CRM-WA14-MB-001

Table of Contents

Product Information and Testing

Product Information

Product Name	WA14 Cell Bank Produced Under cGMP Conditions
Alias	H14
Lot Number	CRM-WA14-MB-001
Parent Material	WA14-WB0119
Depositor	WiCell
Banked by	Waisman Biomanufacturing
Thaw Recommendation	Thaw 1 vial into 3 wells of a 6 well plate.
Culture Platform	Feeder Independent
	Medium: mTeSR1
	Matrix: Matrigel
Protocol	WiCell Feeder Independent Protocol
Passage Number	p21 These cells were cultured for 20 passages prior to freeze, 5 of them in mTeSR1/Matrigel. One number (+1) is added to the passage number at freeze so that the number on the vial best represents the overall passage number of the cells at thaw.
Date Vialed	15-December-2012
Vial Label	WA14 Master Cell Bank Lot #: CRM-WA14-MB-001 Vialed: 15DEC2012 p21 Store in LN_2
Biosafety and Use Information	Appropriate biosafety precautions should be followed when working with these cells. The end user is responsible for ensuring that the cells are handled and stored in an appropriate manner. WiCell is not responsible for damages or injuries that may result from the use of these cells.

Testing Performed by Waisman Biomanufacturing and WiCell

Test Description	Test Provider	Test Method	Test Specification	Result
Sterility Test per 21 CFR 610.12	Apptec	30744/A	No Growth	Pass
Bacteriostatis and Fungistatis	Apptec	30736	Test samples did not demonstrate bacteriostatic/fungistatic activity No Inhibition Detected	Pass
Karyotype by G Band	WiCell	SOP-CH-003	Report Result	See Report
Flow Cytometry for ESC Marker Express Oct-3/4, SSEA-1/3/4, TRA-1-60, TRA-1-81	WiCell	SOP-CH-024	Oct-4/SSEA-4>80% Other Markers Report Results	Pass
Identity by STR	UW Molecular Diagnostics Laboratory	PowerPlex 16HS System by Promega	Matches WA14 reference STR	Pass
Endotoxin	Waisman Clinical Biomanufacturing Facility	QCP-013	<10 EU/ml	Pass
Post-Thaw Viable Cell Recovery	Waisman Clinical Biomanufacturing Facility	QCP-003	Report Results	Pass
Detection of Mycoplasma by PTC Method	Apptec	30055	Not Detected	Pass
Adventitious Virus-In vitro assay MRC5, Vero and 3T3 cell lines	Apptec	32869	Not Detected	Pass
Adventitious Virus-In Vivo assays; suckling and adult mice, and embryonated chicken eggs	Apptec	30194	Not Detected	Pass
Retrovirus by PERT testing	Apptec	30357	Not Detected	Pass

©2013 WiCell Research Institute The material provided under this certificate has been subjected to the tests specified and the results and data described herein are accurate based on WiCell's reasonable knowledge and belief. Appropriate Biosafety Level practices and universal precautions should always be used with this material. For clarity, the foregoing is governed solely by WiCell's Terms and Conditions of Service, which can be found at http://www.wicell.org/privacyandterms.

Product Information and Testing

Murine Pathogens (MAP)	Apptec	30001	Not Detected	Pass
HLA profile[1]	UW Molecular Diagnostics Laboratory	AlleleSEQR Kits by Abbott	Consistent with known profile	Pass
Bovine pathogens[1]	BioReliance	032901	No contamination detected	Pass
Porcine pathogens[1]	BioReliance	033901	No contamination detected	Pass
Retrovirus by thin section EM[1]	WuXi Apptec	30610	No contamination detected when cultured without MEFs	Pass
Co-cultivation with Mus Dunni Cells and PG4 S+L- assay[1]	WuXi Apptec	30201	No contamination detected	Pass
HIV 1&2 by PCR[1]	BioReliance	105010	Negative	Pass
HTLV 1&2 by PCR[1]	BioReliance	105013	Negative	Pass
HBV by PCR[1]	BioReliance	105042	Negative	Pass
HCV by PCR[1]	BioReliance	105025	Negative	Pass
CMV by PCR[1]	BioReliance	105012	Negative	Pass
EBV by PCR[1]	BioReliance	105011	Negative	Pass
HHV-6 by PCR[1]	BioReliance	105020	Negative	Pass
HHV-7 by PCR[1]	BioReliance	105029	Negative	Pass
HHV-8 by PCR[1]	BioReliance	105056	Negative	Pass
HP B19 by PCR[1]	BioReliance	105037	Negative	Pass
Comparative Genome Hybridization[1]	WiCell Research Institute	SOP-CH-308 SOP-CH-309 SOP-CH-310	Report - no specification	See report
Gene Expression Profile[1]	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[1]	American Red Cross	ABO/rH System	Report Blood type	O+

[1] Test was performed on WA14-MCB-01.

Amendment(s):

Reason for Amendment	Date
Updated CoA to include copyright information.	09-Jan-2013
Original CoA	13-May-2013

Date of Lot Release	Quality Assurance Approval
13-May-2013	1/10/2014 X AMC AMC Quality Assurance Signed by: [redacted]

©2013 WiCell Research Institute The material provided under this certificate has been subjected to the tests specified and the results and data described herein are accurate based on WiCell's reasonable knowledge and belief. Appropriate Biosafety Level practices and universal precautions should always be used with this material. For clarity, the foregoing is governed solely by WiCell's Terms and Conditions of Service, which can be found at http://www.wicell.org/privacyandterms.

Test Facility:
[redacted]

This report is [illegible] for use [illegible] used for advertising [illegible] party [illegible] of client without written permission of [illegible] to the sponsor's product.

WuXi AppTec

Report Number
919723
Page 1 of 1

Waisman Clinical Biomanufacturing Facility
[redacted]

January 22, 2013
P.O. #: [redacted]

STERILITY TEST REPORT

Sample Information: CRM-WA14-MB-001

Date Received: January 04, 2013
Date in Test: January 08, 2013
Date Completed: January 22, 2013

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

TEST PARAMETERS	PRODUCT	
Approximate Volume Tested	1.0 mL	1.0 mL
Number Tested	10	10
Type of Media	SCD	FTM
Media Volume	200 mL	200 mL
Incubation Period	14 Days	14 Days
Incubation Temperature	20 °C to 25 °C	30 °C to 35 °C
RESULTS	10 NEGATIVE	10 NEGATIVE

[redacted] 01-23-13
QA Reviewer Date

[redacted] 01-22-13
Technical Reviewer Date

Testing conducted in accordance with current Good Manufacturing Practices.

[redacted]

Reference: FO1-QCP-028 #19174 JJ 28 Jan 2013 ROM 30 Jan 2013

Test Facility: [redacted]

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

WuXi AppTec

Waisman Clinical Biomanufacturing Facility
[redacted]

January 10, 2013
P.O. #: [redacted]

GENERAL MICROBIOLOGY TEST REPORT

Sample Information: CRM-WA14-MB-001

Date Received: January 04, 2013
Date in Test: January 07, 2013
Date Completed: January 10, 2013

Test Information:
Test Code: 30736
Sterility Method Suitability (Bacteriostasis / Fungistasis)
Immersion, USP / EP
Procedure #: BS210WSM.204
Media Volume: 20 mL
Volume Tested: 0.1 mL

SCD	*B. subtilis* ATCC 6633	*C. albicans* ATCC 10231	*A. brasiliensis* ATCC 16404
Test Sample	Positive	Positive	Positive
Inoculated Control	Positive	Positive	Positive
Inoculum Level (CFU)	59	79	14
RESULTS	Pass	Pass	Pass

FTM	*S. aureus* ATCC 6538	*P. aeruginosa* ATCC 9027	*C. sporogenes* ATCC 11437
Test Sample	Positive	Positive	Positive
Inoculated Control	Positive	Positive	Positive
Inoculum Level (CFU)	26	35	35
RESULTS	Pass	Pass	Pass

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

Note 1: Product volume to media volume ratio is equivalent to test ratio employed for sterility testing. Reference Sterility Test Report(s): 919723

[redacted] 01-11-13
QA Reviewer Date

[redacted] 01-11-13
Technical Reviewer Date

Testing conducted in accordance with current Good Manufacturing Practices.

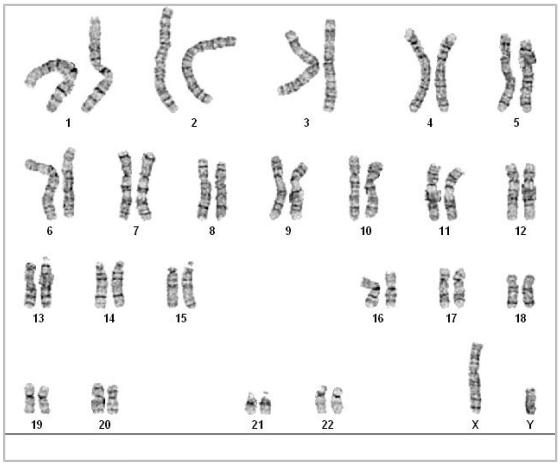
[redacted]

Reference: FOI-QCP-028X 19174 KOM 18Jan2013 JJ 18Jan2013 KOM 30Jan2013

Chromosome Analysis Report: 009611

Date Reported: Friday, January 04, 2013
Cell Line: CRM-WA14-MB-001 10682
Passage#: 21
Date of Sample: 12/28/2012
Specimen: hESC
Results: 46,XY

Cell Line Gender: Male
Reason for Testing: GMP testing
Investigator: [redacted], WiCell CDM



Cell: 11
Slide: 2
Slide Type: Karyotype

Total Counted: 20
Total Analyzed: 8
Total Karyotyped: 4
Band Resolution: 450 - 550

Interpretation:

This is a normal karyotype. No clonal abnormalities were detected at the stated band level of resolution.

Completed by: [redacted] CG(ASCP)
Reviewed and Interpreted by: [redacted], PhD, FACMG

A signed copy of this report is available upon request.

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".

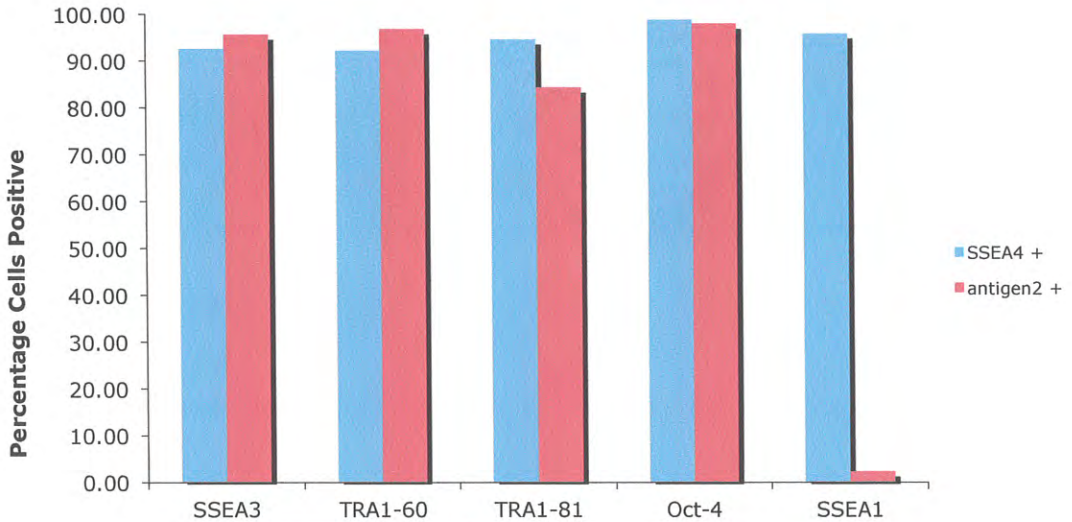
This assay was conducted solely for listed investigator/institution. The results may not be relied upon by any other party without the prior written consent of the Director of the WiCell Cytogenetics Laboratory. The results of this assay are for research use only. If the results of this assay are to be used for any other purpose, contact the Director of the WiCell Cytogenetics Laboratory.

