Certificate of Analysis

Thaw and Culture Details

Cell Line Name	**WA09**	
WiCell Lot Number	**WIC-WA09-MB-004**	
Parent Material	WA09-WB0090	
Provider/Client	WiCell	
Banked By	Waisman Biomanufacturing	
Thaw and Culture Recommendations	WiCell recommends thawing 1 vial into 10 wells of 6 well plates using mTeSR™1 and Matrigel®.	
Protocol	WiCell Feeder Independent Pluripotent Stem Cell Protocol	
Culture Platform Prior to Freeze	Medium: mTeSR™1	Matrix: Matrigel®
Passage Number	p28 Cells were cultured for 27 passages prior to freeze. Plated cells at thaw should be labeled passage 28.	
Date Vialed	29-June-2023	
Vial Label	WiCell WA09 Master Cell Bank Lot #: WIC-WA09-MB-004 Vialed: 29 JUN 2023 Store in LN2 Passage 28	
Biosafety and Use Information	Appropriate biosafety precautions should be followed when working with these cells. The end user is responsible for ensuring that the cells are handled and stored in an appropriate manner. WiCell is not responsible for damages or injuries that may result from the use of these cells. Cells distributed by WiCell are intended for research purposes only and are not intended for use in humans.	
Compliance Statement	The lot listed above has been manufactured and tested in compliance with current Good Manufacturing Practices for phase 1 and clinical trial materials.	

Certificate of Analysis

Lot Release Testing Results

Test Description	Test Provider	Test Method	Test Specification	Result
Karyotype	WiCell	Karyotype by G Band (20 Metaphase spreads)	Normal with no recurrent nonclonals; No Clonal Abnormalities	Pass
Viability	Waisman Biomanufacturing	Post Thaw % Viability and Post Thaw Viable Cell Count	≥ 70% Viable ≥7x10^5 Viable Cells/mL	Pass
Identity by STR	WiCell	PowerPlex 16 HS System by Promega™	Matches STR Profile of Parental Lot	Pass
Sterility	Charles River Protocol #GP-V660 or equivalent	Protocol #GP-V660; Direct Inoculation Method Sterility Testing <USP71>	No Growth	Pass
Undifferentiated Status	WiCell	Flow Cytometry for ESC Marker Expression	>80% OCT4/SSEA4 Double Positive	Pass
Qualification of Test Article for the Detection of Agar Cultivable and Non-Cultivable Mycoplasma including Mycoplasmastasis Testing in accordance with USP/EP/PTC/JP	Charles River	Protocol #GP-V611.22	No Inhibition	Pass
In vitro Adventitious Agent Testing	Charles River	Protocol #GP-V602.22; Cell line testing on 3 cell lines	Not Detected	Pass
In vivo Adventitious Agent Testing	Charles River	Protocol #CRLPR-424; Inoculation of suckling and adult mice, guinea pigs, and embryonated chicken eggs	Not Detected	Pass

Certificate of Analysis

Lot Release Testing Results

Test Description	Test Provider	Test Method	Test Specification	Result
In vitro Test for Bovine Adventitious Viral Agents	Charles River	Protocol #GPV615.3; Modified 9CFR	Not Detected	Pass
Test for Porcine Adventitious Viral Agents	Charles River	Protocol #GPV727.2; Modified 9 CFR	Not Detected	Pass
Test for Murine Leukemia Viruses Co-Cultivation Assay with MiCI1 (S+L-) Focus Induction and Incubation	Charles River	Protocol #GPV600.1	Not Detected	Pass
Ultrastructural Evaluation of Cell Cultures for Viral Particles, with Characterization and Tabulation of Retrovirus-like Particles	Charles River	Protocol #PAI-1	Not Detected	Pass
PCR-Based Reverse Transcription Assay (PBRT)	Charles River	Protocol #GP-V692	Not Detected	Pass
Mouse Antibody Production (MAP) Test with LCMV Challenge	Charles River	Protocol #CRL-PR-31	Not Detected	Pass

Certificate of Analysis

Cell Line Testing Results

The following tests were performed on the cell line.
[1]This test was performed on a related lot. All other tests were performed on WA09-MCB-01, a master cell bank.
Please see the individual test reports for results of each test.

Test Description	Test Provider	Test Method	Test Specification	Result
HLA profile	UW Molecular Diagnostics Laboratory	AlleleSEQR Kits by Abbott	Consistent with known profile	Pass
Bacteriastasis & Fungistasis[1]	Charles River	Protocol #GP-V717	No Inhibition	Pass
HIV 1&2 by PCR	Covance	Not available	Negative	Pass
HTLV 1&2 by PCR	BioReliance	105013	Negative	Pass
HTLV 2 by PCR	Covance	Not available	Negative	Pass
HBV by PCR	Covance	Not available	Negative	Pass
HCV by PCR	Covance	Not available	Negative	Pass
CMV by PCR	BioReliance	105012	Negative	Pass
EBV by PCR	Covance	Not available	Negative	Pass
HHV-6 by PCR	BioReliance	105020	Negative	Pass
HHV-7 by PCR	Covance	Not available	Negative	Pass
HHV-8 by PCR	Covance	Not available	Negative	Pass
HP B19 by PCR	Covance	Not available	Negative	Pass

Certificate of Analysis

Cell Line Testing Results

The following tests were performed on the cell line.
[1]This test was performed on a related lot. All other tests were performed on WA09-MCB-01, a master cell bank.
Please see the individual test reports for results of each test.

Test Description	Test Provider	Test Method	Test Specification	Result
Comparative Genome Hybridization	WiCell	SOP-CH-308 SOP-CH-309 SOP-CH-310	Report - no specification	See report
Gene Expression Profile	UW Gene Expression Center	SOP-CH-321 SOP-CH-322 SOP-CH-333 SOP-CH-311	Report - no specification	See report
ABO and rH typing	American Red Cross	ABO/rH System	Report Blood type	Pass; A+

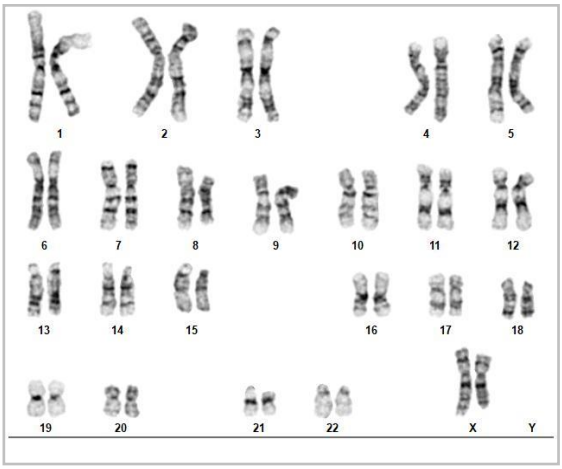
Approval

Approval Date	WiCell Quality Assurance Approval
05-JANUARY-2024	2/19/2024 X JKG JKG WiCell Quality Assurance Signed by: Gay, Jenna

Chromosome Analysis Report: 097827

Date Reported: Friday, July 14, 2023
Cell Line: WIC-WA09-MB-004
Submitted Passage #: 28
Date of Sample: 7/7/2023
Specimen: Human ESC
Results: 46,XX

Cell Line Sex: Female
Reason for Testing: LOT_RELEASE
Investigator: WiCell Stem Cell Bank, WiCell



Cell: 64
Slide: G02
Slide Type: Karyotype

Total Counted: 20
Total Analyzed: 8
Total Karyogrammed: 4
Band Resolution: 475 - 575

Interpretation:
This is a normal karyotype; no clonal abnormalities were detected at the stated band level of resolution.

Completed by: Kate Bird, CG(ASCP)
Reviewed and Interpreted by: Vanessa Horner, PhD, FACMG

For internal use only

Date:_____ **Sent By:**____ **Sent To:**_____ **QC Review By:** ____

Limitations: This assay allows for microscopic visualization of numerical and structural chromosome abnormalities. The size of structural abnormality that can be detected is >3-10Mb, dependent upon the G-band resolution obtained from this specimen. For the purposes of this report, band level is defined as the number of G-bands per haploid genome. It is documented here as "band level", i.e., the range of bands determined from the four karyograms in this assay. Detection of heterogeneity of clonal cell populations in this specimen (i.e.,mosaicism) is limited by the number of metaphase cells examined, documented here as "# of cells counted".

Short Tandem Repeat

Requestor: WiCell Stem Cell Bank, WiCell
Samples Received: 07Jul23, 29Jun23, 23Jun23
STR Amplification Date: 09Jul23

Sample Name	WIC-WA09-MB-004 p28	[redacted]	
WiCell CTR No.[1]	97827	97757	97709
FGA	26, 28	[redacted]	
TPOX	10, 11		
D8S1179	8, 14		
vWA	17, 17		
Amelogenin	X, X		
Penta_D	9, 13		
CSF1PO	11, 11		
D16S539	12, 13		
D7S820	9, 11		
D13S317	9, 9		
D5S818	11, 12		
Penta_E	11, 14		
D18S51	13, 13		
D21S11	30, 30		
TH01	9.3, 9.3		
D3S1358	13, 16		
Allelic Polymorphisms	24	28	26
Matches*	See Matches Comment	See Matches Comment	73192, 72296, 72297, 72643, 97621
Comments			

[redacted]
Identifying information has been redacted to protect donor confidentiality. If more information is required, please contact info@wicell.org

**Note: The STR profile of the following sample is a 100% match for the given sample/samples unless otherwise specified.*

[1] CTR No.: Characterization Test Request Number; also known as a laboratory accessioning number.

Short Tandem Repeat

Requestor: WiCell Stem Cell Bank, WiCell
Samples Received: 07Jul23, 29Jun23, 23Jun23
STR Amplification Date: 09Jul23

Assay Description: STR analysis is performed using the PowerPlex 16 HS System by Promega™. Results are reported as 13 CODIS STR markers, Amelogenin for gender determination and two low-stutter, highly discriminating pentanucleotide STR markers.

Results: The genotypic profiles comprise a range of 24 - 28 allelic polymorphisms across the 15 STR loci analyzed.

Interpretation: 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 suggests that the cells submitted correspond to the cell lines as named and were not contaminated with any other human cells or a significant amount of mouse feeder layer cells.

Sensitivity: Sensitivity limits for detection of STR polymorphisms unique to either this or other human cell lines is ~2-4%.

Matches:
Sample 97827 is a 100% match to 97437, 97371, 97171, 96184, 96183, 95823, 95822, 93654, 93595, 92908 and additional profiles. Additional matches can be provided upon request.

Sample 97757 is a 100% match to 96488, 96463, 94744, 94743, 93806, 86570, 86550, 82881, 82204, 82128 and additional profiles. Additional matches can be provided upon request.

