_\_\_\_

**Date:** \_\_\_\_\_

**Sent To:** \_\_\_\_\_

**Results Recorded:** \_\_\_\_\_

**Report Date:** February 15, 2010

### Case Details:

**Cell Line:** WIC-WA09-MB-001(5530)

**Passage #:** 58

**Date Completed:** 2/4/2010

**Cell Line Gender:** Female

**Investigator:** National Stem Cell Bank

**Specimen:** hESC on Matrigel

**Date of Sample:** 1/27/2010

**Tests, Reason for:** BMP genetic stability study- EOP testing

**Results:** 46,XX

Completed by [REDACTED] CG(ASCP), on 2/4/2010

Reviewed and interpreted by [REDACTED] PhD, FACMG, on 2/4/2010

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



**Cell:** S01-04

**Slide:** B-20

**Slide Type:** Karyotyping

**# of Cells Counted:** 20

**# of Cells Karyotyped:** 4

**# of Cells Analyzed:** 8

**Band Level:** 400-500

**Results Transmitted by Fax / Email / Post**

**Sent By:** \_\_\_\_\_

**QC Review By:** \_\_\_\_\_

**Date:** \_\_\_\_\_

**Sent To:** \_\_\_\_\_

**Results Recorded:** \_\_\_\_\_

**Report Date:** March 26, 2010

**Case Details:**

**Cell Line:** WIC-WA09-MB-001 (7855)

**Passage #:** 68

**Date Completed:** 3/26/2010

**Cell Line Gender:** Female

**Investigator:** cGMP

**Specimen:** hESC on Matrigel

**Date of Sample:** 3/22/2010

**Tests, Reason for:** End of production study

**Results:** 46,XX

Completed by [REDACTED], CG(ASCP), on 3/25/2010

Reviewed and interpreted by [REDACTED] PhD, FACMG, on 3/26/2010

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



**Cell:** S01-04

**Slide:** B-17

**Slide Type:** Karyotyping

**# of Cells Counted:** 20

**# of Cells Karyotyped:** 4

**# of Cells Analyzed:** 8

**Band Level:** 450-525

**Results Transmitted by Fax / Email / Post**

**Sent By:** \_\_\_\_\_

**QC Review By:** \_\_\_\_\_

**Date:** \_\_\_\_\_

**Sent To:** \_\_\_\_\_

**Results Recorded:** \_\_\_\_\_

## Short Tandem Repeat Analysis\*

**Sample Report: 7855-STR**

UW HLA#: 62816