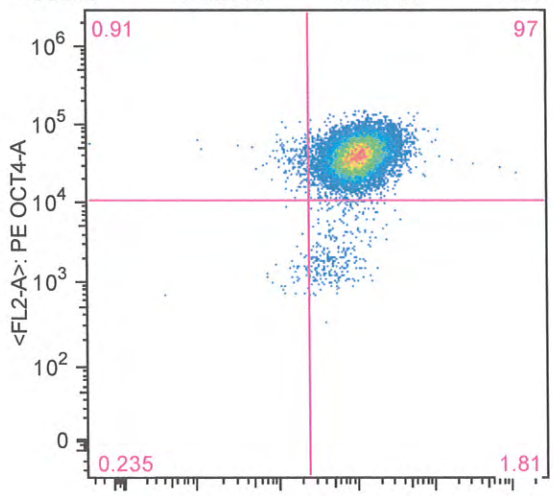
Flow Cytometry Characterization Report

Cell Line:	CRM-WA14-MB-001	Date:	1/6/13
Passage:	22	Acquisition:	1/4/13
Sample ID:	10682	File Creation:	N/A 09Jan13JC6
		File Submission:	N/A 09Jan13 JC6

antigen2:	SSEA4 - antigen2 +	SSEA4 + antigen2 +	SSEA4 + antigen2 -	SSEA4 - antigen2 -	ALL SSEA4 +	ALL antigen2 +	Verification: should be 100%
SSEA3	5.25	90.40	2.18	2.13	92.58	95.65	**99.96**
TRA1-60	7.08	89.70	2.53	0.65	92.23	96.78	**99.96**
TRA1-81	4.26	80.10	14.50	1.21	94.60	84.36	**100.07**
Oct-4	0.92	97.00	1.81	0.24	98.81	97.92	**99.96**
SSEA1	0.34	1.95	93.90	3.80	95.85	2.29	**99.99**

Percent analyzable events: 42.60%
#wells submitted: 6
Total cells recovered: 9.384×10^6





Report prepared By [redacted] Date: 01/08/13

QA review By/Date [redacted] 09Jan13

UW Health

University of Wisconsin
Hospital and Clinics

Histocompatibility/Molecular Diagnostics Laboratory

[redacted]

Short Tandem Repeat Analysis*

Sample Report: **CRM-WA14-MB-001 #1-4**
(4 vials; 1.5E6 cells/ vial)

Requestor: Waisman Biomanufacturing Institute

Test Date: 02/06/13

UWHC Group MR# 2537201
UWHC Acc#
1) 130320484
2) 130320486
3) 130320487
4) 130320488

File Name: 130207 blb

Sample Date: 02/01/13
Received Date: 02/01/13

Report Date: 02/14/13

Sample Names: (labels on tube)
CRM-WA14-MB-001

Description: Human Embryonic Stem Cells

DNA Extracted by UWHC Molecular Diagnostics

Locus	Repeat #	STR Genotype Vial#1	STR Genotype Vial#2	STR Genotype Vial#3	STR Genotype Vial#4
D16S539	5, 8-15	11,13	11,13	11,13	11,13
D7S820	6-14	10,11	10,11	10,11	10,11
D13S317	7-15	11,11	11,11	11,11	11,11
D5S818	7-15	11,13	11,13	11,13	11,13
CSF1PO	6-15	11,12	11,12	11,12	11,12
TPOX	6-13	8,8	8,8	8,8	8,8
Amelogenin	NA	X,Y	X,Y	X,Y	X,Y
TH01	5-11	6,7	6,7	6,7	6,7
vWA	11, 13-21	15,16	15,16	15,16	15,16

Comments: Based on 4 CRM-WA14-MB-001 vials of cells dated and received on 02/01/13 from Waisman Biomanufacturing Institute, these samples (UWHC MR# 2537201; UWHC Acc# 130320484, 130320486, 130320487 and 130320488) all exactly match the STR profile of the human stem cell line WA14 comprising 14 allelic polymorphisms across the 8 STR loci reported. No STR polymorphisms other than those corresponding to the human WA14 stem cell line were detected and the concentration of DNA required to achieve an acceptable STR genotype (signal/ noise) was equivalent to that required for the standard procedure (~1 ng/amplification reaction) from human genomic DNA. These results suggest that each of the 4 CRM-WA14-MB-001 vials of cells submitted correspond to the WA14 human stem cell line and they were not contaminated with any other human stem cells or a significant amount of mouse feeder layer cells. Sensitivity limits for detection of STR polymorphisms unique to either this or other human stem cell lines is ~5%.

[redacted]
Molecular Diagnostics Laboratory

[redacted]
Molecular Diagnostics Laboratory

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

File: Final STR Report

Attached to FoI-QCP-094 #19333 JS 20 Feb 2013 JL 2/20/13

LAL TEST FOR BACTERIAL ENDOTOXIN VIA KINETIC TURBIDIMETRIC ASSAY

DATE: 04 Apr 2013 Project: CRM01

Plate Reader last calibrated: 03/06/13 Calibration Due: 04/06/13

SAMPLES TESTED:

	PRODUCT NAME	LOT NUMBER	DILUTION SCHEME AND VOLUMES USED
1	WA14 McB	CRM-WA14-MB-001	1:100 – 100µL of 1:10 diluted supernatant into 900µL LAL water 1:1000 – 100µL of 1:100 into 900µL LAL Water } 100µL per well
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			

NA JS 04Apr2013

QA Issued [redacted] Date 03/04/13 Log # 19447

LAL TEST FOR BACTERIAL ENDOTOXIN VIA KINETIC TURBIDIMETRIC ASSAY

RESULTS: Attach computer report.

WA14 MCB:

1:100 = < 0.5000 EU/mL

1:1000 = < 5.0000 EU/mL

= < 0.5 EU/mL

ASSAY EVALUATION:

Discuss the passing or failing of acceptance criteria below.

Assay passed based on:

4-point curve

R-value = -0.9997

All % CV's = ≤ 10%

All spike recoveries are between 50-200%

1:100 = 131%; 1:1000 = 116%

COMMENTS: Attach sheets as needed

NA

Operator Signature: [redacted] Date: 04 Apr 2013

Reviewer Signature: [redacted] Date: 04/05/2013

QA Issued [redacted] Date 03/04/13 Log # 19447

File Name:	398213040400.plt	Lab Name:	Waisman Center QC
Assay Date:	4/4/2013 5:24:53 PM	Onset OD:	0.05
Collection Mode:	Kinetic Turbidimetric	Wavelength Filter:	340
Type of Curve Fit:	Linear Regression, Avg. Replicates	Reader:	BioTek
Polynomial Order:	N/A	Temperature:	36.9 : 37.0 °C
Serial Number:	153982	Operator:	[redacted]

Standard Set	Range	R-Value	Acceptable	Slope	Y-Intercept
STD1:STD4	5 : 0.005	-0.9997	YES	-0.2470	2.9388

LAL Water Lot #:	99732177	Exp:	FEB2015
LAL Lot #:	D1732L	Exp:	DEC2015
Endotoxin Lot #:	EX11872	Exp:	4/4/13
BG120 Lot #:	DBK0143	Exp:	APR2016
Accessory 1:	N/A	Exp:	N/A
Accessory 2:	N/A	Exp:	N/A
Accessory 3:	N/A	Exp:	N/A
Comments:			

Product Information for Standard Set STD1:STD4

Product Name:	WA14 MCB 1	**Test Conc/Dil:**	1:100 mL/mL
Identifier:	SPL1	**Endotoxin Limit:**	No Limit
Product Code:	N/A	**Pass:**	YES
Product Lot Number:	CRM-WA14-MB-001		
Sample Description:			

Well Layout	Reaction Time	Mean Reaction Time	Endotoxin Value	Mean Endotoxin Value	(RT) CV%
A5	>3360.0	3360.0	<0.5000 EU/mL	<0.5000 EU/mL	0.00
A6	>3360.0		<0.5000		
A7	>3360.0		<0.5000		

Criteria Computations:

Name:	SPL1
Condition:	CV(SPL1.RTIME) < 10%
Status:	VALID

Spike Identifier:	SPK1
Theoretical Spike Value:	0.5
Spike Recovery:	50 <= SPK1.RY <= 200 : VALID

Well Layout	Reaction Time	Mean Reaction Time	Endotoxin Value	Mean Endotoxin Value	(RT) CV%	Spike Recovery %
A8	978.8	963.3	0.6166 EU/mL	0.6576 EU/mL	1.66	131
A9	970.0		0.6395			
A10	941.3		0.7223			

Criteria Computations:

Name:	SPK1
Condition:	CV(SPK1.RTIME) < 10% AND 50 <= SPK1.RY <= 200
Status:	VALID

Analyst: [redacted] Reviewer: [redacted]
Date: Date:

File Name:	398213040400.plt	Lab Name:	Waisman Center QC
Assay Date:	4/4/2013 5:24:53 PM	Onset OD:	0.05
Collection Mode:	Kinetic Turbidimetric	Wavelength Filter:	340
Type of Curve Fit:	Linear Regression, Avg. Replicates	Reader:	BioTek
Polynomial Order:	N/A	Temperature:	36.9 : 37.0 °C
Serial Number:	153982	Operator:	[redacted]

Standard Set	Range	R-Value	Acceptable	Slope	Y-Intercept
STD1:STD4	5 : 0.005	-0.9997	YES	-0.2470	2.9388

LAL Water Lot #:	99732177	Exp:	FEB2015
LAL Lot #:	D1732L	Exp:	DEC2015
Endotoxin Lot #:	EX11872	Exp:	4/4/13
BG120 Lot #:	DBK0143	Exp:	APR2016
Accessory 1:	N/A	Exp:	N/A
Accessory 2:	N/A	Exp:	N/A
Accessory 3:	N/A	Exp:	N/A
Comments:			

Product Name:	WA14 MCB 2	**Test Conc/Dil:**	1:1000 mL/mL
Identifier:	SPL2	**Endotoxin Limit:**	No Limit
Product Code:	N/A	**Pass:**	YES
Product Lot Number:	CRM-WA14-MB-001		
Sample Description:			

Well Layout	Reaction Time	Mean Reaction Time	Endotoxin Value	Mean Endotoxin Value	(RT) CV%
B5	>3360.0	3360.0	<5.0000 EU/mL	<5.0000 EU/mL	0.00
B6	>3360.0		<5.0000		
B7	>3360.0		<5.0000		

Criteria Computations:

Name:	SPL2
Condition:	CV(SPL2.RTIME) < 10%
Status:	VALID

Spike Identifier:	SPK2
Theoretical Spike Value:	0.5
Spike Recovery:	50 <= SPK2.RY <= 200 : VALID

Well Layout	Reaction Time	Mean Reaction Time	Endotoxin Value	Mean Endotoxin Value	(RT) CV%	Spike Recovery %
B8	997.5	991.6	0.5710 EU/mL	0.5848 EU/mL	0.80	116
B9	996.9		0.5724			
B10	980.5		0.6123			

Criteria Computations:

Name:	SPK2
Condition:	CV(SPK2.RTIME) < 10% AND 50 <= SPK2.RY <= 200
Status:	VALID

Analyst: [redacted] Reviewer: [redacted]

Date: Date:

File Name:	398213040400.plt	Lab Name:	Waisman Center QC
Assay Date:	4/4/2013 5:24:53 PM	Onset OD:	0.05
Collection Mode:	Kinetic Turbidimetric	Wavelength Filter:	340
Type of Curve Fit:	Linear Regression, Avg. Replicates	Reader:	BioTek
Polynomial Order:	N/A	Temperature:	36.9 : 37.0 °C
Serial Number:	153982	Operator:	[redacted]

Standard Set	Range	R-Value	Acceptable	Slope	Y-Intercept
STD1:STD4	5 : 0.005	-0.9997	YES	-0.2470	2.9388

LAL Water Lot #:	99732177	Exp:	FEB2015
LAL Lot #:	D1732L	Exp:	DEC2015
Endotoxin Lot #:	EX11872	Exp:	4/4/13
BG120 Lot #:	DBK0143	Exp:	APR2016
Accessory 1:	N/A	Exp:	N/A
Accessory 2:	N/A	Exp:	N/A
Accessory 3:	N/A	Exp:	N/A
Comments:			

Standards and Controls Information

Ident.	Expected Concentration	Well Layout	Reaction Time	Mean Reaction Time	Standard Deviation	CV%	CV < 10%	Calculated Value
STD1	5 EU/mL	A1	587.4	591.9	6.25	1.06	VALID	4.7248
		A2	600.7					
		A3	587.5					
STD2	0.5 EU/mL	B1	997.5	1005.4	9.43	0.94	VALID	0.5531
		B2	1018.6					
		B3	1000.0					
STD3	0.05 EU/mL	C1	1817.5	1835.0	18.14	0.99	VALID	0.0484
		C2	1860.0					
		C3	1827.5					
STD4	0.005 EU/mL	D1	3300.0	3225.0	111.41	3.45	VALID	<0.0049
		D2	3307.5					
		D3	3067.5					
CTRL1		F1	>3360.0	3360.0	0.00	0.00	VALID	<0.0050
		F2	>3360.0					
		F3	>3360.0					

Analyst: [redacted]
Date:

Reviewer: [redacted]
Date:

File Name:	398213040400.plt	Lab Name:	Waisman Center QC
Assay Date:	4/4/2013 5:24:53 PM	Onset OD:	0.05
Collection Mode:	Kinetic Turbidimetric	Wavelength Filter:	340
Type of Curve Fit:	Linear Regression, Avg. Replicates	Reader:	BioTek
Polynomial Order:	N/A	Temperature:	36.9 : 37.0 °C
Serial Number:	153982	Operator:	[redacted]

Standard Set	Range	R-Value	Acceptable	Slope	Y-Intercept
STD1:STD4	5 : 0.005	-0.9997	YES	-0.2470	2.9388