7/10/2023 X Justin Hobson Tech #1 Characterization Signed by: Hobson, Justin	7/11/2023 X Amber Kuhn Tech #2 Characterization Signed by: Kuhn, Amber	7/11/2023 X Andy Arntz QA Review Quality Assurance Signed by: Arntz, Andy

charles river

REPORT OF ANALYSIS

Sterility Testing of Final Containers and Biological Products (Direct Method)

Client: University of Wisconsin
1500 Highland Avenue
Madison, WI 53705

Testing Facility: Charles River Laboratories
358 Technology Drive
Malvern, PA 19355

Protocol Number: GP-V660

Protocol Effective Date: 14AUG2023

On-Test Date: 18AUG2023

Results:

Sample Identification	Sample Number	Assay Result
WIC-WA09-MB-004	418948	No Growth

Comments:

None

Exception Document(s):

None

NOTE: Refer to the final page for the certification statement, final electronic signatures, and revision history.

Attach to F01-QCP-028 – 42380 JS 09-07-23 BA 10/11/2023

Sample Preparation: Sample was used as submitted by client and consisted of four vials each filled with approximately 1 mL of sample material. One approximately 0.5 mL aliquot per vial of sample was inoculated into individual vessels containing 100 mL of Soybean Casein Digest Medium (SCDM). This procedure was repeated for Fluid Thioglycollate Medium (FTM).

STERILITY TEST DATA SHEET # Vessels with Growth / Total # Vessels			
Culture	**Inoculum**	**# Vessels**	**Day 14**
SCDM / Sample	418948	4	0/4
SCDM / -Control	None	2	0/2
FTM / Sample	418948	4	0/4
FTM / -Control	None	2	0/2

Attach to F01-QCP-028 - 42380 JS 09-07-23 BA 10/24/2023

charles river

Certification:

This report summarizes testing performed at the Charles River Laboratories, Inc., PA-Biologics facility, which is operated in compliance with the applicable requirements of the U.S. Food and Drug Administration's Good Manufacturing Practice regulations as found in Title 21 CFR Parts 210 and 211. There were no deviations from the test method(s) that impacted the quality or integrity of the test result(s).

Study Lead Signer Name:	Danielle Tubiello	**Date:**	06 Sep 2023 12:03
Quality Assurance Signer Name:	Taina Rosario	**Date:**	06 Sep 2023 14:02

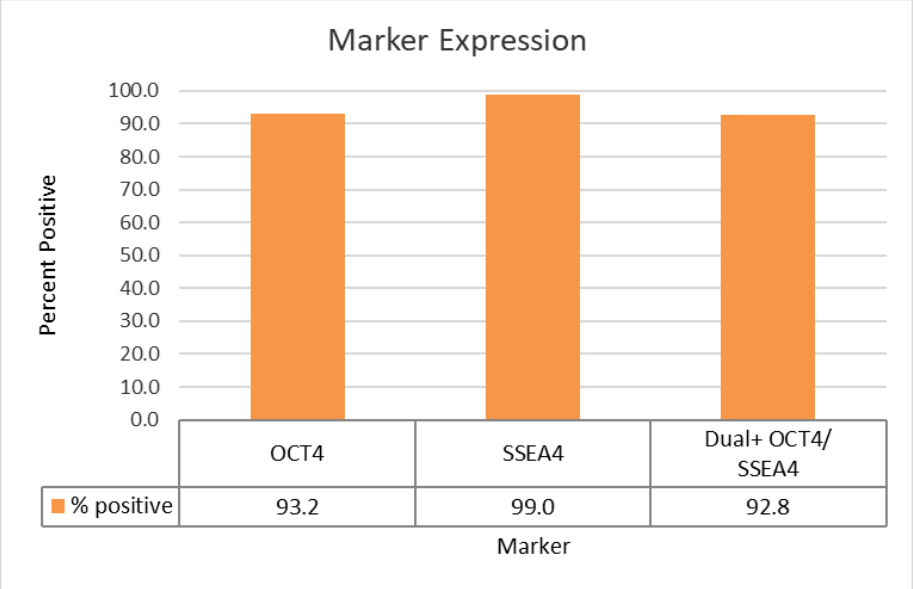
The above approval(s) were signed via the Labware LIMS electronic signature. Signers are aware that their electronic signature is the legally binding equivalent of their handwritten signature.

Attach to F01-QCP-028 - #2380 JS 09-07-23 BA 6/24/2023

Sample Information

Sample/Cell Line Name	WIC-WA09-MB-004
WiCell Sample ID/CTR Number	97930
Passage Number at Assessment	28
Assessment Date	17-Jul-23

Results



Interpretation

The cell line is 92.8% undifferentiated based on the dual expression of Oct4/SSEA4.

Approvals

7/17/2023	7/17/2023	7/19/2023
X Justin Hobson	X James Johnson	X Hunter Hefti
Tech #1 Characterization Signed by: Hobson, Justin	Tech #2 Characterization Signed by: Johnson, James	QA Review Quality Assurance Signed by: Hefti, Hunter

charles river

REPORT OF ANALYSIS

Testing for the Presence of Agar Cultivable and Non-cultivable Mycoplasmas in Accordance with the European Pharmacopoeia, the United States Pharmacopoeia and the Japanese Pharmacopoeia Guidelines including Mycoplasmastasis Testing

Client:	University of Wisconsin 1500 Highland Avenue Madison, WI 53705-2274
Testing Facility:	Charles River Laboratories 466 Devon Park Drive Wayne PA, 19087
Protocol Number:	GP-V611.22
Protocol Effective Date:	29DEC2021
On-Test Date:	05SEP2023

Results:

Sample Identification	Sample Number	Assay Result	
		Mycoplasma:	Mycoplasmastasis:
WIC-WA09-MB-004	422559	Not Detected	No Inhibition

Comments:

None

Exception Document(s):

None

NOTE: Refer to final page for the certification statement, final electronic signatures, and revision history.

RPT-231003-014
Version 1

Attach to F01-QCP-028 - 42388 JJ 24OCT23 BA 10/24/2023

Attach to F01-QCP-028 – 423887 JS 24OCT23 BA 10/24/2023

Sample Preparation: The sample was used as supplied by the client.

TABLE 1

MYCOPLASMA TEST RESULTS
AGAR CULTIVABLE
(Agar Plates Observed on Day 28; Broth Cultures Observed on Day 21)

Inoculum	Broth (0 HR)	Initial Plates (0 HR)	Passage 1 (3 Days)	Passage 2 (7 Days)	Passage 3 (14 Days)	Passage 4 (21 Days)
	MIC[a]	MIC	MIC	MIC	MIC	MIC
422559	–	–	–	–	–	–
Neg. Control	–	–	–	–	–	–
422559 + *M. pneumoniae*	+	+	–[b]	+	+	+
422559 + *M. orale*	+	+	+	+	–[b]	–[b]
M. pneumoniae	+	+	–[b]	+	+	+
M. orale	+	+	+	+	–[b]	–[b]

MIC = Microaerophilic Incubation
Medium A = Mycoplasma Broth Base, Noble Agar, Arginine, Glucose, Phenol Red, Horse Serum, Yeast Extract, MEM Vitamins, and Penicillin G

[a] No color change was observed in the sample or negative control broth vessels.

[b] Although no viable colonies could be isolated from the 0.2 mL aliquots inoculated onto these agar plates this does not affect the assay results as alternative plates inoculated with 0.2 mL aliquots removed from this vessel and streaked onto agar plates during alternative passages resulted in the presence of isolated colonies. Therefore, the assay is still valid as there was recovery of the organism from alternative passages.

TABLE 2 – Inhibition COLONY COUNTS FOR SPIKED SAMPLES DAY 14 (19SEP2023)		
Sample	Microaerophilic	
	CFU/0.2mL	Average CFU/0.2mL
422559 + *M. pneumoniae*	29, 26, 36, 33	31
422559 + *M. orale*	24, 20, 17, 26	22
M. pneumoniae	28, 25, 26, 23	26
M. orale	17, 24, 14, 25	20

TABLE 3 MYCOPLASMA TEST RESULTS AGAR NON-CULTIVABLE	
Inoculum	Indicator Cell Culture
	Cell Substrate
422559	–
Negative Control	–
M. orale	+
M. hyorhinis	+

Cell Substrate = Vero76
Stain Method = Hoechst

Attach to F01-QCP-028 – 42388 JS 24OCT23 BA 10/24/2023

charles river

Certification:
This report summarizes testing performed at the Charles River Laboratories, Inc., PA-Biologics facility, which is operated in compliance with the applicable requirements of the U.S. Food and Drug Administration's Good Manufacturing Practice regulations as found in Title 21 CFR Parts 210 and 211. There were no deviations from the test method(s) that impacted the quality or integrity of the test result(s).

Study Lead Signer Name:	Ashley Morgan	**Date:**	03 Oct 2023 13:04
Quality Assurance Signer Name:	Shanique Doyle	**Date:**	05 Oct 2023 16:59

The above approval(s) were signed via the Labware LIMS electronic signature. Signers are aware that their electronic signature is the legally binding equivalent of their handwritten signature.

Attach to F01-QCP-028 – 42388 JS 24OCT23 BA 10/24/2023

charles river

REPORT OF ANALYSIS

In Vitro Adventitious Virus Assay: MRC-5, Vero 76, and HeLa Cells (28 Days)

Client: University of Wisconsin
1500 Highland Avenue
Madison, WI 53705

Testing Facility: Charles River Laboratories
466 Devon Park Drive
Wayne, PA 19087

Protocol Number: GP-V602.22

Protocol Effective Date: 31JAN2020

On-Test Date: 07SEP2023

Results:

Sample Identification	Sample Number	Assay Result Adventitious Viral Agents
WIC-WA09-MB-004	422558	Not Detected

Comments:

None

Exception Document(s):

None

NOTE: Refer to the Final Page for the certification statement, final electronic signature, and revision history.

RPT-231009-078
Version 1

Attach to F01-QCP-028 -42388 JS 24OCT23 SH 10/24/2023

TISSUE CULTURE SAFETY TESTING (*In vitro*)				
Inoculum	**Test**	**MRC-5**	**Vero 76**	**HeLa**
Negative	**CPE**[a]	–[b]	–	–
422558	**CPE**	–	–	–
Positive[c]	**CPE**	+[d]	+	+
Negative	**HAD**[e]	–	–	–
422558	**HAD**	–	–	–
Positive[c]	**HAD**	+	+	+
Negative	**HA**[f]	–	–	–
422558	**HA**	–	–	–
Positive[c]	**HA**	–	+	–

[a] Observations for cytopathogenic effect

[b] Negative result obtained

[c] MRC-5, Vero 76, HeLa cells inoculated with Bovine Parainfluenza 3

[d] Positive result obtained

[e] Hemadsorption Test

[f] Hemagglutination Test

Attach to F01-QCP-028 -42388 JS 24OCT23 BA 10/24/2023

charles river

Certification:
This report summarizes testing performed at the Charles River Laboratories, Inc., PA-Biologics facility, which is operated in compliance with the applicable requirements of the U.S. Food and Drug Administration's Good Manufacturing Practice regulations as found in Title 21 CFR Parts 210 and 211. There were no deviations from the test method(s) that impacted the quality or integrity of the test result(s).