Sample Date: 03/26/10

Received Date: 03/26/10

Requestor: WiCell Research Institute

Test Date: 03/30/10

File Name: 100331

Report Date: 04/06/10

**Sample Name:** (label on tube) **7855-STR****Description:** DNA Extracted by WiCell  
252.4 ug/mL; 260/280 = 1.97

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

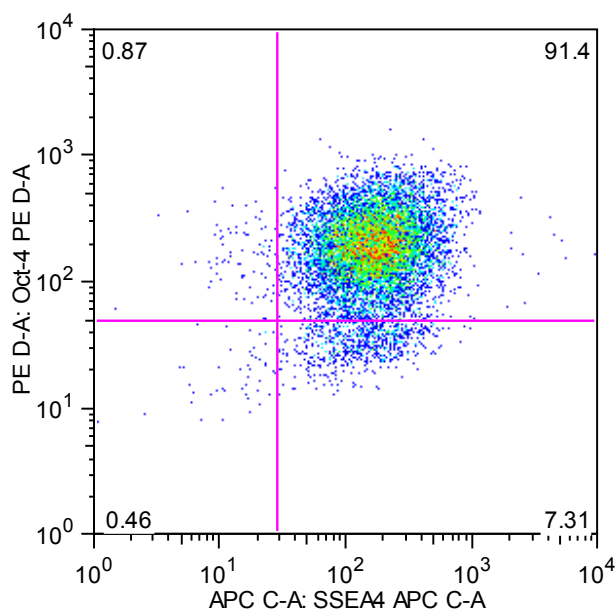
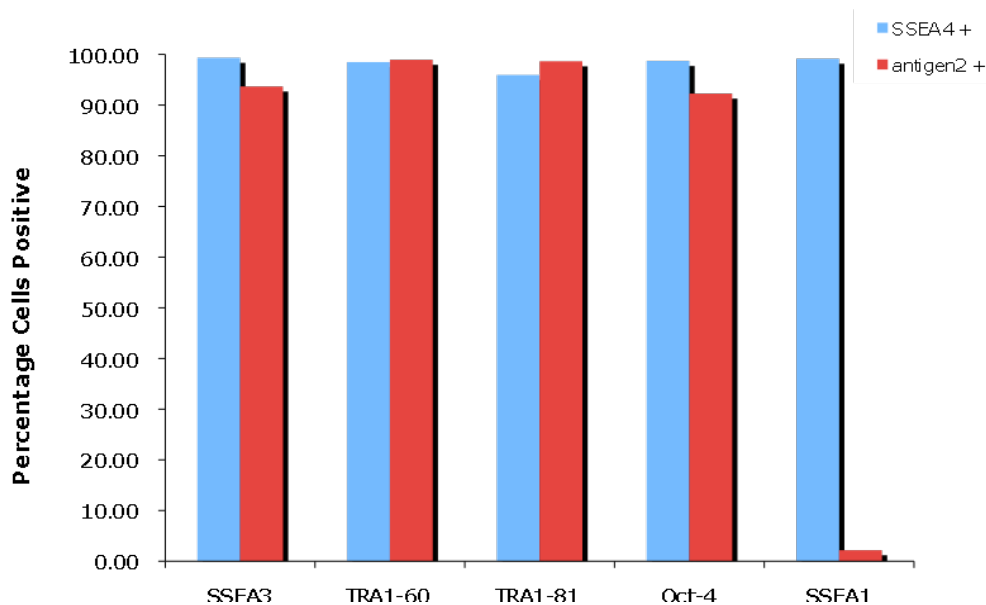
**Comments:** Based on the DNA 7855-STR dated and received on 03/26/10 from WI Cell, this sample (UW HLA# 62816) matches exactly the STR profile of the human stem cell line H9 comprising 12 allelic polymorphisms across the 8 STR loci analyzed. No STR polymorphisms other than those corresponding to the human H9 stem cell line were detected and the concentration of DNA required to achieve an acceptable STR genotype (signal/noise) was equivalent to that required for the standard procedure (~1 ng/amplification reaction) from human genomic DNA. These results suggest that the 7855-STR DNA sample submitted corresponds to the H9 stem cell line and it was not contaminated with any other human stem cells or a significant amount of mouse feeder layer cells. Sensitivity limits for detection of STR polymorphisms unique to either this or other human stem cell lines is estimated to be ~5%.

HLA/Molecular Diagnostics Laboratory

HLA/Molecular Diagnostics Laboratory

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

<u>antigen2:</u>	<u>SSEA4 - antigen2 +</u>	<u>SSEA4 + antigen2 +</u>	<u>SSEA4 + antigen2 -</u>	<u>SSEA4 - antigen2 -</u>	<u>ALL SSEA4 +</u>	<u>ALL antigen2 +</u>
SSEA3	0.07	93.60	5.73	0.60	99.33	93.67
TRA1-60	1.44	97.50	0.99	0.09	98.49	98.94
TRA1-81	3.74	94.90	1.04	0.28	95.94	98.64
Oct-4	0.87	91.40	7.31	0.46	98.71	92.27
SSEA1	0.02	2.15	97.00	0.82	99.15	2.17