LAL Water Lot #:	99732177	Exp:	FEB2015
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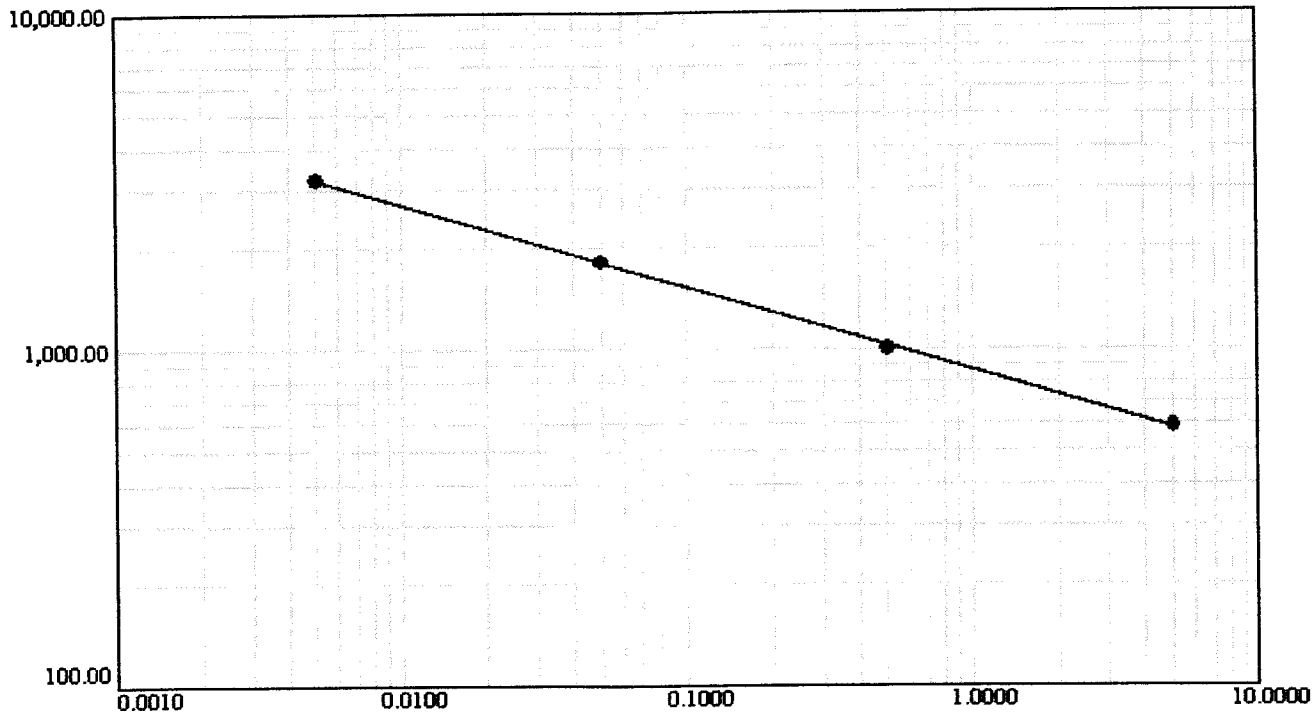
BG120 Lot #:	DBK0143	Exp:	APR2016
Accessory 1:	N/A	Exp:	N/A
Accessory 2:	N/A	Exp:	N/A
Accessory 3:	N/A	Exp:	N/A
Comments:			



X-Axis: Concentration (EU/ml) **Y-Axis:** Reaction Time (sec.)

Equation: Log(RT) = 2.9388 + -0.2470 * Log (EU)

Analyst: [redacted] Reviewer: [redacted]

Date: Date:

File Name:	398213040400.plt	Lab Name:	Waisman Center QC
Assay Date:	4/4/2013 5:24:53 PM	Onset OD:	0.05
Collection Mode:	Kinetic Turbidimetric	Wavelength Filter:	340
Type of Curve Fit:	Linear Regression, Avg. Replicates	Reader:	BioTek
Polynomial Order:	N/A	Temperature:	36.9 : 37.0 °C
Serial Number:	153982	Operator:	[redacted]

Standard Set	Range	R-Value	Acceptable	Slope	Y-Intercept
STD1:STD4	5 : 0.005	-0.9997	YES	-0.2470	2.9388

LAL Water Lot #:	99732177	Exp:	FEB2015
LAL Lot #:	D1732L	Exp:	DEC2015
Endotoxin Lot #:	EX11872	Exp:	4/4/13
BG120 Lot #:	DBK0143	Exp:	APR2016
Accessory 1:	N/A	Exp:	N/A
Accessory 2:	N/A	Exp:	N/A
Accessory 3:	N/A	Exp:	N/A
Comments:			

Plate Criteria Computations:

Condition:

Status: VALID

Analyst: [redacted]

Date:

Reviewer: [redacted]

Date:

File Name:	398213040400.plt	Lab Name:	Waisman Center QC
Assay Date:	4/4/2013 5:24:53 PM	Onset OD:	0.05
Collection Mode:	Kinetic Turbidimetric	Wavelength Filter:	340
Type of Curve Fit:	Linear Regression, Avg. Replicates	Reader:	BioTek
Polynomial Order:	N/A	Temperature:	36.9 : 37.0 °C
Serial Number:	153982	Operator:	[redacted]

Standard Set	Range	R-Value	Acceptable	Slope	Y-Intercept
STD1:STD4	5 : 0.005	-0.9997	YES	-0.2470	2.9388

LAL Water Lot #:	99732177	Exp:	FEB2015
LAL Lot #:	D1732L	Exp:	DEC2015
Endotoxin Lot #:	EX11872	Exp:	4/4/13
BG120 Lot #:	DBK0143	Exp:	APR2016
Accessory 1:	N/A	Exp:	N/A
Accessory 2:	N/A	Exp:	N/A
Accessory 3:	N/A	Exp:	N/A
Comments:			

Well Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD1 STD1 5.0000	STD1 STD1 5.0000	STD1 STD1 5.0000		SPL1 /A14 MCB 0.0100	SPL1 /A14 MCB 0.0100	SPL1 /A14 MCB 0.0100	SPK1 /A14 MCB 0.5000	SPK1 /A14 MCB 0.5000	SPK1 /A14 MCB 0.5000		
B	STD2 STD2 0.5000	STD2 STD2 0.5000	STD2 STD2 0.5000		SPL2 /A14 MCB 0.0010	SPL2 /A14 MCB 0.0010	SPL2 /A14 MCB 0.0010	SPK2 /A14 MCB 0.5000	SPK2 /A14 MCB 0.5000	SPK2 /A14 MCB 0.5000		
C	STD3 STD3 0.0500	STD3 STD3 0.0500	STD3 STD3 0.0500									
D	STD4 STD4 0.0050	STD4 STD4 0.0050	STD4 STD4 0.0050									
E												
F	CTRL1 CTRL1	CTRL1 CTRL1	CTRL1 CTRL1									
G												
H												

Analyst: [redacted] Reviewer: [redacted]

Date: Date:

File Name:	398213040400.plt	Lab Name:	Waisman Center QC
Assay Date:	4/4/2013 5:24:53 PM	Onset OD:	0.05
Collection Mode:	Kinetic Turbidimetric	Wavelength Filter:	340
Type of Curve Fit:	Linear Regression, Avg. Replicates	Reader:	BioTek
Polynomial Order:	N/A	Temperature:	36.9 : 37.0 °C
Serial Number:	153982	Operator:	[redacted]

Standard Set	Range	R-Value	Acceptable	Slope	Y-Intercept
STD1:STD4	5 : 0.005	-0.9997	YES	-0.2470	2.9388

LAL Water Lot #:	99732177	Exp:	FEB2015
LAL Lot #:	D1732L	Exp:	DEC2015
Endotoxin Lot #:	EX11872	Exp:	4/4/13
BG120 Lot #:	DBK0143	Exp:	APR2016
Accessory 1:	N/A	Exp:	N/A
Accessory 2:	N/A	Exp:	N/A
Accessory 3:	N/A	Exp:	N/A
Comments:			

Products

Group	Name	Lot Number	Reaction Time	CV%	CV < 10%	Endotoxin Value	Spike Rcvy %
SPL1	WA14 MCB 1	CRM-WA14-MB-001	>3360.0; >3360.0; >3360.0	0.00	VALID	<0.5000 EU/mL	
SPK1			978.8; 970.0; 941.3	1.66	VALID	0.6576 EU/mL	131
SPL2	WA14 MCB 2	CRM-WA14-MB-001	>3360.0; >3360.0; >3360.0	0.00	VALID	<5.0000 EU/mL	
SPK2			997.5; 996.9; 980.5	0.80	VALID	0.5848 EU/mL	116

Analyst: [redacted]
Date:

Reviewer: [redacted]
Date:

398213040400.plt

File Name:	398213040400.plt	Lab Name:	Waisman Center QC
Assay Date:	4/4/2013 5:24:53 PM	Onset OD:	0.05
Collection Mode:	Kinetic Turbidimetric	Wavelength Filter:	340
Type of Curve Fit:	Linear Regression, Avg. Replicates	Reader:	BioTek
Polynomial Order:	N/A	Temperature:	36.9 : 37.0 °C
Serial Number:	153982	Operator:	[redacted]

Standard Set	Range	R-Value	Acceptable	Slope	Y-Intercept
STD1:STD4	5 : 0.005	-0.9997	YES	-0.2470	2.9388

LAL Water Lot #:	99732177	Exp:	FEB2015
LAL Lot #:	D1732L	Exp:	DEC2015
Endotoxin Lot #:	EX11872	Exp:	4/4/13
BG120 Lot #:	DBK0143	Exp:	APR2016
Accessory 1:	N/A	Exp:	N/A
Accessory 2:	N/A	Exp:	N/A
Accessory 3:	N/A	Exp:	N/A
Comments:			

Time to Reach Onset OD

	1	2	3	4	5	6	7	8	9	10	11	12
A	587.4	600.7	587.5		>3,360.0	>3,360.0	>3,360.0	978.8	970.0	941.3		
B	997.5	1.018.6	1,000.0		>3,360.0	>3,360.0	>3,360.0	997.5	996.9	980.5		
C	1.817.5	1.860.0	1,827.5									
D	3.300.0	3.307.5	3,067.5									
E												
F	>3.360.0	>3.360.0	>3,360.0									
G												
H												

Analyst: [redacted]
Date:

Reviewer: [redacted]
Date:

WuXi AppTec

FINAL STUDY REPORT

STUDY TITLE:	**MYCOPLASMA DETECTION: "POINTS TO CONSIDER"**
PROTOCOL NUMBER:	30055-8
TEST ARTICLE IDENTIFICATION:	CRM-WA14-MB-001
PERFORMING LABORATORY:	WuXi AppTec, Inc. [redacted]
SPONSOR:	Waisman Biomanufacturing [redacted]
STUDY NUMBER:	181035
CLIENT MNEMONIC:	WSM01
RESULT SUMMARY:	Considered **negative** for mycoplasma contamination

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[redacted]

QUALITY ASSURANCE UNIT SUMMARY

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

Critical Phase	Date	Study Director	Management
Reading	02/19/13	02/19/13	03/14/13
Final Report	03/13/13	03/13/13	03/14/13

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

Quality Assurance Auditor: [redacted] Date: 3/14/13

GOOD LABORATORY PRACTICES STATEMENT

The study referenced in this report was conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice (GLP) regulations set forth in 21 CFR part 58.

The studies not performed by or under the direction of WuXi AppTec, Inc., are exempt from this Good Laboratory Practice Statement and include characterization and stability of the test compound(s)/test article.

Study Director: [redacted] Date: 3-14-13

Professional Personnel Involved:

[redacted]

Vice President of Process Improvement and Operations
Manager, Mycoplasma Testing Laboratory
Associate Study Director
Client Relations Manager

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[redacted]

1.0 PURPOSE

To demonstrate that a test article consisting of a cell bank, production or seed lots, or raw materials is free of mycoplasmal contamination, according to "Points to Consider" criteria.

2.0 TEST FACILITY: WuXi AppTec, Inc.

[redacted]

3.0 SCHEDULING

TEST ARTICLE RECEIVED:	01/29/13
INITIATION DATE:	01/31/13
COMPLETION DATE:	03/14/13

4.0 TEST ARTICLE IDENTIFICATION

Test Article Name:	CRM-WA14-MB-001
General Description:	WA14 GMP testing samples, cells and medium suspension
Number of Aliquots used:	1 x 15 mL
Storage Conditions:	Ultracold (< -60 °C)

5.0 TEST ARTICLE CHARACTERIZATION

The Sponsor was responsible for all test article characterization data as specified in the GLP regulations. The identity, strength, stability, purity, and chemical composition of the test article were solely the responsibility of the Sponsor. The Sponsor was responsible for supplying to the testing laboratory results of these determinations and any others that may have directly impacted the testing performed by the testing laboratory, prior to initiation of testing.

Furthermore, it was the responsibility of the Sponsor to ensure that the test article submitted for testing was representative of the final product that was subjected to materials characterization. Any special requirements for handling or storage were arranged in advance of receipt and the test article was received in good condition.

6.0 SAMPLE STORAGE

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

7.0 SAFETY

Appropriate routine safety procedures were followed in handling the test article, unless more cautious procedures were specified by the Sponsor. All applicable WuXi AppTec safety policies and procedures were observed during the performance of the test.