Study Lead Signer Name:	John Poling	**Date:**	09 Oct 2023 16:55
Quality Assurance Signer Name:	Gbemisola Olaoye	**Date:**	10 Oct 2023 16:08

The above approval(s) were signed via the Labware LIMS electronic signature. Signers are aware that their electronic signature is the legally binding equivalent of their handwritten signature.

Attach to F01-QCP-028 - 42388 JD 24 OCT 23 BA 10/24/2023

charles river

Final Report/Certificate of Analysis, Version I

Title: Detection Of Inapparent Viruses In A Biologic Sample per EU and US Regulations

Protocol Number: PR-424-5

Protocol Effective Date: 17AUG2023

1.0 Client

University of Wisconsin
1500 Highland Avenue
Madison, WI 53705-2274
USA

2.0 Test Article Identity: WIC-WA09-MB-004

CR ID #: 419161

3.0 Test Facility

Charles River Laboratories/In Vivo Biosafety
299 Ballardvale St.
Wilmington, MA 01887

4.0 Lab Initiation date: 05-Sep-2023

5.0 Lab Completion date: 16-Nov-2023

6.0 References

This method is designed to adhere to the following regulatory guidelines:

Guidance for Industry, February 2010, Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications

European Pharmacopoeia 2.6.16, Tests for Extraneous Agents in Viral Vaccines for Human Use

Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals as recommended by the US FDA Center for Biologics Evaluation and Research (1993)

International Conference on Harmonization, Guidance for Industry Q5A (R1): Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin (1999)

7.0 Procedure

All animals and eggs assigned to this protocol were obtained from Charles River production facilities on which routine health monitoring was performed. The Test Article was received from the Client and was inoculated via multiple routes into guinea pigs (Hartley, 350-450 grams), mice (PWM, CD-1, 15-20 grams, and suckling <24 hours old) and embryonated chicken eggs (10-11 days for allantoic fluid inoculation and 6-7 days for yolk sac inoculation); the hosts were monitored. After the completion of the prescribed observation period, survival percentages were determined. Guinea pigs were submitted for gross necropsy. Appropriate specimens from the suckling mice and embryonated eggs were processed, and hemagglutination testing was performed on allantoic and yolk sac fluids. Additionally, homogenates or pools from primary inoculation groups of suckling mice and embryonated chicken eggs were passaged into secondary inoculation groups of mice and eggs. The secondary inoculation groups were monitored. The survival percentage of each secondary inoculation group was determined at the completion of the observation period and hemagglutination testing was performed on the designated specimens.

Attach to F01-QCP-028 – 42381 JS 11-22-23 K 11/22/23

8.0 Test Article Preparation

A low-speed centrifugation (1500 x g for 10 minutes) was performed on the test article prior to inoculation for the guinea pigs' phase due to the sample aliquots being turbid with visible debris

9.0 Unexpected Results

None.

10.0 Deviations

Major deviation PR 1766343 was initiated as the results for hemagglutination (HA) testing of the yolk sac passage fluid samples were not documented in the study specific batch records. The samples were discarded after HA testing completed. This required that the yolk sac passage phase be repeated.

11.0 Results

11.1 Survival Summary

	Number of hosts inoculation	Number of hosts to survive 24 hours*	Number of hosts to survive observation period	% survival *
Guinea Pigs	6	6	6	100
Post Weaning Mice	20	20	19 **	95
Post Weaning Mice Subpassage	5	5	5	100
Suckling Mice	20	20	20	100
Suckling Mice Passage	20	20	20	100
Embryonated Eggs via Allantoic Fluid Route	10	10	10	100
Embryonated Eggs via Allantoic Fluid Route - Passage	10	10	10	100
Embryonated Eggs via Yolk Sac Route	10	10	9	90
Embryonated Eggs via Yolk Sac Route – Passage ***	10	9	9	100
Embryonated Eggs via Yolk Sac Route - Passage Repeat	10	10	9	90

*Death within the first day of inoculation is attributed to mechanical injury and not included in the survival summary.

**As per protocol, carcass was submitted for necropsy and tissue collection; necropsy examination showed no evidence of viral infection: collected tissues processed and inoculated into additional mice (subpassage). See Table 11.1 for Survival Summary.

***See Section 10.0 Deviations.

Hemagglutination Assay

Hemagglutination Assay (HA)	No agglutination observed - Negative

Attach to F01-QCP-028 – 4238/ JS 11-22-23 JK 11/22/23

11.2 Conclusion:

In all cases, the survival rates for test article-inoculated animals and embryonated eggs were 80% or higher. There were no gross abnormalities indicative of viral infection in the guinea pigs that were submitted for necropsy. Animals showed no signs of viral infection or transmissible disease. Embryos in eggs inoculated with the test article appeared healthy at the conclusion of the observation period and hemagglutinating activity was not detected in fluid from test article-inoculated eggs. Therefore, infectious virus was not detected in the test article, **WIC-WA09-MB-004**.

Attach to F01-QCP-028 - 42381 JS 11-22-23 & 11/22/23

charles river

Certification:

This report summarizes testing performed at the Charles River Laboratories, Inc. , In Vivo Biosafety facility in Wilmington, MA which is operated in compliance with the applicable requirements of the U .S. Food and Drug Administration's Good Manufacturing Practice regulations as found in Title 21 CFR Parts 210 and 211. There were no deviations from the test method(s) that impacted the quality or integrity of the test result(s).

Study Lead Signer Name:	Eric Scola	**Date:**	22 Nov 2023 08:51
Quality Assurance Signer Name:	Devayani Patil	**Date:**	22 Nov 2023 11:21

The above approval(s) were signed via the Labware LIMS electronic signature. Signers are aware that their electronic signature is the legally binding equivalent of their handwritten signature.

Attach to F01-QCP-028 – 42381 JS 11-22-23 SI 11-22-23

charles river

REPORT OF ANALYSIS

In vitro Test for Bovine Adventitious Viral Agents (Modified 9 CFR) in Products Other than Bovine Serum

Client: University of Wisconsin
1500 Highland Avenue
Madison, WI 53705-2274

Testing Facility: Charles River Laboratories
466 Devon Park Drive
Wayne, PA 19087

Protocol Number: GP-V615.3

Protocol Effective Date: 04APR2022

On-Test Date: 06SEP2023

Results:

Sample Identification	Sample Number	Assay Result Bovine Adventitious Viral Agents
WIC-WA09-MB-004	422556	Not Detected

Comments:

None

Exception Document(s):

None

NOTE: Refer to the final page for the certification statement, final electronic signatures, and revision history.

Attach to F01-QCP-028 – 42388 JS 24OCT23 BAJ 10/24/2023

TABLE 1 OBSERVATIONS FOR IMMUNOFLUORESCENCE IN INDICATOR CELLS ON DAYS 7, 14, AND 21	
Inoculum	Indicator Cell Line
	Bovine Turbinate
422556	-[a]
Negative Control	-
Bovine Adenovirus	+[b]
Bovine Parvovirus	+
Bovine Respiratory Syncytial Virus	+
Bovine Viral Diarrhea Virus, non-cytopathic NY-1 Strain	+
Rabies Virus	+
Reovirus	+
Inoculum	**Vero 76**
422556	-
Negative Control	-
Bovine Respiratory Syncytial Virus	+
Bluetongue Virus	+
Rabies Virus	+
Reovirus	+

[a] Negative result obtained

[b] Positive result obtained

Attach to F01-QCP-028 – Y2388 JS 24OCT23 BA 10/24/2023

TABLE 2 OBSERVATIONS FOR HEMADSORPTION		
Inoculum	**Indicator Cells**	
	BT	**Vero**
422556	-[a]	-
Positive Control PI3[b] (Day 0)	+[c]	+
Negative Control	-	-

[a] Negative result obtained
[b] Bovine Parainfluenza Virus 3
[c] Positive result obtained

TABLE 3 OBSERVATIONS FOR CYTOPATHOLOGY		
Inoculum	**Indicator Cells**	
	BT	**Vero**
422556	-[a]	-
Positive Controls:		
Parainfluenza 3	+[b]	+
Bovine Viral Diarrhea Virus, non-cytopathic NY-1 Strain	-	NT[c]
Bovine Parvovirus	+	NT
Bovine Adenovirus	+	NT
Bovine Respiratory Syncytial Virus	+	-
Bluetongue Virus	NT	-
Reovirus	-	-
Rabies Virus	-	-
Negative Controls	-	-

[a] Negative result obtained
[b] Positive result obtained
[c] Not tested

Attach to F01-QCP-028 – 42388 JS 24OCT23 PSA 10/24/2023

charles river

Certification:
This report summarizes testing performed at the Charles River Laboratories, Inc., PA-Biologics facility, which is operated in compliance with the applicable requirements of the U.S. Food and Drug Administration's Good Manufacturing Practice regulations as found in Title 21 CFR Parts 210 and 211. There were no deviations from the test method(s) that impacted the quality or integrity of the test result(s).

Study Lead Signer Name:	Lindsey Allebach	**Date:**	05 Oct 2023 15:29
Quality Assurance Signer Name:	Philip Bell	**Date:**	10 Oct 2023 13:31

The above approval(s) were signed via the Labware LIMS electronic signature. Signers are aware that their electronic signature is the legally binding equivalent of their handwritten signature.

Attach to F01-QCP-028 – 42388 JS 24OCT23 PBA 10/24/2023

charles river

REPORT OF ANALYSIS

Test for Porcine Adventitious Viral Agents (Modified 9 CFR) in T-75cm^2 Flasks

Client:	University of Wisconsin 1500 Highland Avenue Madison, WI 53705
Testing Facility:	Charles River Laboratories 466 Devon Park Drive Wayne, PA 19087
Protocol Number:	GP-V727.2
Protocol Effective Date:	19JUL2021
On-Test Date:	06SEP2023

Results:

Sample Identification	Sample Number	Assay Result Porcine Adventitious Viral Agents
WIC-WA09-MB-004	422557	Not Detected

Comments:

None

Exception Document(s):

None

NOTE: Refer to the final page for the certification statement, final electronic signatures, and revision history.

Attach to F01-QCP-028 – 42388 JS 10 OCT 23 BA 10/24/2023

Sample Preparation: Prior to inoculation, sample was thawed and centrifuged to ensure that no cells were present.