CD29-  
,3f\_test.fcs  
Event Count: 10233

**Report Date:** 4/11/2010

**Case Details:**

**Cell Line:** WIC-WA09-MB-001-C-p68 (Female)

**Reference:** WA01-MCB-03-S.5-p26(3) (Male)

**Investigator:** cGMP

**Specimen:** hESC on Matrigel

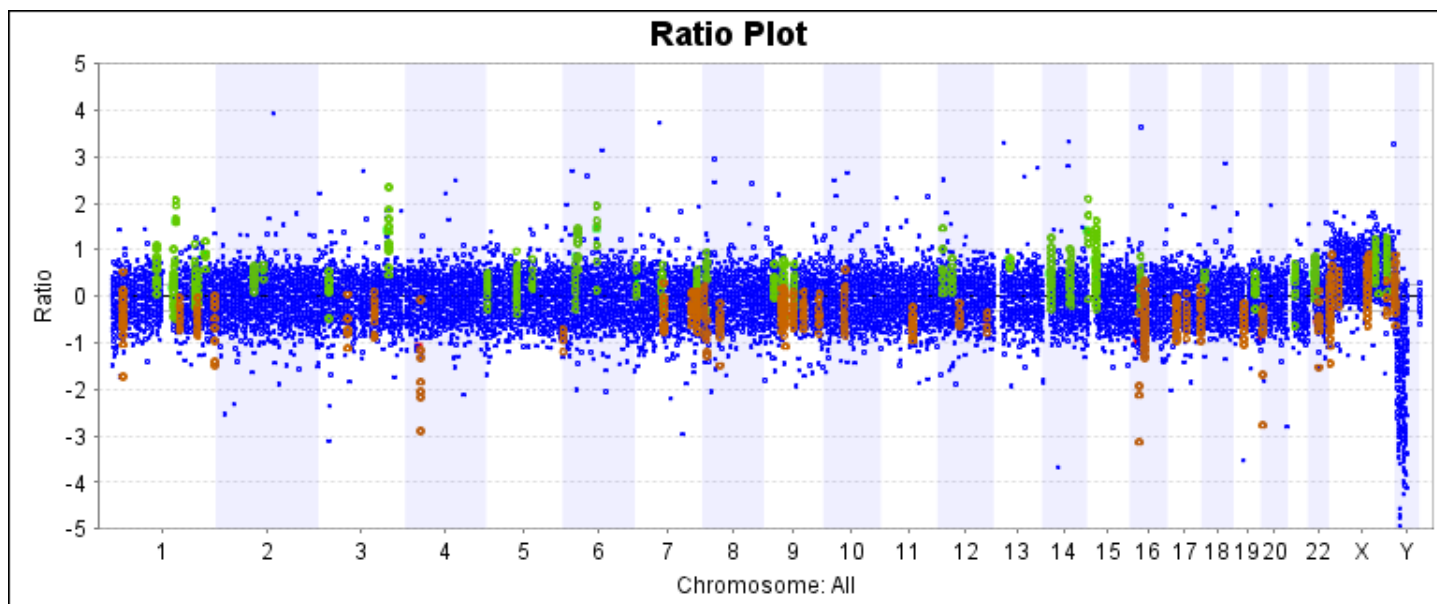
**Date of Sample:** 3/22/2010

**Reason for Testing:** End of production study

**GEO Accession #:** N/A

**aCGH Results:**

Results are given in the attached Excel spreadsheet labeled "report." There were 101 copy number gains and losses identified by modified circular binary segmentation<sup>1</sup>. 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 CGH Fusion™ 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<sup>2,3</sup> or may be related to differential gene expression that is monitored in the NSCB characterization protocol and the ISCI study<sup>4</sup>. Changes associated with karyotype abnormalities and/or previously reported publications<sup>2,5</sup> 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 <sup>3</sup>	2 of 2
Published Copy Number Changes <sup>5,6</sup>	2 of 8
Reference DNA Copy Number Changes <sup>2</sup>	41 of 43
Select Differentially Expressed Genes	0 of 88

These results are consistent with karyotype results [46,XX] as reported in 001657-032210 7855-KAR.

Test sample gain or loss is consistent with the opposite gender reference standard. Additional analysis of this data was performed using different ratio settings and different window averaging.

**Results Completed By:** [REDACTED] MS, CG(ASCP)<sup>CM</sup>  
**Reviewed and Interpreted By:** [REDACTED], PhD, FACMG

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**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<sup>TM</sup>, SignalMap<sup>TM</sup>, OneClickCGH (RBS v1.0)<sup>TM</sup>, OneClickFusion (RBS v1.0)<sup>TM</sup>
- 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<sup>TM</sup>, 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](mailto:cytogenetics@wicell.org) to request further testing.

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**Results Transmitted by Fax / Email / Post**  
**Sent By:** \_\_\_\_\_

**Date:** \_\_\_\_\_  
**Sent To:** \_\_\_\_\_

**Report Date:** May 31, 2010

### Case Details:

**Cell Line:** WIC-WA09-MB-001 (8716)

**Passage #:** 78

**Date Completed:** 5/31/2010

**Cell Line Gender:** Female

**Investigator:** WiCell Stem Cell Bank

**Specimen:** hESC on Matrigel

**Date of Sample:** 5/20/2010

**Tests, Reason for:** EOP Testing

**Results:** 46,XX

Completed by [REDACTED], CG(ASCP), on 5/28/2010

Reviewed and interpreted by [REDACTED] PhD, FACMG, on 5/31/2010

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



**Cell:** S01-03

**Slide:** B-11

**Slide Type:** Karyotyping

**# of Cells Counted:** 20

**# of Cells Karyotyped:** 4

**# of Cells Analyzed:** 8

**Band Level:** 500-575

Preliminary results were communicated by Seth Taapken on 5/28/10.

**Results Transmitted by Fax / Email / Post**

**Sent By:** \_\_\_\_\_

**QC Review By:** \_\_\_\_\_

**Date:** \_\_\_\_\_

**Sent To:** \_\_\_\_\_

**Results Recorded:** \_\_\_\_\_