8.0 EXPERIMENTAL DESIGN

8.1 Experimental Summary

Whereas no single test is capable of detecting all mycoplasmal strains, freedom from mycoplasmal contamination may be demonstrated by the use of both an indirect and direct procedure.

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The indirect method of detection allows visualization of mycoplasma, particularly non-cultivable strains, by growing the mycoplasma on an indicator cell line and then staining using a DNA-binding fluorochrome stain. The indicator cell line should be easy to grow, have a large cytoplasmic to nuclear area ratio and support the growth of a broad spectrum of mycoplasma species. The African green monkey kidney cell line, Vero, fits this description and was used in this assay. The assay was performed with negative and positive controls. Both a strongly cyto-adsorbing (*M. hyorhinis*) and a poorly cyto-adsorbing (*M. orale*) mycoplasma species were used as positive controls. Staining the cultures with DNA binding fluorochrome allows for the detection of mycoplasma based on the staining pattern observed. Only the cell nuclei demonstrate fluorescence in the negative cultures but nuclear and extra-nuclear fluorescence is observed in positive cultures.

Direct cultivation is a sensitive and specific method for the detection of mycoplasma. The agar and broth media employed supply nutrients necessary for the growth of cultivable mycoplasmas. These media also supply a source of carbon and energy, and favorable growth conditions. The direct assay was performed with both negative and positive controls. A fermentative mycoplasma (*M. pneumoniae)* and a non-fermentative mycoplasma *(M. orale)* were used as positive controls. The procedure employed in this study is based on the protocol described in the 1993 Attachment # 2 to the "Points To Consider" document, as recommended by the FDA, Center for Biologics Evaluation and Research (CBER).

8.2 Justification for Selection of the Test System

Contamination of cell cultures by mycoplasma is a common occurrence and is capable of altering normal cell structure and function. Among other things, mycoplasma may affect cell antigenicity, interfere with virus replication, and mimic viral actions. Testing for the presence of mycoplasma for cell lines used to produce biologicals is recommended by the FDA, Center for Biologics Evaluation and Research (CBER) under "Points to Consider."

8.3 PROTOCOL AMENDMENTS/DEVIATIONS

There were no amendments or deviations that occurred during the course of this study.

9.0 IDENTIFICATION OF THE TEST SYSTEM

9.1 Test Sample Preparation

The test article, one tube containing 15 mL, was thawed at 37 ± 2 °C and 1:5 and 1:10 dilutions were prepared using sterile phosphate buffered saline (PBS).

1 mL of the undiluted test article, the 1:5, and 1:10 dilutions were then inoculated onto each of two coverslips (per sample/dilution) containing Vero cells. The coverslips were incubated for 1-2 hours at 36 ± 1 °C / 5 - 10% CO_2 and then 2 mL of EMEM, 8% fetal bovine serum (FBS) was added to each coverslip. The coverslips were again incubated at 36 ± 1 °C / 5 - 10% CO_2. After three days of incubation, the coverslips were fixed, stained, and then read using an epifluorescent microscope.

0.2 mL of the undiluted test article was then inoculated onto each of two SP-4 agar plates, and 10 mL was inoculated into a 75 cm^2 flask containing 50 mL of SP-4 broth. The plates were incubated anaerobically at 36 ± 1 °C for a minimum of 14 days.

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The broth flask was incubated aerobically at 36 ± 1 °C, and subcultured onto each of two SP-4 agar plates (0.2 mL/plate) on Days 3, 7, and 14. These subculture plates were incubated anaerobically at 36 ± 1 °C for a minimum of 14 days. The broth flask was read each working day for 14 days. The SP-4 agar plates (Day 0) were read after 14 days of incubation. The SP-4 broth subculture plates (Days 3, 7, and 14) were read after 14 days incubation.

9.2 Controls and Reference Materials

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

9.2.2 Positive Controls

a. Indirect Assay

a.1 Strongly cyto-adsorbing species - *M. hyorhinis* GDL (ATCC #23839) at 100 or fewer colony forming units (CFU) per inoculum.

a.2 Poorly cyto-adsorbing species - *M. orale* (ATCC #23714) at 100 or fewer CFU per inoculum.

b. Direct Assay

b.1 Nonfermentative mycoplasma species - *M. orale* (ATCC #23714) at 100 or fewer CFU per inoculum.

b.2 Fermentative mycoplasma species - *M. pneumoniae* FH (ATCC #15531) at 100 or fewer CFU per inoculum.

9.2.3 Control Preparation

a. Negative Controls

a.1 1 mL of sterile SP-4 broth was inoculated onto each of two coverslips containing Vero cells to serve as the negative control in the indirect assay.

a.2 0.2 mL of SP-4 broth was inoculated onto each of two SP-4 agar plates to serve as the negative control in the direct assay. 10 mL of SP-4 broth was inoculated into a 75 cm^2 flask containing 50 mL of SP-4 broth to serve as the negative control in the direct assay.

b. Positive Controls

b.1 *M. hyorhinis, M. orale,* and *M. pneumoniae* were diluted to ≤ 100 CFU per inoculum in sterile SP-4 broth. 1 mL of *M. hyorhinis* and *M. orale* at ≤ 100 CFU/mL was inoculated onto each of two coverslips containing Vero cells. These coverslips served as the positive controls in the indirect assay.

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b.2 The coverslips were incubated for 1-2 hours at 36 ± 1 °C / 5 - 10% CO_2 and then 2 mL of EMEM, 8% fetal bovine serum (FBS) was added to each coverslip. The coverslips were again incubated at 36 ± 1 °C / 5 - 10% CO_2. After three days of incubation, the cell cultures were fixed, stained, and then read using an epifluorescent microscope.

b.3 0.2 mL of *M. orale* and *M. pneumoniae* at ≤ 100 CFU/plate was inoculated onto each of two SP-4 agar plates. 10 mL of *M. orale* and *M. pneumoniae* at ≤ 10 CFU/mL (≤ 100 CFU/inoculum) were each inoculated into a 75 cm^2 flask containing 50 mL of sterile SP-4 broth.

b.4 The agar plates were incubated anaerobically at 36 ± 1 °C for 14 days. The broth cultures were incubated aerobically at 36 ± 1 °C for a minimum of 14 days and were read each working day for 14 days. On Days 3, 7, and 14, 0.2 mL from each broth culture flask was subcultured onto each of two SP-4 agar plates. These subculture plates were incubated anaerobically at 36 ± 1 °C. The subculture plates were observed microscopically for the presence of mycoplasma colonies after a minimum of 14 days incubation.

c. See Section 17.0, Results, for the results of these controls.

10.0 TEST EVALUATION

10.1 Indirect Assay

Hoechst stain will bind to most DNA containing organisms and organelles present in the culture; this includes indicator cell nuclei, prokaryotes including mycoplasma and cell debris. The source of DNA is determined by the staining pattern. The indicator cell nuclei fluoresce brightly and are generally 10-20 μm in diameter. Mycoplasma fluorescence is less intense, is extra-nuclear and typically appears as small round bodies approximately 0.3 μm in diameter.

10.2 Direct Assay

Change in color or turbidity of broth culture can be an indicator of the presence of mycoplasma growth. Fermentative mycoplasma produce acid from the carbohydrates in the medium causing the pH of the medium to drop and the broth to turn yellow in color. Nonfermentative mycoplasma produce ammonia by arginine hydrolysis causing the pH to rise and the broth to turn red. In general, growth of mycoplasma can cause the broth to become turbid. These changes must be confirmed by agar plate subculture or DNA-staining since changes in the appearance of the broth culture can also be caused by the properties of the inoculum.

Mycoplasma colonies grow down into the agar causing the center of the colony to appear opaque and the peripheral surface growth to appear translucent. These "fried-egg" colonies vary in size, 10-500 μm, and can be readily observed unstained using a light microscope.

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[redacted]

10.3 Indirect Assay and Direct Assay Results Interpretation

If:	Test Article				
Mycoplasmal fluorescence	-	+	+/-	+/-	-
Broth (Color change or turbidity change)	-	+/-	+/-	+/-	+*
Agar - Day 0 (at least one plate)	-	+/-	+/-	+	-
Agar - Day 3, 7, 14 (at least one plate on one day)	-	+/-	+	+/-	-
Then: Overall Final Result	**-**	**+**	**+**	**+**	**-**

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

10.4 Positive Results

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

10.5 Negative Results

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

11.0 ASSAY VALIDITY

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

11.1 Indirect Assay

Detection of Mycoplasma Contamination by Indirect Assay

Controls	Mycoplasma Fluorescence Observed (At least one coverslip required for the evaluation)
Negative Control	-
**M. hyorhinis* (≤ 100 CFU)	+
**M. orale* (≤ 100 CFU)	+

*In at least one coverslip

11.2 Direct Assay

Detection of Mycoplasma Contamination by Direct Assay

	Negative Control	M. [illegible]	M. orale
Broth (Color change or turbidity change)	-	+ / -	+ / -
Agar Day 0 (at least one plate)	-	+	+
Agar Day 3, 7, 14 (at least one plate on one day)	-	+	+
Results	-	+	+

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[redacted]

12.0 METHOD FOR CONTROL OF BIAS: Not applicable.

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

14.0 STATISTICAL METHODS
The results of this study were qualitative; therefore no statistical analysis was required.

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

16.0 TEST ARTICLE DISPOSITION
Unused test samples remain in the storage area until all testing is completed. Once completed, the remaining samples were discarded or returned as requested by the Sponsor.

17.0 RESULTS

Indirect Assay and Direct Assay Results

Sample	Indirect	Direct		
		Day 0 Agar Slants	Broth Flasks And Associated Subculture Plates	Overall
Test Article	Negative	Negative	Negative	Negative
Negative Control	Negative	Negative	Negative	Negative
M. hyorhinis	Positive			Positive
M. orale	Positive	Positive	Positive	Positive
M. pneumoniae		Positive	Positive	Positive

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

18.0 ANALYSIS AND CONCLUSION

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

18.2 These findings indicate that the test article is considered **negative** for the presence of mycoplasma contamination.

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[redacted]

19.0 REFERENCES

19.1 Barile, Michael F. and McGarrity, Gerard J. (1983). "Isolation of Mycoplasmas from Cell Culture by Agar and Broth Techniques." Methods in Mycoplasmology, Vol II, ed. J.G. Tully and S. Razin. (New York: Academic Press) pp. 159-165.

19.2 Del Giudice, Richard A. and Tully, Joseph G. 1996. "Isolation of Mycoplasma from Cell Cultures by Axenic Cultivation Techniques," ed. J.G. Tully and S. Razin, Molecular and Diagnostic Procedures in Mycoplasmology, Vol. II (New York: Academic Press).

19.3 McGarrity, Gerard J. and Barile, Michael F. 1983. "Use of Indicator Cell Lines for Recovery and Identification of Cell Culture Mycoplasmas," ed. J.G. Tully and S. Razin, Methods in Mycoplasmology, Vol. II (New York: Academic Press).

19.4 Masover, Gerald and Frances Becker. 1996. "Detection of Mycoplasma by DNA Staining and Fluorescent Antibody Methodology," ed. J.G. Tully and S. Razin, Molecular and Diagnostic Procedures in Mycoplasmology, Vol. II (New York: Academic Press).

19.5 Schmidt, Nathalie J. and Emmons, Richard W. 1989. "Cell Culture Procedures for Diagnostic Virology," ed. Nathalie J. Schmidt and Richard W. Emmons, 6th ed., Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections (Washington: American Public Health Association).

19.6 U.S. Food and Drug Administration (FDA) Center for Biologics Evaluation and Research (CBER). 1993. "Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals."

19.7 WuXi AppTec SOP: MTL-30055, Mycoplasma Detection "Points to Consider"

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[redacted]

FINAL STUDY REPORT

STUDY TITLE: ***In Vitro*** **Assay for Adventitious Virus Contaminants: MRC-5, VERO and NIH/3T3 Cells (Extended Duration) with Hemadsorption and Hemagglutination Endpoints (GLP)**

TEST PROTOCOL NUMBER: 32869.00

TEST ARTICLE IDENTIFICATION	WUXI APPTEC ACCESSION NUMBER
CRM-WA14-MB-001	13-000225

SPONSOR: [redacted]

PERFORMING LABORATORY: WuXi AppTec, Inc. [redacted]

WUXI APPTEC ACCESSION NUMBER	RESULTS
13-000225	The presence of adventitious virus was not detected in the test article.

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[redacted]

JL 3/12/13

QUALITY ASSURANCE UNIT SUMMARY

STUDY: *In Vitro* Assay for Adventitious Virus Contaminants: MRC-5, VERO and NIH/3T3 Cells (Extended Duration) with Hemadsorption and Hemagglutination Endpoints (GLP)

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

Phase Inspected	Date
Step 4.7.3 Passage 1, observed test article pass (NIH/3T3 cells).	February 10, 2013

[redacted] 07MAR13
Quality Assurance Date

GOOD LABORATORY PRACTICES STATEMENT

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

[redacted] 07MAR13
Study Director Date

Professional Personnel involved in Study:

[redacted]
Chief Scientist of Virology and VP of Service Development
Director of Virology Operations - Virology & Viral Clearance
Study Director, Virology/Operations
Senior Director of Operations

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[redacted]

√LL 3/12/13

1.0 PURPOSE

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

2.0 SPONSOR:

Waisman Biomanufacturing
[redacted]

3.0 TEST FACILITY:

WuXi AppTec, Inc.
[redacted]

4.0 SCHEDULING

DATE SAMPLES RECEIVED: January 29, 2013
STUDY INITIATION DATE: February 1, 2013
STUDY COMPLETION DATE: See page 2 for Study Director's signature and date.