TABLE 1
OBSERVATIONS FOR IMMUNOFLUORESCENCE IN INDICATOR CELLS ON DAYS 7, 14, AND 21

Inoculum	Indicator Cell Line
	Bovine Turbinate
422557	-[a]
Negative Control	-
Bovine Viral Diarrhea Virus	+[b]
Pseudorabies Virus	+
Rabies Virus	+
Inoculum	**Vero 76**
422557	-
Negative Control	-
Reovirus	+
Pseudorabies Virus	+
Rabies Virus	+
Inoculum	**MA104**
422557	-
Negative Control	-
Porcine Reproductive and Respiratory Syndrome Virus	+
Inoculum	**St Neb**
422557	-
Negative Control	-
Porcine Parvovirus	+
Transmissible Gastroenteritis Virus	+
Inoculum	**PK-15**
422557	-
Negative Control	-
Porcine Adenovirus	+
Inoculum	**HCT-8**
422557	-
Negative Control	-
Porcine Hemagglutinating Encephalomyelitis Virus	+

[a] Negative result obtained
[b] Positive result obtained

Attach to F01-QCP-028 -47388 JS 10 OCT 23 BD 10/24/2023

TABLE 2 OBSERVATIONS FOR HEMADSORPTION		
Inoculum	Indicator Cell Line	
	Bovine Turbinate	Vero 76
422557	-[a]	-
Positive Control PI3[b] Day 0	+[c]	+
Positive Control PI3 Day 14	+	+
Negative Control	-	-

[a] Negative result obtained
[b] Bovine Parainfluenza Virus 3
[c] Positive result obtained

TABLE 3 OBSERVATIONS FOR CYTOPATHOGENIC EFFECT IN INDICATOR CELLS FOR SAMPLE AND NEGATIVE CONTROLS		
Indicator Cell Line	Inoculum	
	422557	Negative Control
Bovine Turbinate	-[a]	-
Vero 76	-	-
MA104	-	-
ST Neb	-	-
PK-15	-	-
HCT-8	-	-

[a] Negative result obtained

Attach to F01-QCP-028 – 42388 JS 10 OCT 23 BA 10/24/2023

TABLE 4 OBSERVATIONS FOR CYTOPATHOGENIC EFFECT IN INDICATOR CELLS FOR POSITIVE CONTROLS		
Indicator Cell Line	**Virus**	**Result**
Bovine Turbinate	**Bovine Viral Diarrhea Virus**	-[a]
	Pseudorabies Virus	+[b]
	Rabies Virus	-
	Bovine Parainfluenza 3	+
Vero 76	**Bovine Parainfluenza 3**	+
	Encephalomyocarditis Virus	+
	Reovirus	+
	Pseudorabies Virus	+
	Rabies Virus	-
ST Neb	**Encephalomyocarditis Virus**	+
	Porcine Parvovirus	+
	Transmissible Gastroenteritis Virus	+
MA104	**Porcine Reproductive and Respiratory Syndrome Virus**	+
PK-15	**Porcine Adenovirus**	+
HCT-8	**Porcine Hemagglutinating Encephalomyelitis Virus**	-

[a] Negative result obtained
[b] Positive result obtained

Attach to F01-QCP-028 – 42388 55 10 OCT 23 BA 10/24/2023

charles river

Certification:

This report summarizes testing performed at the Charles River Laboratories, Inc., PA-Biologics facility, which is operated in compliance with the applicable requirements of the U.S. Food and Drug Administration's Good Manufacturing Practice regulations as found in Title 21 CFR Parts 210 and 211. There were no deviations from the test method(s) that impacted the quality or integrity of the test result(s).

Study Lead Signer Name:	Timothy Starosta	**Date:**	09 Oct 2023 14:04
Quality Assurance Signer Name:	Pajtim Dumani	**Date:**	10 Oct 2023 10:48

The above approval(s) were signed via the Labware LIMS electronic signature. Signers are aware that their electronic signature is the legally binding equivalent of their handwritten signature.

Attach to F01-QCP-028 -42388 JS 10 OCT 23 BA 10/24/2023

charles river

REPORT OF ANALYSIS

Extended, Focus Induction Assay for Murine Leukemia Virus with an MiCl1 (S+L-) Detection System

Client:	University of Wisconsin 1500 Highland Avenue Madison, WI 53705-2274
Testing Facility:	Charles River Laboratories 466 Devon Park Drive Wayne, PA 19087
Protocol Number:	GP-V600.1
Protocol Effective Date:	30AUG2021
On-Test Date:	08SEP2023

Results:

Sample Identification	Sample Number	Assay Result
WIC-WA09-MB-004	422555	Not Detected

Comments:

None

Exception Document(s):

None

NOTE: Refer to the final page for the certification statement, final electronic signatures, and revision history.

Attach to F01-QCP-028 – 423885 06OCT2023 BA 10/24/2023

S+L- FOCUS ASSAY	
Sample (0.1 mL/well)	**Mean FFU[a]/mL**
DIRECT ASSAY	
Negative Control	ND[b]
422555	ND
Positive Control[c]	7.46×10^{5}[d]
EXTENDED ASSAY	
Negative Control	ND
Extended Negative Control	ND
422555	ND
Positive Control[c]	7.88×10^{5}[d]
Extended Positive Control[e]	1.34×10^{7}[d]

[a] Focus Forming Units
[b] Not Detected
[c] Established range of virus stock titer: $1.61 \times 10^{5} - 1.60 \times 10^{6}$ FFU/mL
[d] Calculated Average Titer
[e] Average titer of the virus stock: 5.07×10^{5} FFU/mL
Virus control in Extended Assay was inoculated with 1.0 mL of 1:1.54×10^{4} virus dilution (approximately 33 FFU/mL)

Attach to F01-QCP-028 - 42388 JS 06 OCT 2023 10/24/2023

charles river

Certification:
This report summarizes testing performed at the Charles River Laboratories, Inc., PA-Biologics facility, which is operated in compliance with the applicable requirements of the U.S. Food and Drug Administration's Good Manufacturing Practice regulations as found in Title 21 CFR Parts 210 and 211. There were no deviations from the test method(s) that impacted the quality or integrity of the test result(s).

Study Lead Signer Name:	Lindsey Allebach	**Date:**	04 Oct 2023 13:53
Quality Assurance Signer Name:	Pajtim Dumani	**Date:**	06 Oct 2023 13:54

The above approval(s) were signed via the Labware LIMS electronic signature. Signers are aware that their electronic signature is the legally binding equivalent of their handwritten signature.

Attach to F01-QCP-028 -42388 JS 06 OCT 2023 DA 10/24/2023

charles river

FINAL REPORT

Testing Facility Study No.:	EM-23.486
PA Biologics LIMS Study Number:	SDY-14787
LIMS Testing Aliquot Number:	423186
Client Sample ID:	WIC-WA09-MB-004
Protocol No.:	1
Protocol Title:	Ultrastructural Evaluation of Cell Cultures for Viral Particles, with Characterization and Tabulation of Retrovirus-like Particles
Providing Laboratory:	Charles River Laboratories, Inc., PA Biologics 466 Devon Park Dr. Wayne, PA 19087 USA
Study Sponsor:	University of Wisconsin

Prepared By:

Charles River Laboratories, Inc.

4025 Stirrup Creek Dr., Suite 150

Durham, NC 27703 USA

Attach to F01-QCP-028 - 42559 JS 16NOV23 BA 11/16/2023

QUALITY ASSURANCE STATEMENT

Study Number: EM-23.486

This study has been audited by Quality Assurance in accordance with the applicable Good Laboratory Practice regulations to the extent possible (a Test Article/Test System is not included, and a Study Director was not assigned). Reports were submitted in accordance with SOPs as follows:

		Dates Findings Submitted to:	
Date(s) of Audit	**Phase(s) Audited**	**Project Scientist**	**Testing Facility Management**
14Nov2023	Data Review - Histology/EM	15Nov2023	15Nov2023
14Nov2023	Final Report	15Nov2023	15Nov2023

In addition to the abovementioned audits, -process based- and/or routine facility audits were also conducted during the course of this study. Audit findings, if any, specific to this study were reported by Quality Assurance to the Project Scientist and Testing Facility Management and listed as a Phase Audit on this Quality Assurance Statement.

The study has been reviewed to assure that it accurately describes the materials and methods, and that the reported results accurately reflect the raw data.

All electronic signatures appear at the end of the document upon finalization.

I. PURPOSE/JUSTIFICATION

The objectives of this study were:

A. To evaluate cell cultures for all particles with virus-like morphology.

B. To characterize and to determine numbers and locations of A-type, B-type, C-type, D-type, and R-type retrovirus-like particles in cell cultures.

II. TESTING FACILITY

Charles River Laboratories, Inc.
4025 Stirrup Creek Dr., Suite 150
Durham, NC 27703 USA

III. PROVIDING LABORATORY

Charles River Laboratories, Inc.,
PA Biologics
358 Technology Dr.
Malvern, PA 19355 USA

Or

Charles River Laboratories, Inc.,
PA Biologics
466 Devon Park Dr.
Wayne, PA 19087 USA

IV. RESPONSIBLE PERSONNEL

Gara L. Creamer	Project Scientist
Bradley Savard	Electron Microscopist
Sirena Hudgins	EM Laboratory Manager

V. TEST SCHEDULE

The study was initiated on 06-Oct-2023.

VI. TEST SAMPLE AND NEGATIVE CONTROL

One vial, containing a pelleted cell culture, was shipped via overnight carrier from Charles River Laboratories, Inc., PA Biologics and received on 26-Sep-2023. According to the Electron Microscopy Sample Submission Form from Charles River Laboratories, Inc., PA Biologics, the sample was fixed in 2% glutaraldehyde in 0.1 M sodium cacodylate buffer. Specimens in glutaraldehyde are stable up to a year[1]. The container was labeled to identify the test sample.

[1] Hayat, M. 1981. Fixation for Electron Microscopy. New York: Academic Press. p.147, 207

VII. EXPERIMENTAL PROCEDURES

A. Sample Processing

The cell pellet was processed for transmission electron microscopy (TEM) and embedded in Spurr's epoxy resin following the Testing Facility's current Standard Operating Procedures. A sample consisting of water and agar from the same containers of water and agar used in processing the test sample was processed concurrently as a negative control.

Thin sections of test sample were cut at 70-90 nm, mounted on copper grids, stained with methanolic uranyl acetate and Reynold's lead citrate, and examined by TEM.

B. Transmission Electron Microscopy

Two hundred cells were evaluated for the presence of any type of particle with virus-like morphology.

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

Each of the 200 cells was evaluated for particles with A-, B-, C-, D- and R-type retrovirus-like morphology.

Digital images were taken to document the typical appearance of the sample. The images and a digital image inventory are included with this electron microscopy report.

VIII. EVALUATION OF THE TEST RESULTS

A. Transmission Electron Microscopy

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

B. Digital Image Inventory

Image Numbers: 23.486-1_001, 23.486-1_002

IX. STATISTICAL EVALUATION

No statistical analysis was performed on the study data.

X. COMPUTERIZED SYSTEMS

Critical computerized systems used in the study by the Testing Facility are listed below. All computerized systems used in the conduct of this study have been validated; when a particular system has not satisfied all requirements, appropriate administrative and procedural controls were implemented to assure the quality and integrity of data.

System Name	Description of Data Collected and/or Analyzed
AMT XR16M Active Vu Digital Camera	Collection of TEM digital images
M-Files	Reporting and collection of 21 CFR Part 11 compliant signatures
DocuSign™	Collection of 21 CFR Part 11 compliant signatures

XI. STORAGE AND ARCHIVING

All study specific raw data, blocks, grids, documentation, protocol, digital images, and electronic data from this study were submitted for short-term archiving at the Testing Facility no later than the date of final report issue, then transferred to a long-term archive within 60 days.