5.0 TEST ARTICLE CHARACTERIZATION

Determinations of strength, homogeneity, purity, and stability of the test article are solely the responsibility of the Sponsor. The Sponsor is responsible for supplying to the testing laboratory results of these determinations and any others that may directly impact the testing performed by the testing laboratory, prior to initiation of testing.

6.0 TEST ARTICLE IDENTIFICATION: CRM-WA14-MB-001

7.0 TEST SYSTEM DESCRIPTION

Utilization of mammalian cells in the manufacture of biologicals leads to the potential risk of contamination by adventitious viruses.[1-6] Many human and animal viruses share this designation. These viruses vary widely in their pathogenicity but account for significant morbidity and mortality.[7,8] The choice of cell lines used in this assay is dictated by the 1993 Points to Consider[2] and ICH Q5A[3] guidelines.

Introduction of test article cells and/or culture fluids derived from such cells to a human embryonic cell line (MRC-5), a simian kidney cell line (VERO), and a murine fibroblast cell line (NIH/3T3) allows the detection of a wide range of animal and human viruses, including picornaviruses (poliovirus, coxsackievirus groups A and B, echovirus, and rhinovirus), orthomyxoviruses (influenza), paramyxoviruses (parainfluenza, mumps, and measles), herpesviruses (herpes simplex and cytomegalovirus), adenoviruses, and reoviruses.[1]

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[redacted]

√LL 3/12/13

Inoculated indicator cell cultures are examined at least twice a week for at least 28 days and compared to the positive controls for the development of characteristic changes in morphology attributable to the growth of viral agents. Since orthomyxo- and paramyxoviruses may replicate in MRC-5, VERO or NIH/3T3 cells with the development of little or no cytopathic effects,[1] the presence of these viruses is detected by their ability to adsorb erythrocytes to the surface of infected cells.[7] This hemadsorption assay is performed at the conclusion of the observation period on day 28 or later. In addition, a hemagglutination assay is performed at the conclusion of the observation period of 28 days or longer.

8.0 EXPERIMENTAL DESIGN

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

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

8.2 MRC-5, VERO and NIH/3T3 cell monolayers were inoculated with disrupted, clarified test article lysate. A total of 0.2 mL of test article was used as inoculum per well for each cell line. Six wells were inoculated per cell line.

8.3 The positive cultures were established: MRC-5 cells were inoculated with encephalomyocarditis virus (EMC), VERO cells were inoculated with adenovirus type 5 (Ad 5) and NIH/3T3 cells were inoculated with herpes simplex type 1 virus (HSV-1).

8.4 Inoculated indicator cell cultures were incubated at 37±2°C in a humidified atmosphere of 5±2% CO_2 and observed at least twice a week for 28 days for changes in morphology. The cells were observed for evidence of inclusion bodies, cell rounding, abnormal number of/or presence of giant cells, or any other cytopathology attributable to an extraneous agent.[8,9]

8.5 Maintenance medium was replaced as required to support cell health. Subcultivation was performed on days 7, 14, and 21.[2,9]

8.6 On day 28, the hemadsorption assay was performed:[1] A volume of 0.5 mL of chicken, guinea pig, and human type O erythrocytes, which had been previously washed 3 times in buffered saline and resuspended to yield a 0.5% suspension, was added separately to the test article-inoculated and control monolayers. The replicate cultures were incubated at 2-8°C and 20-25°C for 30-45 minutes and observed macroscopically and microscopically for adsorption of erythrocytes to the monolayers.

8.7 On day 28, conditioned media from each culture (negative control and test article cultures) is harvested and assayed for hemagglutination activity (HA) with suspensions of chicken, guinea pig and human erythrocytes. Replicate plates are incubated at 2-8°C or 37±2°C for 1-2 hours and HA activity is determined.

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9.0 TEST ARTICLE PREPARATION

On January 29, 2013, WuXi AppTec, Inc. received 1 tube containing 6.0 ml of "WA14 GMP testing samples, cells and medium suspension," frozen in dry ice and designated for use in this assay. The test article was stored at ≤ -60°C until the assay was initiated.

On the day of inoculation, February 03, 2013, the test article was thawed using a 37±2°C waterbath and was subjected to one additional freeze/thaw cycle using a dry ice/ethanol bath and a 37±2°C waterbath. The test article was clarified by low-speed centrifugation and the supernatant was then inoculated as per step 8.2 in the experimental design section

10.0 POSITIVE CONTROLS

10.1 Positive control inocula were virus stocks, which have met the criteria set forth in an internal SOP.

Positive controls for CPE were:

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

10.2 The positive control for hemadsorption was one set of VERO negative control cultures infected with parainfluenza type 3 (PI3) virus.

11.0 NEGATIVE CONTROLS

MRC-5, VERO and NIH/3T3 cultures inoculated with EMEM served as the negative control cultures for CPE and hemadsorption.

12.0 ASSAY VALIDITY

The test is considered valid when the following conditions are met: the positive control cultures must demonstrate characteristic cytopathic changes, the positive control for hemadsorption must demonstrate hemadsorption with RBCs, the positive control for hemagglutination must demonstrate hemagglutination with RBCs, and the negative control cultures must be negative for viral cytopathic changes, hemadsorption and hemagglutination.

13.0 TEST EVALUATION

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

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

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14.0 RESULTS

The test was valid. MRC-5 cultures infected with EMC were positive; VERO cultures infected with Ad 5 were positive, and NIH/3T3 cultures infected with HSV-1 were positive. Cell line negative control cultures showed no morphologic changes over the 28-day test period (Table 1).

VERO negative cultures infected with PI3 virus were positive for hemadsorption when used as positive controls run on day 28 of the assay. Negative control cultures showed no morphologic changes and no hemadsorption activity (Table 2).

MRC-5, VERO and NIH/3T3 cultures inoculated with the test article did not demonstrate changes that would be expected with viral contamination (Table 1). The test article-inoculated cell cultures did not induce hemadsorption activity (Table 2). In addition, the test article-inoculated cell cultures did not induce hemagglutination (Table 3). Thus, the presence of adventitious virus was not detected in the test article.

TABLE 1: Detection of Viruses by Observation of Viral Cytopathic Changes

Culture Inoculum	Cell Line		
	MRC-5	VERO	NIH/3T3
Accession #13-000225	-	-	-
EMEM (Negative Control)	-	-	-
Encephalomyocarditis (Positive Control)	+	NA	NA
Adenovirus type 5 (Positive Control)	NA	+	NA
Herpes Simplex Type 1 (Positive Control)	NA	NA	+

Legend:

- \- Negative reaction indicating absence of viral agent
- \+ Positive reaction indicating presence of viral agent

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TABLE 2: Hemadsorption Activity of Indicator Cell Lines

Culture Inoculum	Cell Line	Day of Test	2 - 8°C			20 – 25°C		
			C	GP	H	C	GP	H
Accession #13-000225	**MRC-5**	**28**	-	-	-	-	-	-
	VERO	**28**	-	-	-	-	-	-
	NIH/3T3	**28**	-	-	-	-	-	-
EMEM (Negative Control)	MRC-5	28	-	-	-	-	-	-
	VERO	28	-	-	-	-	-	-
	NIH/3T3	28	-	-	-	-	-	-
Parainfluenza Type 3 Virus (Positive Control 1:10 Dilution)	VERO	28	+	+	+	+	+	+
Parainfluenza Type 3 Virus (Positive Control 1:20 Dilution)	VERO	28	+	+	+	+	+	+

Legend:

C	Chicken erythrocytes
GP	Guinea pig erythrocytes
H	Human type O erythrocytes
-	Negative reaction indicating absence of viral agent
+	Positive reaction indicating presence of viral agent

TABLE 3: Hemagglutination Activity of Indicator Cell Lines

Sample	Supernatant From	2 - 8°C			37 ± 2°C		
		C	GP	H	C	GP	H
Accession #13-000225	**MRC-5**	-	-	-	-	-	-
	VERO	-	-	-	-	-	-
	NIH/3T3	-	-	-	-	-	-
Negative Control Supernatant	MRC-5	-	-	-	-	-	-
	VERO	-	-	-	-	-	-
	NIH/3T3	-	-	-	-	-	-
Virus Stock	Influenza A	+	+	+	+	+	+

Legend:

C	Chicken erythrocytes
GP	Guinea pig erythrocytes
H	Human type O erythrocytes
-	Negative reaction indicating absence of viral agent
+	Positive reaction indicating presence of viral agent

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15.0 CONCLUSION

Evidence of adventitious virus contamination was not detected in the test article.

16.0 STATISTICAL DATA ANALYSIS

Statistical analysis of the data was not required.

17.0 DEVIATIONS / AMENDMENTS

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

No amendments to the protocol were generated.

18.0 RECORD RETENTION

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

19.0 REFERENCES

1. Jacobs JP, McGrath DI, Garrett AJ, and Schild GC (1981). "Guidelines for the acceptability, management, and testing of serially propagated human diploid cells for the production of live virus vaccines for use in man." *J Biol Stand* 9: 331-342

2. "Points To Consider In The Characterization Of Cell Lines Used To Produce Biologicals" (1993). Center For Biologics Evaluation And Research Food And Drug Administration

3. International Conference on Harmonisation of Technical Requirements for Registration of Pharamceuticals for Human Use. Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin. ICH Q5A (R1). 23 September 1999

4. Quality of Biotechnological Products: Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products. ICH Topic Q5D. March 1998

5. Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use. US Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research. February 28, 1997

6. Committee for Medicinal Products for Human Use (CHMP). Guideline on Development, Production, Characterisation and Specifications for Monoclonal Antibodies and Related Products. 18 December 2008

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LOT-QCP-028- 19177 JS 11Mar2013

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

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

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

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E-FR-1-13-000225.30194

FINAL STUDY REPORT

STUDY TITLE: ***In Vivo*** **Assay for Viral Contaminants: European and US/FDA Test (GLP)**

TEST PROTOCOL NUMBER: 30194.02

TEST ARTICLE IDENTIFICATION	WUXI APPTEC ACCESSION NUMBER
CRM-WA14-MB-001	13-000225

SPONSOR: [redacted]

PERFORMING LABORATORY: WuXi AppTec, Inc.
[redacted]

WUXI APPTEC ACCESSION NUMBER	RESULTS
13-000225	No evidence of viral contamination was detected in the test article by inoculation of embryonated hen eggs.

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[redacted]

QUALITY ASSURANCE UNIT SUMMARY

STUDY: In Vivo Assay for Viral Contaminants: European and US/FDA Test (GLP)

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

Phase Inspected	Date
Step 4.6.5 Inoculation of test article via the yolk sac route.	February 25, 2013

[redacted]
Quality Assurance

02Apr13
Date

GOOD LABORATORY PRACTICES STATEMENT

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

[redacted]
Study Director

03Apr13
Date

Professional Personnel involved in study:

[redacted] Laura Schina, Kathleen Kenney, Babatunde Sholanke, Albert Guerrini

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[redacted]

1.0 PURPOSE

The purpose of this assay is to detect the presence of adventitious viral agents in a test article by inoculation of embryonated hen eggs, mice (suckling and adult), and guinea pigs.

2.0 SPONSOR:

Waisman Biomanufacturing
[redacted]

3.0 TEST FACILITY:

WuXi AppTec, Inc.
[redacted]

4.0 SCHEDULING

DATE SAMPLES RECEIVED: January 29, 2013
STUDY INITIATION DATE: January 31, 2013
STUDY COMPLETION DATE: See page 2 for Study Director's signature and date.

5.0 TEST ARTICLE CHARACTERIZATION

Determinations of strength, homogeneity, purity and stability of the test article are solely the responsibility of the Sponsor. The Sponsor is responsible for supplying to the testing laboratory results of these determinations and any others that may directly impact the testing performed by the testing laboratory, prior to initiation of testing.

6.0 TEST ARTICLE IDENTIFICATION: CRM-WA14-MB-001

7.0 TEST SYSTEM DESCRIPTION

Utilization of mammalian cells in the manufacture of biologicals leads to the risk of contamination by adventitious viruses.[1,2] This study is designed to detect murine and non-murine viruses that are not detected by the Mouse Antibody Production Test or *in vitro* culture studies. This study is designed to fulfill the European requirements for *in vivo* viral contaminant testing and the requirements for in vivo viral contaminant testing as described in the "Points to Consider from the FDA" for US/FDA requirements.[6]

The test article is inoculated into the following test systems: adult mice, suckling mice, quinea pigs, and embryonated hen's eggs. Adult mice are susceptible to a number of used agents, including coxsackie viruses and members of the flavivirus group (St. Louis encephalitis virus and Japanese encephalitis virus).[3] Suckling mice are susceptible to a wide range of viruses including togaviruses, bunyaviruses, flaviruses, picornaviruses (poliovirus, coxsackievirus groups A & B, echovirus) and herpes viruses (herpes simplex). Guinea pigs are susceptible hosts for a number of viral agents, including paramyxoviruses (Sendai) and reoviruses.