Electronic data generated by the Testing Facility were archived as noted above, except for reporting files stored on M-Files® and deviations which were archived at the Charles River Laboratories facility location in Wilmington, MA.

XII. COMPLIANCE STATEMENT AND REPORT APPROVAL

The study was performed in accordance with the U.S. Department of Health and Human Services, Food and Drug Administration (FDA), United States Code of Federal Regulations, Title 21, Part 58: Good Laboratory Practice (GLP) for Nonclinical Laboratory Studies and as accepted by Regulatory Authorities throughout the European Union (OECD Principles of Good Laboratory Practice), Japan (MHLW), and other countries that are signatories to the OECD Mutual Acceptance of Data Agreement.

This study conforms with the above Good Laboratory Practice regulations to the extent possible for Transmission Electron Microscopy work.

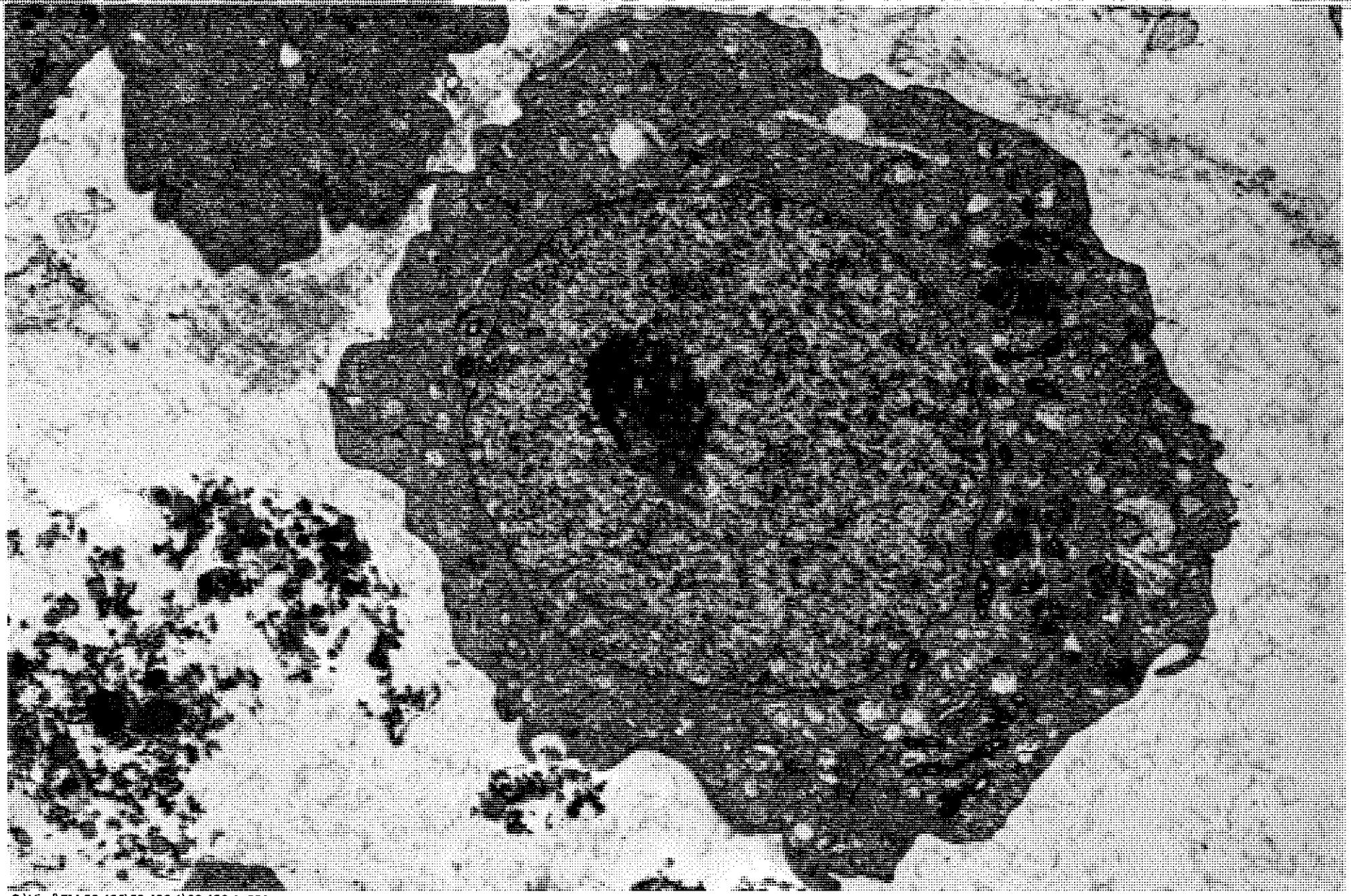
A Study Director was not assigned; therefore, the Project Scientist is responsible for the technical conduct of the electron microscopy work performed as well as for the documentation and reporting of deviations and results.

This study was conducted in accordance with the procedures described herein. All deviations authorized/acknowledged by the Project Scientist are documented in the Study Records. The report represents an accurate and complete record of the results obtained. There were no deviations from the above regulations that affected the overall integrity of the study or the interpretation of the study results and conclusions.

Approval:

Gara L. Creamer, B.S.
Project Scientist

All electronic signatures appear at the end of the document upon finalization.



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Viral
SCO-0143
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12:08:31 10-Nov-2023
TEM Mode: Imaging
Microscopist: Savard, Bradley
Camera: BIOSPR16, Exposure(ms): 3200 Gain: 1, Bin: 1
Gamma: 0.90, No Sharpening, Normal Contrast

2 µm
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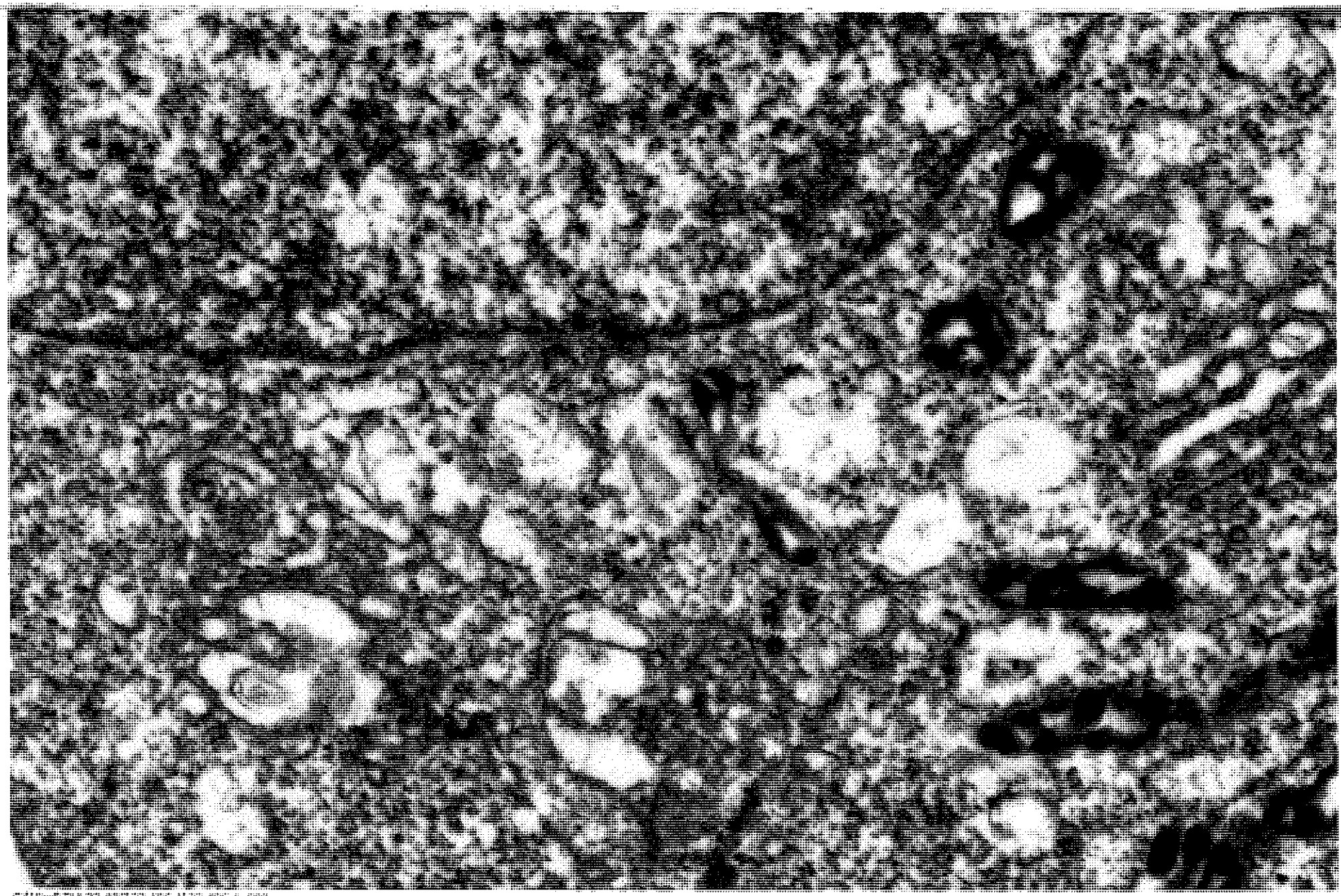
Direct Mag: 2000x
Charles River Labs, Inc.

Attach to F01-QCP-028 – 42559 JS 16 Nov 23 BA 11/16/2023



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12:15:44 10-Nov-2023
TEM Mode: Imaging
Microscopist: Savard, Bradley
Camera: BIOSPR16, Exposure(ms): 3200 Gain: 1, Bin: 1
Gamma: 1.00, No Sharpening, Normal Contrast

500 nm
HV=80.0kV
Direct Mag: 10000x
Charles River Labs, Inc.

Attach to F01-QCP-028 - 42559 JS 16NOV23 BA 11/16/2023

Secondary Signature Page

Process Protocol Effective Date: 12-Jan-2023

Charles River Laboratories Inc., PA Biologics LIMS Study Number: SDY-14787

Testing Facility Study Number: EM-23.486

Client Sample ID: WIC-WA09-MB-004

LIMS Testing Aliquot Number: 423186

Sponsor: University of Wisconsin

Sponsor Approval Date: 24-Apr-2023

The signature below indicates that Testing Facility Management approves the Project Scientist indicated below. The signature of the Project Scientist below indicates their acknowledgement and approval of the process protocol.

Approvals:

Schantel Bouknight, DVM, PhD, DACVP
Testing Facility Management

Gara L. Creamer, B.S.
Project Scientist

All electronic signatures appear at the end of the document upon finalization.

Attach to **F01-QCP-028** – 42559 JS 16NOV23 BA 11/16/2023

SIGNATURE(S) FOR DOCUMENT: EM-23.486 - SSP

TFM Approval:	I approve this document.
Name:	**Bouknight, Schantel A**
Bouknight, Schantel A	05-Oct-2023 16:37:03 (UTC+00:00)
Electronically Signed in M-Files	Timestamp

Project Scientist Approval:	I approve this document.
Name:	**Creamer, Gara**
Creamer, Gara	06-Oct-2023 18:49:13 (UTC+00:00)
Electronically Signed in M-Files	Timestamp

Attach to F01-QCP-028 – 42359 JS 16 NOV 23 BA 11/16/2023

charles river

REPORT OF ANALYSIS

PCR-Based Reverse Transcriptase Assay (PBRT) Using Fluorescent Probe Technology

Client: University of Wisconsin
1500 Highland Avenue
Madison, WI 53705-2274 USA

Testing Facility: Charles River Laboratories
466 Devon Park Drive
Wayne, PA 19087

Protocol Number: GP-V692

Protocol Effective Date: 12JAN2022

On-Test Date: 06SEP2023

Results:

Sample Identification	Sample Number	Assay Result 1:100 Dilution	Assay Result 1:1,000 Dilution
WIC-WA09-MB-004	422554	Not Detected	Not Detected

Comments: None

Exception Document(s): None

Validity Criteria: Please refer to the Data Evaluation and Assay criteria sections within *GP-V692 PCR-Based Reverse Transcriptase Assay (PBRT) Using Fluorescent Probe Technology,*

- All controls must meet the assay criteria as stated in GP-V692.
- Mv1Lu cell culture supernatant is the negative control for the assay, is considered background and therefore, RT activity is not calculated for this value.
- C_T values that are "undetermined" indicate no detectable amplification and therefore, these values are not used to calculate RT activity.
- If the Mv1Lu cell culture supernatant has no detectable amplification, a C_T value of 40.00 is used to evaluate positivity.

NOTE: Refer to the final page for the certification statement, final electronic signatures, and revision history

Attach to F01-QCP-028 - 42388 JS 02 OCT 2023 BA 10/24/2023

TABLE 1
DETERMINATION OF REVERSE TRANSCRIPTASE ACTIVITY IN SAMPLE

Standard pUnits[a]	Average C_T VALUE [b]	Correlation Coefficient (R)
4.0×10^9	19.10	R = 0.9899
8.0×10^8	21.66	
1.6×10^8	23.26	*The correlation coefficient (R value) must be ≥ 0.9800.*
3.2×10^7	25.84	
6.4×10^6	27.84	
1.28×10^6	30.84	

Negative Control	Average C_T VALUE [b]	Expected Result	Result
Mv1Lu	Undetermined	Background	Background

Sample Spikes	Average C_T VALUE [b]	Expected Result	Result
422554 (1:100) + SMRV	22.28	Positive	Positive
422554 (1:1000) + SMRV	22.19	Positive	Positive
422554 (1:100) + TEK-1	28.76	Positive	Positive
422554 (1:1000) + TEK-1	29.97	Positive	Positive

Determining If RT Activity Has Been Detected in a Replicate Sample:

All sample dilutions are tested in triplicate reactions. Any replicate whose CT value is greater than 3 cycles from the Mv1Lu negative control CT average, is considered as a positive indication of RT activity. *For example:*

If Mv1Lu has an "undetermined" C_T value and a sample's replicate has a C_T value of 38.26:
40.00 - 38.26 = 1.74 or **No RT activity detected**, *however,*
If Mv1Lu has an "undetermined" C_T value and a sample's replicate has a C_T value of 35.12:
40.00 - 35.12 = 4.88 or **RT activity detected**

Sample	C_T			Result: If any of 3 replicates results in RT activity, the sample is considered positive for RT activity.
	Rep 1	Rep 2	Rep 3	
422554 (1:100)	Undetermined	Undetermined	Undetermined	3 of 3 Replicates: RT Activity Not Detected
	Not Detected	Not Detected	Not Detected	
422554 (1:1000)	Undetermined	Undetermined	38.36	3 of 3 Replicates: RT Activity Not Detected
	Not Detected	Not Detected	Not Detected	

[a] pUnits = Moloney Murine Leukemia virus (MuLV) reverse transcriptase - equivalent units of activity.
[b] All reactions were tested in triplicate. The average CT value refers to the arithmetic mean of three CT replicate values.

RPT-230912-043
Version 1

Attach to F01-QCP-028 – 42388 JS 02 OCT 2023 BA 01/24/2023

charles river

Certification:
This report summarizes testing performed at the Charles River Laboratories, Inc., PA-Biologics facility, which is operated in compliance with the applicable requirements of the U.S. Food and Drug Administration's Good Manufacturing Practice regulations as found in Title 21 CFR Parts 210 and 211. There were no deviations from the test method(s) that impacted the quality or integrity of the test result(s).

Study Lead Signer Name:	Ashley Callaghan	**Date:**	13 Sep 2023 12:30
Quality Assurance Signer Name:	Pajtim Dumani	**Date:**	19 Sep 2023 15:29

The above approval(s) were signed via the Labware LIMS electronic signature. Signers are aware that their electronic signature is the legally binding equivalent of their handwritten signature.

Attach to F01-QCP-028 - 42388 JJ 02 OCT 2023 BA 10/24/2023

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Final Report/Certificate of Analysis, Version I

Title: Mouse Antibody Production Test with LCMV Challenge

Protocol Number: PR-31-15

Protocol Effective Date: 07FEB2023

1.0 Client

University of Wisconsin
1500 Highland Avenue
Madison, WI 53705-2274
USA

2.0 Test Article Identity: WIC-WA09-MB-004

CR ID #: 419163

3.0 Test Facility

Charles River Laboratories/In Vivo Biosafety
299 Ballardvale St.
Wilmington, MA 01887

4.0 Lab Initiation date: 28-Aug-2023

5.0 Lab Completion date: 04-Oct-2023

6.0 References

This method is designed to adhere to the following regulatory guidelines:

Guidance for Industry, Characterization and Qualification of Cell Substrates and other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications (Feb 2010)

Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals as recommended by the US FDA Center for Biologics Evaluation and Research (1993)

International Conference on Harmonisation, Guidance for Industry Q5A (R1): Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin (1999)

7.0 Procedure

CD-1 mice (3-8 weeks old) are obtained from a Charles River facility on which routine health monitoring is performed. Ten mice are inoculated with the test article and three mice are inoculated with a saline control (Hanks' Balanced Salt Solution). All mice are observed daily for clinical signs. Two test article inoculated mice, and one control article inoculated mouse are assigned to the LCMV challenge. The remaining mice are monitored for at least 28 days post-inoculation for clinical signs. At the end of the observation period, the animals are euthanized, and blood samples are collected from each animal. Serum is submitted for serology testing against a standard panel of viruses known to infect mice.

8.0 Unexpected Results

None

9.0 Deviations

No critical/major deviations occurred that affected the quality or integrity of the test, or interpretation of the results.

Attach to F01-QCP-028 – 42381 JS 10-12-23 RJA 10/24/2023

10.0 Results

10.1 Serology

Name	Results
Sendai virus (SEND)	Negative
Pneumonia virus of mice (PVM)	Negative
Mouse hepatitis virus (MHV)	Negative
Minute virus of mice (MVM)	Negative
Mouse parvovirus, Non-Structural Protein 1 (PARV NS1)	Negative
Mouse Parvovirus (MPV)	Negative
Theiler's murine encephalomyelitis virus (GDVII)	Negative
Reovirus Type 3 (REO)	Negative
Epizootic diarrhea of infant mice (EDIM)	Negative
Mouse pneumonitis virus (K)	Negative
Ectromelia (ECTRO)	Negative
Polyoma virus (POLY)	Negative
Mouse adenovirus (MAV 1& 2)	Negative
Lymphocytic choriomeningitis virus (LCMV)	Negative
Mouse cytomegalovirus (MCMV)	Negative
Mouse thymic virus (MTLV)	Negative
Hantaan virus (HANT)	Negative
Prospect Hill Virus (PHV)	Negative
Lactate Dehydrogenase Elevating Virus (LDV)	Negative

10.2 LCMV Challenge: Test article inoculated animals must be euthanized or die within the 6-9 day period prescribed by protocol: Test Article inoculated mice met criteria for negative test.

10.3 Conclusion: Viral contamination was not detected in the test article, WIC-WA09-MB-004.

Attach to F01-QCP-028 - 42381 JS 10-12-23 BA 10/24/2023

charles river

Certification:

This report summarizes testing performed at the Charles River Laboratories, Inc. , In Vivo Biosafety facility in Wilmington, MA which is operated in compliance with the applicable requirements of the U .S. Food and Drug Administration's Good Manufacturing Practice regulations as found in Title 21 CFR Parts 210 and 211. There were no deviations from the test method(s) that impacted the quality or integrity of the test result(s).

Study Lead Signer Name:	Leslie Scola	**Date:**	11 Oct 2023 11:28
Quality Assurance Signer Name:	Devayani Patil	**Date:**	11 Oct 2023 11:50

The above approval(s) were signed via the Labware LIMS electronic signature. Signers are aware that their electronic signature is the legally binding equivalent of their handwritten signature.

Attach to F01-QCP-028 -42381 JS 10-12-23 BA 10/24/2023

National Stem Cell Bank Testing Report

HLA

Cell Line	H9
Lot Number	H9 MCB.1
Passage Number	P24
Report Number/ File Name	6185-HLA (H9 MCB.1)
Date of Report	8/16/07
SOPs Followed	SOP-CH-303B
QA Review By/On	EM 8/17/07

Notes:

WA09-MCB-1

UW Health
University of Wisconsin Hospital and Clinics

Histocompatibility/Molecular Diagnostics Laboratory
D4/231, (608) 263-8815
600 Highland Avenue
Madison, WI 53792-2472

Date: 06/29/2007 17:03:46

To: WiCell Research Institute
510 Charmany Dr. Suite 59
Madison, WI 53719

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, H9 P43	DQB SSP		0201/24/26/34/90	3503/13	0401/9N	1501				
56256 /	A,B,C Seq	06/15/2007	0301/7-9/17	4427/12	0704/11	1601				
06/15/2007	DRB Seq	06/20/2007								

David F. Lorentzen, Manager
HLA/Molecular Diagnostics Laboratory
6-29-07
Date

David I. Watkins, PhD, Director
HLA/Molecular Diagnostics Laboratory
7/02/07
Date

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

[I:\db\tx\ttmaster\vttmreport.frx]

charles river

REPORT OF ANALYSIS
Version 1

Bacteriostasis and Fungistasis Final Product (Direct Method)

Client:	Waisman Biomanufacturing 1500 Highland Avenue Madison, WI 53705
Protocol Number:	GP-V717
Protocol Effective Date:	09FEB2016
On-Test Date:	03JUN2022

Results:

Sample Identification	Sample Number	Assay Result
WIC-WA09-MB-002	315147	No Inhibition

Comments:
None

Exception Document(s):
None

This report summarizes testing performed at the Charles River Laboratories, Inc., PA-Biologics facility, which is operated in compliance with the applicable requirements of the U.S. Food and Drug Administration's Good Manufacturing Practice regulations as found in Title 21 CFR Parts 210 and 211. There were no deviations from the test method(s) that impacted the quality or integrity of the test result(s).