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Embryonated eggs are inoculated by 4 routes: the chorioallantoic membrane (CAM), the amniotic cavity, the yolk sac and the allantois. Inoculation by the CAM route favors detection of viruses such as herpes simplex virus, vaccinia, or variola, which produce plaques or pocks on the chorioallantois.[5] Orthomyxo- (influenza virus) and paramyxoviruses (parainfluenza, mumps, measles) replicate more efficiently in the respiratory tract of the embryo (inoculum introduced into the amniotic cavity is swallowed by the embryo and enters the respiratory tract, thereby enhancing the possibility of viral propagation), or the entodermal cells of the allantois, and thus detection of these viruses is favored when the test article is inoculated by the amniotic and allantoic routes.[4,5] The yolk sac route favors propagation of herpes viruses, rickettsiae, mycoplasma and bacteria.[4,5] Subpassage of materials from inoculated suckling mice and inoculated embryonated eggs into new test systems serves to increase the sensitivity of the assay since viral agents present in the original inoculum would be amplified through this serial passage.

8.0 EXPERIMENTAL DESIGN

The test article will be maintained according to the Sponsor's instructions. When the test article consists of cells, typically a suspension of 10^7 cells/ml will be prepared as inoculum; test article cells will be disrupted by performing a total of 2 freeze-thaws to produce a lysate, which will be then clarified by low-speed centrifugation. If the test article consists of supernatant fluids or purified vector products, it will be used directly. Virus vectors can be tested by distributing a specific testing level over a certain number of animals and/or eggs per route of inoculation or by bringing a Sponsor's designated volume up to the required testing volume. Sample material will be typically filtered through a 0.45-µm cellulose acetate low protein-binding filter prior to inoculation.

8.1 Embryonated Eggs

The embryonated egg testing will be performed at WuXi AppTec's Philadelphia, PA facility.

A minimum of 5 eggs will serve as delivery controls. These eggs will be candled each working day for viability and will be chilled at the end of the incubation period. These eggs will not be harvested or examined after being chilled.

For allantoic cavity (CAS) inoculations, the allantoic cavity of at least ten (10) embryonated hen eggs (9-11 days old) will be inoculated with 0.5 ml of 0.45-µm filtered test article. The embryonated eggs will be incubated at 37-38°C for at least 5 days. The embryos will be candled each working day for viability. Embryos that die prior to the end of the incubation period will be chilled and later examined with all other embryos at the end of the incubation period. The passage 1 (P1) allantoic fluids will be harvested, pooled, and stored at or below -60°C until assayed for hemagglutination activity (HA) or until subpassaged into a second set of at least ten (10) eggs (9-11 days old). Prior to inoculating the second set of eggs, the subpassaged material will be clarified by low-speed centrifugation and filtered through a 0.45-µm filter. The second set of eggs will be incubated under the same conditions as the first set. The passage 2 (P2) allantoic fluids will be harvested, pooled, and stored at or below -60°C until assayed for HA, after which time the embryos will be examined. To establish negative controls, this procedure will be run in parallel for two additional sets of embryonated eggs using 0.45-µm filtered Eagle's Minimal Essential Medium (EMEM) as the initial inoculum.

[redacted]

For amniotic (AM) inoculations, at least ten (10) embryonated hen eggs (9-11 days old) will be inoculated by introduction of 0.1 ml of 0.45-µm filtered test article into the amniotic cavity. To establish negative controls, a second set of at least ten (10) embryonated eggs will be inoculated with 0.1 ml of 0.45-µm filtered EMEM. The eggs will be incubated at 37-38°C for at least 5 days. The embryos will be candled each working day for viability. Embryos that die prior to the end of the incubation period will be chilled and later examined with all the other embryos at the end of the incubation period. After at least 5 days of incubation, the allantoic fluids will be harvested and pooled. A portion of the pooled allantoic fluids will be frozen and stored at or below -60°C until assayed for hemagglutination activity (HA).

The HA assay will be performed in microtiter plates by making serial two-fold dilutions of the allantoic fluids collected from the negative controls, test article-inoculated eggs, and of an influenza type A viral stock. Washed chicken, guinea pig, and human erythrocytes will be added separately as 0.5% suspensions, and replicate plates will be observed for HA after incubation for 1-2 hours at 2 temperatures (2-8°C and 37±2°C).

For chorioallantoic membrane (CAM) inoculations, at least ten (10) embryonated hen eggs (9 to 11 days) old will be inoculated by the introduction of 0.1 ml of 0.45-µm filtered test article onto the chorioallantoic membrane. To establish negative controls, a second set of at least ten (10) embryonated eggs will be inoculated similarly by the introduction of 0.1 ml of 0.45-µm filtered EMEM onto the CAM. The eggs will be incubated at 37-38°C for at least 5 days. The embryos will be candled each working day for viability. Embryos that die prior to the end of the incubation period will be chilled and later examined with all the other embryos at the end of the incubation period. At the end of the incubation period, the upper portion of the shell will be cut away, the CAM will be observed for pock or plaque formation, and the embryos will be examined.

For yolk sac (ys) inoculations, the yolk sac of at least ten (10) embryonated eggs (9-11 days old) will be inoculated with 0.5 ml of 0.45-µm filtered test article. The eggs will be incubated at 37-38°C for at least 5 days. The embryos will be candled each working day for viability. Embryos that die prior to the end of the incubation period will be chilled and later examined with all the other embryos at the end of the incubation period. Then the P1 yolk sacs will be harvested, washed, and pooled. A 10% yolk sac suspension will be prepared and stored at or below -60°C until subpassaged into a second set of at least ten (10) eggs (9-11 days old). Prior to inoculating the second set of eggs or for the HA, the subpassaged material will be clarified by and filtered through a 0.45-µm filter. The second set of eggs will be incubated under the same conditions as the first set, after which time the embryos will be examined. To establish negative controls, this procedure will be run in parallel for two additional sets of embryonated eggs using 0.45-µm filtered EMEM as the initial inoculum.

8.2 Materials

8.2.1 Embryonated Hen Eggs

Source:	SPAFAS or other certified vendor
Grade:	Specific pathogen-free (SPF)
Age:	9-11 days

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[redacted]

9.0 TEST ARTICLE PREPARATION

On January 29, 2013, WuXi AppTec, Inc. received 1 tube containing 20 mL of "WA14 GMP testing samples, cells and medium suspension," frozen in dry ice and designated for use in this assay. The test article was stored at ≤ -60°C until the assay was initiated.

10.0 POSITIVE CONTROLS

Stock influenza type A virus served as the positive control for the hemagglutination assay.

11.0 NEGATIVE CONTROLS

Embryonated eggs inoculated with EMEM served as negative controls. A minimum of 5 embryonated eggs served as delivery controls. Procedural information (including age of control system, inoculation amounts and routes, filtration procedures and incubation conditions) for negative controls was the same as that for the corresponding test systems.

For the HA assay, pooled allantoic fluids harvested from the negative control embryonated eggs and EMEM alone served as negative controls.

12.0 ASSAY VALIDITY

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

12.1 At least 80% of negative control embryos must survive the test period in good health (after subtracting those embryos that die due to trauma or bacterial contamination).

12.2 Negative control samples must not hemagglutinate any species of red blood cells; the positive control must hemagglutinate all species of red blood cells.

13.0 TEST EVALUATION

A test article is considered negative for the presence of detectable adventitious viral contaminants if the following criteria are met.

13.1 At least 80% of the test article-inoculated embryos must survive the test period (after subtracting those embryos that die due to trauma or bacterial contamination) and must be normal in appearance.

13.2 The allantoic fluids collected from the test article-inoculated embryos must not produce hemagglutination.

If any of these criteria are not met, the Study Director, in consultation with Quality Assurance, will determine based on the study observations and other relevant information whether the test article is positive for adventitious virus or if additional testing is required to determine a root cause.

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14.0 RESULTS

The test was valid. At least 80% of negative control embryos survived the test period in good health (after subtracting those embryos that die due to trauma or bacterial contamination). Negative control samples did not hemagglutinate any species of red blood cells, and the positive controls hemagglutinated all 3 species of red blood cells.

Allantoic Inoculation:

Eight (8) of twelve (12) embryonated eggs inoculated with test article via the allantoic cavity survived the test period and were normal when observed at the conclusion of the observation period. Four (4) embryonated eggs died from apparent trauma on day 1 of the test period and were hemorrhagic when observed. Upon subpassage of the P1 pooled allantoic fluids, twelve (12) of twelve (12) embryonated eggs in the second set survived the test period and were normal when observed at the conclusion of the observation period (Table 1). Neither P1 nor P2 pooled allantoic fluids hemagglutinated the 3 species of erythrocytes (Table 4).

TABLE 1: Effects of Inoculum on Viability of the Allantoic Inoculated Embryonated Eggs

Route of Inoculation	Inoculum	Viable/Total	Death by Trauma[1]
Allantoic	**Accession #13-000225**	**8/12**	**4**
Allantoic	EMEM	7/12	5
Allantoic	**Subpassaged Accession #13-000225**	**12/12**	**NA**
Allantoic	Subpassaged EMEM	10/12	2

Legend: 1 The number of deaths due to trauma. This observation was taken at 24 – 48 hours post-inoculation rather than at the conclusion of the passage time.
NA Not applicable

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Yolk Sac Inoculation:

Ten (10) of twelve (12) embryonated eggs inoculated with test article via the yolk sac survived the test period and were normal when observed at the conclusion of the observation period. Two (2) embryonated eggs died from apparent trauma on day 1 of the test period and were hemorrhagic when observed. Upon subpassage of the P1 10% yolk sac suspension, twelve (12) of twelve (12) embryonated eggs in the second set survived the test period and were normal when observed at the conclusion of the observation period (Table 2).

TABLE 2: Effects of Inoculum on Viability of the Yolk Sac Inoculated Embryonated Eggs

Route of Inoculation	Inoculum	Viable/Total	Death by Trauma[1]
Yolk Sac	**Accession #13-000225**	**10/12**	**2**
Yolk Sac	EMEM	6/12	6
Yolk Sac	**Subpassaged Accession #13-000225**	**12/12**	**NA**
Yolk Sac	Subpassaged EMEM	11/12	1

Legend: [1] The number of deaths due to trauma. This observation was taken at 24 – 48 hours post-inoculation rather than at the conclusion of the passage time.
NA Not applicable

Amniotic Inoculation:

Nine (9) of twelve (12) embryonated eggs inoculated with the test article via the amniotic cavity survived the test period and were normal at the end of the 5-day observation period. Three (3) embryonated eggs died from apparent trauma on day 1 of the test period and were hemorrhagic when observed (Table 3). Pooled allantoic fluids harvested from the nine (9) surviving embryos did not hemagglutinate any of the three species of erythrocytes (Table 4).

Chorioallantoic Membrane Inoculation:

Twelve (12) of twelve (12) embryonated eggs inoculated with the test article via the chorioallantoic membrane (CAM) survived the test period and were normal at the end of the 5-day observation period (Table 3). No lesions were observed on the CAM of the twelve (12) surviving embryos.

TABLE 3: Effects of Inoculum on Viability of Embryonated Eggs

Route of Inoculation	Inoculum	Viable/Total (in Test System)	Death by Trauma[1]
Amniotic	**Accession #13-000225**	**9/12**	**3**
	EMEM	11/12	1
CAM	**Accession #13-000225**	**12/12**	**NA**
	EMEM	12/12	NA

Legend: [1] The number of deaths due to trauma. This observation was taken at 24-48 hours post-inoculation rather than at the conclusion of the passage time.
NA Not applicable

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TABLE 4: Hemagglutination Activity of Allantoic Fluids

Sample	Hemagglutination of Erythrocytes					
	2 - 8°C			37 ± 2°C		
	C	GP	H	C	GP	H
Accession #13-000225 (P1 Allantoic Route)	-	-	-	-	-	-
Accession #13-000225 (P2 Allantoic Route)	-	-	-	-	-	-
Negative control (P1 Allantoic Route)	-	-	-	-	-	-
Negative control (P2 Allantoic Route)	-	-	-	-	-	-
Accession #13-000225 (Amniotic Route)	-	-	-	-	-	-
Negative control (Amniotic Route)	-	-	-	-	-	-
Assay Positive control (Influenza type A)	+	+	+	+	+	+
Assay Negative control (EMEM)	-	-	-	-	-	-

Legend:

P1	Allantoic fluids from embryonated eggs inoculated with test article or EMEM
P2	Subpassage of allantoic fluids from P1 eggs into a new set of embryonated eggs
-	Negative reaction, indicating absence of viral antigen
+	Positive reaction, indicating presence of viral antigen
C	Chicken Red Blood Cells
GP	Guinea Pig Red Blood Cells
H	Human Red Blood Cells

15.0 CONCLUSION

The survival rate of the embryos, excluding those that died from trauma, was greater than 80% for all 4 routes of inoculation. No evidence of viral contamination was detected in the test article-inoculated embryonated hen eggs.