Danielle Tubello — Technical Approval (Print)
Danielle Tubello — (Sign)
08JUN2022 — Date

Vishal Forde — Quality Assurance (Print)
V Forde — (Sign)
10JUN2022 — Date

Revision History

Version 1	**N/A**

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Sample Preparation: Sample was used as submitted by the client. One approximately 0.5 mL aliquot of the sample was inoculated into an individual vessel containing 100 mL of Soybean Casein Digest Medium (SCDM) for each applicable organism. This procedure was repeated for Fluid Thioglycollate Medium (FTM).

BACTERIOSTASIS & FUNGISTASIS TEST DATA SHEET

 # Vessels With Growth / Total # Vessels

Culture	Inoculum	# Vessels	Day 5
315147 / SCDM / +Control	*A. brasiliensis*	1	1/1
315147 / SCDM / +Control	*B. subtilis*	1	1/1
315147 / SCDM / +Control	*C. albicans*	1	1/1
SCDM / +Control	*A. brasiliensis*	2	2/2
SCDM / +Control	*B. subtilis*	2	2/2
SCDM / +Control	*C. albicans*	2	2/2
SCDM / -Control	None	2	0/2
315147 / FTM / +Control	*S. aureus*	1	1/1
315147 / FTM / +Control	*P. aeruginosa*	1	1/1
315147 / FTM / +Control	*C. sporogenes*[a]	1	1/1
FTM / +Control	*S. aureus*	2	2/2
FTM / +Control	*P. aeruginosa*	2	2/2
FTM / +Control	*C. sporogenes*[a]	2	2/2
FTM / -Control	None	2	0/2

[a] *C. sporogenes* (ATCC 11437)

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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	Tracy Freeman
Test Facility	Covance Laboratories Ltd., Otley Road, Harrogate, North Yorkshire HG3 1PY United Kingdom
Study Monitors	Erika Mitchen and Derek Hei
Sponsor	Waisman Clinical BioManufacturing Facility 1500 Highland Ave. University of Wisconsin – Madison Madison, WI 53705 USA
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.

Tracy Freeman
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

Process

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

D Hitchen
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:	Tracy Freeman.
Study Supervisor:	Christina McWee.

STUDY SCHEDULE

The study schedule was as follows:

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

ARCHIVE STATEMENT

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

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

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

CONTENTS

SUMMARY

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

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

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

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

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

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

INTRODUCTION

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

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

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

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

OBJECTIVE

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

MATERIALS

Protocol Adherence

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

Test Article

Identification: H9-MCB.1.

Source: WiCell Research Institute.

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

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

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

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

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

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

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

PCR and QPCR Test Systems

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

Controls

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

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

PROCEDURES

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

RESULTS

Human Immunodeficiency Virus-1 (HIV-1) Results

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

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

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

Human Immunodeficiency Virus-2 (HIV-2) Results

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

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

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

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

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

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

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

Epstein Barr Virus (EBV) Results

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

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

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

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

Human Herpes Virus 7 (HHV-7) Results

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

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

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

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

Human Herpes Virus 8 (HHV-8) Results

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

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

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

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

Hepatitis B Virus (HBV) Results

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

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

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

Human Parvovirus (B19) Results

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

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

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

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

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

Hepatitis C Virus (HCV) Results

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

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

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

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

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

CONCLUSIONS

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

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

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

TABLES

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

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

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

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

Table 2: Results of B19 QPCR

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

U = Undetermined.

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

Table 3: Results of HCV RT-QPCR

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

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

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

APPENDIX

Minor Deviations from the Definitive Protocol

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

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

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

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

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

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

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

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

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

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

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

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

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

ANNEX

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

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

Definitive Protocol

Title	H9-MCB.1: Detection of HIV-1, HIV-2, HTLV-1, HTLV-2, HCMV, EBV, HHV-7, HHV-8 and HBV Sequences using the Polymerase Chain Reaction (PCR) Technique. Detection and Quantification of HHV-6 and B19 Sequences using the Quantitative Polymerase Chain Reaction Technique (QPCR). Detection and Quantification of HCV Sequences using the Reverse Transcriptase Quantitative Polymerase Chain Reaction Technique (RT-QPCR)
Study Director	Tracy Freeman
Testing Facility	Covance Laboratories Ltd., Otley Road, Harrogate, North Yorkshire HG3 1PY United Kingdom
Study Monitors	Erika Mitchen and Derek Hei
Sponsor	Waisman Clinical BioManufacturing Facility 1500 Highland Ave. University of Wisconsin – Madison Madison, WI 53705 USA
Covance Study Number	2823/004
Page Number	1 of 28

COVANCE
THE DEVELOPMENT SERVICES COMPANY

INTRODUCTION

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

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

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

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

OBJECTIVE

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

TEST ARTICLE

Identification: H9-MCB.1.

Source: WiCell Research Institute.

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

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

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

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

PCR AND QPCR TEST SYSTEMS

Controls

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

Blank, sentinel and RNA extraction control:

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

Source: Sigma-Aldrich Company Ltd.

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

Source: Invitrogen Ltd.

PROCEDURES

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

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

Preparation of Test Article for DNA Extraction

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

DNA Extraction

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

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

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

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

Determination of the DNA Concentration of Samples

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

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

Preparation of Test Article for RNA Extraction

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

RNA Extraction

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

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

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

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

Determination of the RNA Concentration of Samples

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

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

PCR Amplification

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

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

Refer to primers section for precise reaction component concentrations.

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

Reverse Transcriptase (RT)-QPCR Amplification

HCV RT-QPCR

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

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

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

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

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

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

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

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

One cycle of:

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

Then 40 cycles of:

95°C for 15 seconds
60°C for 1 minute

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

QPCR Amplification

B19 QPCR

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

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

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

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

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

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

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

One cycle of:

95°C for 10 minutes

Then 40 cycles of:

95°C for 15 seconds
60°C for 1 minute

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

HHV-6 QPCR

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

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

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

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

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

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

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

One cycle of:

95°C for 10 minutes

Then 40 cycles of:

95°C for 15 seconds
60°C for 1 minute

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

PCR Primers

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

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

RT-QPCR and QPCR Primers and Probe

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

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

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

PCR Controls

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

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

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

B19 QPCR Controls

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

HHV-6 QPCR Controls

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

HCV RT-QPCR Controls

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

PCR Thermal Profiles

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

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

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

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

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

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

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

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

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

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

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

HCMV gB1/HCMV gB 2 primers

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

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

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

KS330 233A/KS330 233E primers

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

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

Gel Electrophoresis

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

Southern Blot Hybridisation

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

DATA ANALYSIS

PCR Assay Acceptance Criteria

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

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

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

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

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

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

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

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

PCR Evaluation Criteria

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

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

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

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

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

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

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

QPCR Assay Acceptance Criteria

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

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

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

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

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

QPCR Evaluation Criteria

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

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

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

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

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

RT-QPCR Assay Acceptance Criteria

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

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

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

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

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

RT-QPCR Evaluation Criteria

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

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

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

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

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

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

GLP COMPLIANCE

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

The study will be performed in compliance with*:

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

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

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

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

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

ARCHIVE STATEMENT

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

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

REFERENCES

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

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

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

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

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

APPENDIX 1

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

Definitive Protocol

Amendment(s)*

File note(s)*

Study related correspondence*

Test article description

Test article receipt and utilisation

Metrology#

Records for reagents and stock solutions#

Test article cell culture records*

Work sheets

* where appropriate
# some records held centrally

APPENDIX 2

Draft Report

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

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

The following items will be presented in the Draft Report:

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

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

Final Report

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

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

APPENDIX 3

RESPONSIBLE PERSONNEL AND STUDY SCHEDULE

Study Director	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[1]	C Clare
Resource Management	Estimation
Resource Management	Scheduling

Electronic Access

Study Supervisor[1]	C McWee
Analytical Staff	J McDevitt, N Gahir, S Dwivedi
Study Co-ordinator	G Smith
Project Manager	K Hodgkinson
VP of Biotechnology	C Martin

[1] = Any change documented in study records

PROPOSED DATES

Analytical programme

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

PROTOCOL APPROVAL

Derek Hei
Study Monitor
Waisman Clinical Biomanufacturing Facility

7/11/07

Erika Mitchen
Quality Assurance
WiCell Research Institute

7/13/07

Tracy Freeman
Study Director
Covance Laboratories Ltd

28 June 2007
Date

Daniel Galbraith
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	Tracy Freeman
Testing Facility	Covance Laboratories Ltd., Otley Road, Harrogate, North Yorkshire HG3 1PY United Kingdom
Study Monitors	Erika Mitchen and Derek Hei
Sponsor	Waisman Clinical BioManufacturing Facility 1500 Highland Ave. University of Wisconsin – Madison Madison, WI 53705 USA
Covance Study Number	2823/004
Amendment number	1
Page Number	1 of 3

COVANCE
THE DEVELOPMENT SERVICES COMPANY

This Amendment documents the following:

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

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

OBJECTIVE

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

PROTOCOL APPROVAL

11/2/07

Derek Hei
Study Monitor
Waisman Clinical Biomanufacturing Facility

11/2/07

Erika Mitchen
Quality Assurance
WiCell Research Institute

01 NOV 2007
Date

Tracy Freeman
Study Director
Covance Laboratories Ltd

01 November 2007
Date

Keri Hodgkinson
Project Manager
Covance Laboratories Ltd

Final Report

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

Study Number: AC08DS.105013.BSV

Test Article ID: H9 MCB.1

Sponsor: WiCell Research Institute
505 S. Rosa Road Suite 120
Madison, WI 53719 United States

Authorized Representative: Erika Mitchen

CONCLUSION

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

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

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

STUDY INFORMATION

Test Article: 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
14920 Broschart Road
Rockville, Maryland 20850 USA

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: Xiuli Chen, 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
14920 Broschart Road
Rockville, MD 20850

OBJECTIVE

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

TEST SYSTEM

PCR amplification is performed on test article DNA using HTLV-specific primers. In the presence of HTLV-I/II proviral DNA, these primers produce a 158 bp amplification product. The amplification products are analyzed by agarose gel electrophoresis in the presence of ethidium bromide. Positive results are confirmed and discrimination between HTLV-I and HTLV-II sequences is achieved by restriction endonuclease treatment of the PCR product and analysis of the restriction pattern obtained. The following controls are included in each assay:

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

Positive Controls:

HTLV-I: Negative control DNA spiked with 100 copies of pH750, a plasmid containing a 752 bp fragment from the HTLV-I tax/rex gene
Source: BioReliance

HTLV-II: Negative control DNA spiked with 100 copies of pMAHTII, a plasmid containing a 552 bp fragment from the HTLV-II tax/rex gene
Source: BioReliance

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

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

HTLV-I: Test article extract spiked with 100 copies of pH750.

HTLV-II: Test article extract spiked with 100 copies of pMAHTII.

METHODS

Sample Preparation

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

DNA Amplification

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

RESULTS

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

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

APPROVAL

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

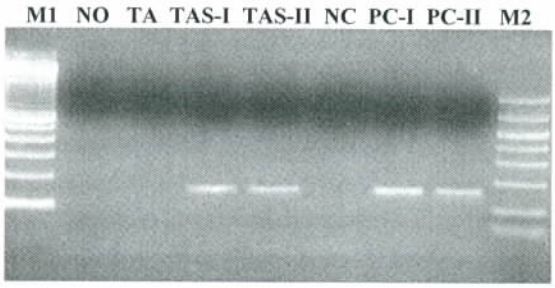
Xiuli Chen

Xiuli Chen, 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: Xiuli Chen, 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.

Marcus Blanchard
QUALITY ASSURANCE

23Oct07
DATE

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
505 S. Rosa Road Suite 120
Madison, WI 53719 United States

Authorized Representative: Erika Mitchen

CONCLUSION

One-half (0.5) µg of DNA isolated from test article H9 MCB.1 (representing approximately 7.5 x 10^4 cells) was analyzed for the presence of human cytomegalovirus (CMV) DNA by the polymerase chain reaction (PCR)[1] technique. The assay can detect 100 copies of CMV in the presence of 0.5 µg of genomic DNA.

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

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

STUDY INFORMATION

Test Article: 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
14920 Broschart Road
Rockville, Maryland 20850

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: Xiuli Chen, 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
14920 Broschart Road
Rockville, Maryland 20850

OBJECTIVE

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

Test System:

PCR amplification is performed on test article DNA using CMV-specific primers. In the presence of CMV, these primers produce a 363 bp amplification product. The amplification products are analyzed by agarose gel electrophoresis in the presence of ethidium bromide. The following controls are included in the assay:

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

METHODS

Sample Preparation

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

DNA Amplification

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

REPEATS

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

RESULTS

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

These results provide evidence that the test article 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.

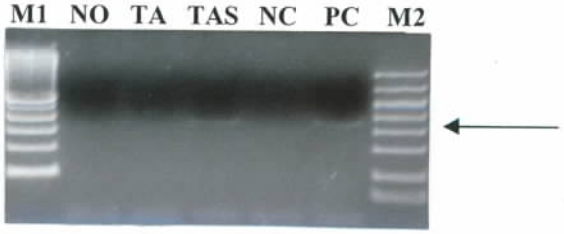
Xiuli Chen

Xiuli Chen, 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: Xiuli Chen, 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.

Ken Daus, 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
505 S. Rosa Road Suite 120
Madison, WI 53719 United States

Authorized Representative: Erika Mitchen

CONCLUSION

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

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

---

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

STUDY INFORMATION

Test Article:	H9 MCB.1 was received by BioReliance on 09/25/2007. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor. Retention of reserve sample from each batch of test article is the responsibility of the sponsor.
Testing Facility:	BioReliance 14920 Broschart Road Rockville, Maryland 20850
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:	Xiuli Chen, 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 14920 Broschart Road Rockville, MD 20850

OBJECTIVE

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

TEST SYSTEM

For detection of HHV-6 viral DNA, PCR amplification is performed on test article DNA using HHV-6-specific primers according to SOP OPBT0924. In the presence of HHV-6A viral sequences, these primers produce a 328 bp amplification product, while the HHV-6A positive control plasmid (pU1102MOD) generates a 299 bp amplification product. In the presence of HHV-6B viral sequences, the primers produce a 553 bp amplification product, while the HHV-6B positive control plasmid (pZ29MOD) generates a 524 bp amplification product. The following controls are included in the assay:

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

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

METHODS

Sample Preparation

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

DNA Amplification

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

RESULTS

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

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

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

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

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

APPROVAL

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

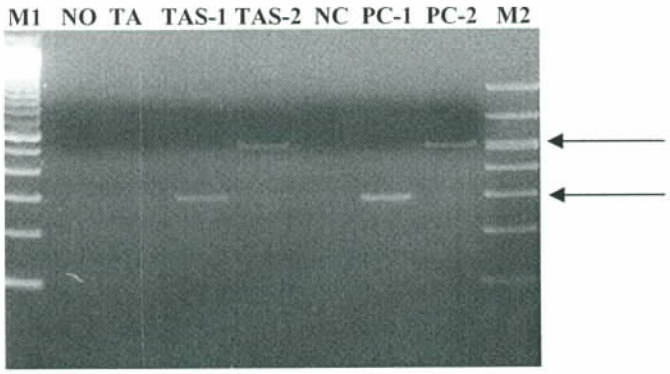
Xiuli Chen

Xiuli Chen, 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: Xiuli Chen, 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.

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	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.

Ken Daus, B.A.
QUALITY ASSURANCE

23 Oct 07
DATE

WiCell CGH Report: 000143 NSCB# 6185

Report Date: *March 13, 2009*

Case Details:

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

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

Investigator: *National Stem Cell Bank*

Specimen: *hES cells on MEFs*

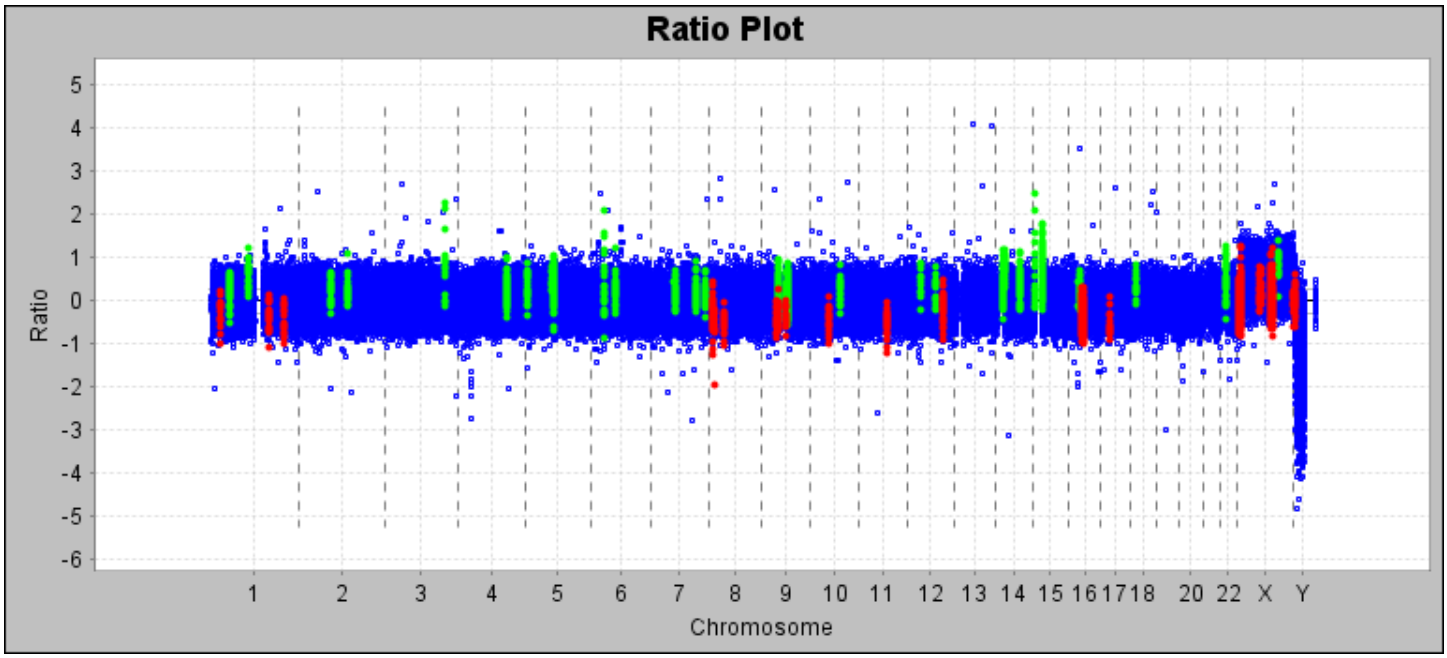
Date of Sample: *7/23/2007*

Reason for Testing: *NSCB MCB Testing*

GEO Accession #: *GSM347606*

Results:

Results are given in the attached excel spreadsheet labeled 'report.' There were 61 copy number gains and losses identified by modified circular binary segmentation[1]. The analysis summary is depicted in the ratio plot below with copy number gains shown in green and losses in red. This data was generated using OneClickCGH™ software.



Interpretation: *The data shown in the table below are derived from the attached Excel spreadsheet labeled "Select". These copy number changes are measures of sensitivity[2, 3] or may be related to differential gene expression that is monitored in the NSCB characterization protocol and the ISCI study[4]. Changes associated with karyotype abnormalities and/or previously reported publications[2, 5] are also listed. Copy number changes designated by an * in "Select" report indicate inconsistency with the reference standard.*

X-chromosome Gains or Losses at Pseudoautosomal Loci[3]	*2 of 2*
Published Copy Number Changes[5,6]	*1 of 8*
Reference DNA Copy Number Changes[2]	*12 of 18*
Select Differentially Expressed Genes	*0 of 45*

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

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

Results Completed By: *Seth Taapken, CLSp(CG)*
Reviewed and Interpreted By: *Karen Dyer Montgomery, PhD, FACMG*

---

aCGH Specifications*:*

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

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

Literature Sources:

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

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

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

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

1. Chip design: 2007-03-02_WiCell_HG18

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

3. QC comments:
   Box plots and distribution graphs are within acceptable range.

4. Expression of ES markers:

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

5. Expression of differentiation markers:

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

American Red Cross

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(215) 451-4000
1-800-GIVE LIFE
www.pleasegiveblood.org

10/25/07

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

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

INSTITUTION: WiCell Research Institute

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

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

DNA MOLECULAR RESULTS:

	Genotype	Predicted Phenotype
ES03-MCB-1 NSCB **1590:**	*ABO*O^1O^1; RHD; RHCE*Ce/Ce*	NSCB **1590:** Group O; RhD+, C+, c-, E-, e+
H9-MCB-1 NSCB **6185:**	*ABO*A^1O^1; RHD; RHCE*cE/ce*	NSCB **6185:** Group A; RhD+, C-, c+, E+, e+
H1-MCB-1 NSCB **9592:**	*ABO*O^1/O^1; RHD; RHCE*Ce/Ce*	NSCB **9592:** Group O; RhD+, C+, c-, E-, e+
HSF1-MCB-1 NSCB **5456:**	*ABO*O^1/O^1; RHD; RHCE*Ce/ce*	NSCB **5456:** Group O; RhD+, C+, c+, E-, e+

COMMENTS: All samples were negative for the *RHD*-inactivating pseudogene and the *RHD-CE-D* hybrid which cause a D- phenotype and are common in African Black ethnic groups.

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

Please Give Blood.