16.0 STATISTICAL DATA ANALYSIS

Statistical analysis of the data is not required.

17.0 DEVIATIONS / AMENDMENTS

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

No amendments to the protocol were generated.

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[redacted]

18.0 RECORD RETENTION

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

19.0 REFERENCES

1. Jacobs, J.P., McGrath, D. I., Garrett, A.J., and Schild, G.C. 1981. "Guidelines for the acceptability, management, and testing of serially propagated human diploid cells for the production of live virus vaccines for use in man." *Journal of Biological Standards* 9: 331-342.

2. Poiley, J.A. 1990. "Methods for the detection of adventitious viruses in cell cultures used in the production of biotechnology products," in Large-Scale Mammalian Cell Culture Technology. ed. Anthony S. Lubiniecki (New York, Basel: Marcel Dekker), 483-494.

3. Lennette, EH and Schmidt, NJ, eds. Diagnostic Procedures for Viral and Rickettsial Infections. (New York: American Public Health Association, Inc., 1969).

4. Belshe, R.B., ed. Textbook of Human Virology. (Littleton, MA: PSG Publishing Company, 1984).

5. Committee for Proprietary Medicinal Products: Ad Hoc Working Party on Biotechnology/ Pharmacy (1989) "Notes To Applicants For Marketing Authorizations On The Production And Quality Control Of Monoclonal Antibodies Of Murine Origin Intended For Use In Man" *Journal of Biological Standards*, Vol. 17:213 – 222).

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

7. US Government Printing Office (FDA) 1996, "Code of Federal Regulations, Title 21, Part 630.35, Subsections (1) and (2)" (no longer enforced)

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WuXi AppTec

E-FR-1-13-000225.30194

FINAL STUDY REPORT

STUDY TITLE: ***In Vivo*** **Assay for Viral Contaminants: European and US/FDA Test (GLP)**

TEST PROTOCOL NUMBER: 30194

TEST ARTICLE IDENTIFICATION	WUXI APPTEC ACCESSION NUMBER
CRM-WA14-MB-001	13-000225

SPONSOR: [redacted]

PERFORMING LABORATORY: WuXi AppTec, Inc.
[redacted]

WUXI APPTEC ACCESSION NUMBER	RESULTS
13-000225	No evidence of viral contamination was detected in the test article.

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[redacted]

WuXi AppTec

FINAL STUDY REPORT

STUDY TITLE:	***In Vivo* Assay for Viral Contaminants: Guinea Pigs, Adult, and Suckling Mice**
PROTOCOL NUMBER:	30194G
TEST ARTICLE IDENTIFICATION:	CRM-WA14-MB-001
PERFORMING LABORATORY:	WuXi AppTec, Inc. [redacted]
SPONSOR:	WuXi AppTec, Inc. - Virology [redacted] On behalf of Waisman Biomanufacturing [redacted]
ACCESSION NUMBER:	13-000225
STUDY NUMBER:	181111
CLIENT MNEMONIC:	QUA03
RESULT SUMMARY:	**No evidence** of adventitious viral agents was detected in the test article.

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[redacted]

QUALITY ASSURANCE UNIT SUMMARY

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

Critical Phase	Date	Study Director	Management
Group I Tissue Harvest and Emulsification	03/04/13	03/07/13	03/22/13
Final Report	03/22/13	03/22/13	03/22/13

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

Quality Assurance Auditor: [redacted] Date: 3/22/13

GOOD LABORATORY PRACTICES STATEMENT

The study referenced in this report was conducted in accordance with US FDA Good Laboratory Practices for Nonclinical Laboratory Studies as found in Title 21 Code of Federal Regulations Part 58, EU Good Laboratory Practice regulations, and applicable ICH Q7a standards.

The studies not performed by or under the direction of WuXi AppTec, Inc., are exempt from this Good Laboratory Practice Statement and include characterization and stability of the test compound(s)/test article.

Study Director: [redacted] Date: 3/22/13

Professional Personnel Involved:

[redacted]

Vice President of Process Improvement and Operations
Study Operations Director
Director, In-Life Operations
Study Director
Client Relations Manager

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1.0 PURPOSE

This test was designed to detect the presence of adventitious viral agents in a test article by the inoculation of adult mice, suckling mice, and guinea pigs.

2.0 TEST FACILITY:

WuXi AppTec, Inc.
[redacted]

3.0 SCHEDULING

DATE SAMPLE RECEIVED:		01/31/13
STUDY INITIATION DATE:		02/01/13
ANIMAL INJECTION DATES:	Adult mice, Guinea pigs, and Suckling mice Group I	02/18/13
	Suckling mice Group II	03/04/13
ANIMAL TERMINATION DATES:	Suckling mice Group I	03/04/13
	Adult mice, Guinea pigs, and Suckling mice Group II	03/18/13
STUDY COMPLETION DATE:		03/22/13

4.0 TEST, NEGATIVE CONTROL ARTICLE AND EMULSIFICATION IDENTIFICATION

The test article was received at WuXi AppTec - Philadelphia. The sample for *in vivo* testing was prepared at the Philadelphia facility and shipped via UPS to WuXi AppTec – St. Paul, where it was tested.

4.1 Test Article Identification

Sample Designation:	CRM-WA14-MB-001
Lot #:	Not Given
General Description:	WA14 GMP testing samples, cells and medium suspension.
Physical State:	Liquid
Concentration:	1 x 10e7 cells/mL
Species of Origin:	Human
Stability (Expiration Date):	Not Applicable
Safety Precautions:	BSL 1
Storage Conditions:	Ultracold (≤ - 60 °C)
Intended Use:	Not Given

4.2 Negative Control Article Identification

The negative control article listed below was purchased and supplied by the test facility for this assay.

Negative Control Article:	EMEM
Lot #:	0000321973
Manufacturer:	Lonza
Physical State:	Liquid
Expiration Date:	10/01/14
Storage Conditions:	Refrigerated
Safety Precautions:	General

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4.3 Emulsification Reagent Identification

The Emulsification reagent listed below was purchased and supplied by the test facility for this assay.

Reagent Name:	EMEM
Lot #:	0000321973
Manufacturer:	Lonza
Physical State:	Liquid
Expiration Date:	10/01/14
Storage Conditions:	Refrigerated

5.0 CHARACTERIZATION

The Sponsor was responsible for all test article characterization data as specified in the GLP regulations. The identity, strength, stability, purity, and chemical composition of the test article were solely the responsibility of the Sponsor. The Sponsor was responsible for supplying to the testing laboratory results of these determinations and any others that may have directly impacted the testing performed by the testing laboratory, prior to initiation of testing. Furthermore, it was the responsibility of the Sponsor to ensure that the test article submitted for testing was representative of the final product that was subjected to materials characterization. Any special requirements for handling or storage were arranged in advance of receipt and the test article was received in good condition.

6.0 SAMPLE STORAGE

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

7.0 EXPERIMENTAL DESIGN

7.1 Experimental Summary

Utilization of mammalian cells in the manufacture of biologicals leads to the risk of contamination by adventitious viruses. This study was designed to detect murine and non-murine viruses that are not detected by the Mouse Antibody Protection Test or *in vitro* culture studies. This study generally followed the recommendations made for the murine requirements for *In Vivo* Viral Contaminant testing as described in FDA 630.35, subsections 1 and 2, as well as European requirements for *in vivo* viral contaminant testing.

Twenty adult mice were inoculated with the prepared test article and five adult mice were inoculated with the negative control article. The animals were observed daily for 28 days for clinical signs of toxicity or a viral infection.

Twenty newborn suckling mice were injected with the prepared test article and 20 newborn suckling mice were injected with the negative control article. The animals were observed daily for clinical signs of toxicity or a viral infection. On Day 14 after injection, the mice were sacrificed and organs were harvested, homogenized, and pooled within the test group or the negative control group. The organs were homogenized with Eagle's Minimal Essential Medium (EMEM) and centrifuged at low speed. The resulting tissue supernatant was used for injection into the second group of newborn mice.

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[redacted]

Twenty newborn suckling mice were injected with the test suckling mice tissue supernatant and 20 mice were injected with the negative control suckling mice tissue supernatant. The tissue supernatants were first filtered through a low-protein binding 0.45 micron filter, loaded into appropriate sized syringes, and prewarmed in a waterbath at 37 ± 2 °C prior to injection. The animals were observed daily for clinical signs of toxicity or a viral infection. On Day 14 after the injections, the mice were sacrificed.

Five adult guinea pigs were injected with the prepared test article and five adult negative control guinea pigs were injected with the negative control article. The animals were observed daily for 28 days for clinical signs of toxicity or a viral infection.

7.2 Justification for Selection of the Test System

Guinea pigs are susceptible to a number of viral agents, including paramyxoviruses (Sendai) and reoviruses. Adult mice are susceptible to a number of viral agents, including coxsackie viruses and members of the flavivirus group (St. Louis encephalitis virus and Japanese encephalitis virus). Newborn suckling mice are susceptible to a wide range of viruses including togaviruses, bunyaviruses, flaviviruses, picornaviruses (poliovirus, coxsackie virus groups A and B, echovirus), and herpes viruses. Subpassage of tissues from inoculated suckling mice into new test systems serves to increase the sensitivity of this assay because any viral agents present in the inoculum would likely be amplified in the new test systems.

7.3 Institutional Animal Care and Use Committee (IACUC)

The protocol and any amendments or procedures involving the care or use of animals on this study were reviewed and approved by WuXi AppTec's IACUC prior to the initiation of such procedures.

IACUC Protocol / Effective Date: 12- 356A / July, 2012

7.4 Safety

Appropriate routine safety procedures were followed in handling the test article. All applicable WuXi AppTec safety policies and procedures were observed during the performance of the test.

7.5 Protocol Amendment/Deviations

7.5.1 Amendment 1

Section 7.3, IACUC protocol number changed to 12- 356A / July, 2012.

The following statement "followed by induction of pneumothorax to ensure death" was removed in Section 10.6 of protocol 30194G because it was not approved in the IACUC protocol 12-356A and was not referenced in the American Veterinary Medical Association's (AVMA) guidelines 2007. The statement removal does not affect the study design or interpretation.

The following word "intracranial (IC)" was replaced with "intracerebral (ICR)" in sections 7.1 and 10.3.3 of protocol 30194G. Also the abbreviation "IC" was replaced with "ICR" throughout the protocol 30194G. The current US and EU guidelines use the terminology "intracerebrally" not "intracranial". The word replacement does not affect the study design or final interpretation.

Section 9.1.4, the total number of mice used in the study is 113 per project. These include 8 dams, 80 newborn suckling mice and 25 adult mice (non-pregnant). The animal number clarification does not affect the study design or final interpretation.

7.5.2 Deviations
There were no deviations that occurred during the course of this study.

8.0 TEST SYSTEM

8.1 Adult Mice

8.1.1 Species/Strain: All of the adult mice used in this study were (*Mus musculus*), Swiss CD-1 strain, specific pathogen free (SPF).

8.1.2 Source: Animals were obtained from Charles River Laboratories, a previously approved vendor of commercial laboratory animals.

8.1.3 Weight Range: The adult mice weights ranged from 15.5 to 19.7 grams at the time of injections and were within the weight limits.

8.1.4 Age: All adult mice were approximately 3 weeks of age at the time of injections.

8.1.5 Number: Twenty-five adult mice were used in this study.

8.1.6 Sex: All of the adult mice used were female.

8.1.7 Animal Identification: Mice were individually identified according to WuXi AppTec SOP: ILS-0112.

8.2 Pregnant and Suckling Mice

8.2.1 Species/Strain: All of the pregnant mice used in this study were (*Mus musculus*), Swiss CD-1 strain, specific pathogen free (SPF).

8.2.2 Source: Animals were obtained from Charles River Laboratories, a previously approved vendor of commercial laboratory animals.

8.2.3 Weight Range: Not applicable.

8.2.4 Age: The suckling mice were within one calendar day of birth at the time of injection.

8.2.5 Number: Eighty suckling mice and eight dams were used in the study.

8.2.6 Sex: Adult animals were female. Suckling mice sex was not determined.

8.2.7 Animal Identification: Animals were identified by cage card according to WuXi AppTec SOP: ILS-0112.

8.3 Guinea Pigs:

8.3.1 Species/Strain: All of the guinea pigs used in this study were albino guinea pig (*Cavia porcellus*), Hartley strain, SPF.

8.3.2 Source: Animals were obtained from Charles River Laboratories, a previously approved vendor of commercial laboratory animals.

8.3.3 Weight Range: All guinea pigs weighed between 329.2 to 364.0 grams at the time of injections.

[redacted]

8.3.4 Age: All guinea pigs were approximately 5 weeks of age at the time of injections.

8.3.5 Number: Ten guinea pigs were used in this study.

8.3.6 Sex: All of the animals used in this study were male.

8.3.7 Animal Identification: Guinea pigs were individually identified according to WuXi AppTec SOP: ILS-0112.

9.0 HUSBANDRY

9.1 Receipt and Acclimation

Adult mice and guinea pigs were received on 02/13/13 and pregnant mice on (02/12/13 and 02/26/13). Animals were acclimated for a minimum of 5 days under the same conditions as the actual test. Each animal was examined for signs of disease and injury prior to entry into the research area.

9.2 Housing

Adult animals were housed in microisolator cages with contact bedding. The pregnant mice were housed with their suckling pups. Housing density complied with the NIH and AAALAC International guidelines for this species.

9.3 Environment

The environmental conditions in the animal rooms were maintained according to WuXi AppTec SOP: ILS-0018, Environmental Conditions in the Animal Facility (current version). The temperature, humidity, air handling, and photo-period will meet or exceed the AAALAC International recommendations for these species. The laboratory and animal rooms will be maintained as limited-access facilities.

9.4 Diet

Mice were supplied with a certified commercial rodent diet. Guinea pigs were supplied with a certified commercial guinea pig diet. No known contaminants were expected to interfere with the test results.

9.5 Water

Potable water was supplied, *ad libitum*, from the local municipal water supply. No known contaminants were expected to interfere with the test results.

10.0 TEST MATERIAL PREPARATION

The 1 x 45 mL tube of frozen test article was thawed in a 37 ± 2 °C waterbath and then was snap-frozen using a dry ice 70% ethanol bath. The test article was placed into a 37 ± 2 °C waterbath to thaw and was vortexed for approximately 1 minute. The cell lysate was clarified by centrifugation at 2000 rpm for ten minutes at 4 °C. The supernatant was harvested aseptically and pooled into a new sterile tube. The supernatant was filtered through a low protein binding 0.45 micron filter and loaded into appropriate syringes. The syringes were prewarmed in a waterbath at 37 ± 2 °C and injected neat.

11.0 TEST ARTICLE ADMINISTRATION

11.1 Selection of Animals

Animals were randomly placed in cages upon receipt and were placed on study as available. Animals considered unsuitable due to poor health or outlying body weight were excluded from the study.

[redacted]

11.2 Inoculation of Guinea Pigs

Five adult guinea pigs were inoculated with a 0.2 mL intramuscular (IM) injection and a 5.0 mL intraperitoneal (IP) injection of the prepared test article. Five adult guinea pigs were inoculated with a 0.2 mL IM injection and a 5.0 mL IP injection of EMEM as a negative control.

11.3 Inoculation of Adult Mice

Twenty adult mice were inoculated with a 0.1 mL IM injection and a 0.5 mL IP injection and a 0.03 mL intracerebral (ICR) injection. The ICR injection of 0.03 mL was done after Isoflurane anesthesia. Five separate negative control animals were injected in the same manner with EMEM.

11.4 Inoculation of Suckling Mice, Group I

Within 1 calendar day of birth, twenty suckling mice were injected with the test article and twenty suckling mice were injected with EMEM. Each test mouse was given a 0.01 mL ICR, a 0.1 mL IP, and a 0.01 mL IM injection of the prepared test article. Each negative control mouse was given a 0.01 mL ICR, a 0.1 mL IP, and a 0.01 mL IM injection of EMEM.

On Day 14 after injection, the suckling mice of Group I were euthanized. Organs were harvested from each animal and pooled within either the test group or the control group. The organs were homogenized in EMEM with a sterile tissue grinder and clarified by low speed centrifugation. The supernatants were used to inoculate Group II suckling mice.

11.5 Passage of Suckling Mice Homogenate, Group II

The prepared tissue supernatants (test and control) were filtered through a low-protein binding 0.45 micron filter and loaded into syringes. The syringes were prewarmed in a waterbath at 37 ± 2 °C prior to injection. Each of twenty suckling mice was given a 0.01 mL ICR, a 0.1 mL IP, and a 0.01 mL IM injection of the prepared test tissue supernatant. Each of twenty suckling mice was given a 0.01 mL ICR, 0.1 mL IP, and a 0.01 mL IM injection of the prepared negative control tissue supernatant.

12.0 OBSERVATIONS

The adult mice and guinea pigs were observed daily for 28 consecutive days for clinical signs of toxicity, infection or mortality. Group I and Group II suckling mice were observed daily for 14 days following injection for clinical signs of toxicity, infection or mortality. Care was taken to minimize stress to the nursing Dam mouse to avoid cannibalization of healthy suckling mice.

13.0 TERMINATION

Following completion of this study, adult mice, suckling mice, and guinea pigs were euthanized by CO_2 asphyxiation.

14.0 EVALUATION CRITERIA

The test article was considered negative for the presence of adventitious viral contaminates if at least 80% of each test group of adult mice, guinea pigs, Group I suckling mice, and Group II suckling mice survive the test period with no adverse clinical signs as described in the protocol.

15.0 VALIDITY

The test was considered valid if at least 80% of each control group of adult mice, guinea pigs, Group I suckling mice, and Group II suckling mice survive the test period in good health without showing clinical signs of toxicity or a viral infection.

16.0 METHOD FOR CONTROL OF BIAS: Not applicable.

17.0 DATA ANALYSIS: Not applicable.

18.0 STATISTICAL METHODS: None used.

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

20.0 COMPLIANCE
The care, housing and handling of the animals were in compliance with:

20.1 AAALAC International and NIH guidelines as reported in the "Guide for the Care and Use of Laboratory Animals," National Research Council – ILAR, Revised 1996.

20.2 (OPRR), "Public Health Service Policy on Humane Care and Use of Laboratory Animals," Health Research Extension Act of 1985 (Public Law 99-158), Revised 1986.

20.3 USDA, Department of Agriculture, Animal and Plant Health Inspection Service, 9 CFR, Parts 1, 2, and 3, Animal Welfare, Final Rule 1989.

21.0 TEST ARTICLE DISPOSITION: Unused test samples remain in the storage area until all testing is completed. Once completed, the remaining samples were discarded or returned as requested by the Sponsor.

22.0 Results

22.1 Negative Control Animals
None of the negative control animals had abnormal clinical signs indicative of toxicity or infection during the test period. Greater than 80% of negative control adult mice, guinea pigs and Group I and II suckling mice survived the test indicating validity requirements were met (Table 1).

22.2 Test Animals
None of the test animals had abnormal clinical signs indicative of toxicity or infection during the test period. Greater than 80% of test adult mice, guinea pigs and Group I and II suckling mice survived the test period indicating evaluation requirements are met (Table 1).

Table 1: Percent Survival and Clinical Signs

Group	% Survival		Clinical Signs	
	Test	Negative Control	Test	Negative Control
Guinea Pigs	100% (5/5)	100% (5/5)	None	None
Adult Mice	100% (20/20)	100% (5/5)	None	None
Suckling Mice, Group I	100% (20/20)	100% (20/20)	None	None
Suckling Mice, Group II	95% (19/20)[1]	95% (19/20)[1]	None	None

[1] Missing pup was presumed cannibalized by the dam.

23.0 CONCLUSION
These findings indicate that under the conditions of the study protocol, **no evidence** of adventitious viral agents was detected in the test article.

[redacted]

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24.0 REFERENCES

24.1 Belshe, R.B., ed. Textbook of Human Virology (Littleton, MA: PSG Publishing Company, 1984).

24.2 Committee for Proprietary Medicinal Products: Ad Hoc Working Party on Biotechnology/Pharmacy (1989) "Notes To Applicants for Marketing Authorizations on the Production and Quality Control of Monoclonal Antibodies of Murine Origin Intended for Use in Man" *Journal of Biological Standards*, Vol. 17:213-222.

24.3 Hay RJ (1985) "ATCC Quality Control Methods for Cell Lines," American Type Culture Collection, Rockville, MD.

24.4 Jacobs, J.P., McGrath, D.I., Garrett, A.J., and Schild, G.C. 1981 "Guidelines for the Acceptability, Management, and Testing of Serially Propagated Human Diploid Cells for the Production of Live Virus Vaccines for Use in Man," *Journal of Biological Standards,* Vol. 9: 331-342.

24.5 Lennette, EH and Schmidt, NJ, eds. Diagnostic Procedures for Viral and Rickettsial Infections (New York: American Public Health Association, Inc., 1969).

24.6 "Production and Quality Control of Monoclonal Antibodies." Directive 75 / 318 / EEC, as amended. Entered in force on July, 1995. Last revised in December, 1994.

24.7 U.S. Food and Drug Administration (FDA) Center for Biologics Evaluation and Research (CBER) 1993 "Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals."

24.8 WuXi AppTec Reference Library Contents, Form ALS-4650-1, (current revision).

24.9 WuXi AppTec SOP: ILS-0136, In Vivo Assay for Viral Contaminants: Testing in Guinea Pigs, Adult and Newborn Mice (US FDA and European Combined Version).

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FINAL STUDY REPORT

STUDY TITLE: **Fluorescent Polymerase Chain Reaction (PCR)-Based Reverse Transcription (F-PBRT) Assay: (GLP)**

TEST PROTOCOL NUMBER: 30357.03

TEST ARTICLE IDENTIFICATION	WUXI APPTEC ACCESSION NUMBER
CRM-WA14-MB-001	13-000225

SPONSOR: [redacted]

PERFORMING LABORATORY: WuXi AppTec, Inc. [redacted]

WUXI APPTEC ACCESSION NUMBER	RESULTS
13-000225	**Negative.** The test article is free of detectable reverse transcriptase activity.

[redacted]

Ref: FO1-QCP-028 #19177 JS 29 Mar 2013 RoM 09 Apr 2013

QUALITY ASSURANCE UNIT SUMMARY

STUDY: Fluorescent Polymerase Chain Reaction (PCR)-Based Reverse Transcription (F-PBRT) Assay: (GLP)

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

Phase Inspected	Date
Step 1.4 Data Analysis	March 25, 2013

[redacted]
Quality Assurance

27MAR13
Date

GOOD LABORATORY PRACTICES STATEMENT

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

[redacted]
Study Director

28Mar13
Date

Professional Personnel involved in study:

[redacted]

[redacted]

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1.0 PURPOSE

The purpose of this assay is to test samples for the presence of retroviral reverse transcriptase activity and for the presence of interfering substances using Taqman technology.

2.0 SPONSOR: Waisman Biomanufacturing [redacted]

3.0 TEST FACILITY: **WuXi AppTec, Inc.** [redacted]

4.0 SCHEDULING

DATE SAMPLES RECEIVED: January 29, 2013
STUDY INITIATION DATE: February 25, 2013
STUDY COMPLETION DATE: See page 2 for Study Director's signature and date.

5.0 TEST ARTICLE CHARACTERIZATION

Determinations of strength, homogeneity, purity, and stability of the test article are solely the responsibility of the Sponsor. The Sponsor is responsible for supplying to the testing laboratory results of these determinations and any others that may directly impact the testing performed by the testing laboratory, prior to initiation of testing.

6.0 TEST ARTICLE IDENTIFICATION: CRM-WA14-MB-001

7.0 TEST SYSTEM DESCRIPTION

Retroviruses possess multiple enzymatic activities, including a reverse transcriptase.[1] Reverse transcriptases (RT) are DNA polymerases capable of utilizing both DNA and RNA as templates, making them unique among the DNA polymerases. These enzymes function in the normal life cycle of retroviruses by synthesizing a double-stranded DNA copy of the viral RNA genome. Retroviruses are widely distributed in nature and are present in the germlines of many mammalian species and also exist as exogenous infectious agents. Therefore the potential exists for these viruses, or virus-derived RT enzymes, to be present in products produced in mammalian cells or animals (such as embryonated hen eggs). Historically, assays for reverse transcriptases utilized artificial template-primer combinations such as oligo-dT/poly-dA. These assays, while useful and still available, are not as sensitive as more recently developed assays. The PCR-based reverse transcriptase assay, or PBRT, is approximately 10^5-10^6 times more sensitive than a conventional RT assay and is capable of detecting the activity of as few as 10-

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100 molecules of reverse transcriptase.[2,3,4] This technology is also frequently referred to as a PCR-enhanced reverse transcriptase (PERT) assay. Utilizing real-time PCR, specifically Taqman technology, the F-PBRT (Fluorescent-PCR Based Reverse Transcription) assay maintains the same sensitivity and specificity as traditional PBRT or PERT assays while significantly decreasing the time and labor required in completing the assay.

The F-PBRT assay uses RNA isolated from a plant virus (brome mosaic virus, BMV) as a template, BMV specific oligonucleotide primers, and a fluorescent probe. In the first portion of the reaction cDNA is synthesized from the BMV RNA via any RT molecules (or positive control) that may be present in the sample.[5] In the PCR amplification stage of the assay, the fluorescent probe labeled with both a reporter and a quencher dye anneals specifically to the synthesized cDNA between the forward and reverse primers. The PCR reaction uses the 5' to 3' nuclease activity of the taq DNA polymerase to cleave the reporter dye from the probe resulting in increased fluorescence when RT synthesized cDNA is present.

The potential exists for non-retroviral polymerases, such as mammalian DNA polymerases alpha and gamma,[6] to yield "false-positive" results in this assay. Procedures are included in the assay to eliminate these false-positive signals, thereby ensuring that the polymerase activity detected is retroviral in origin.

It is important to point out that a positive signal from this F-PBRT assay does not necessarily indicate the presence of infectious retroviral particles, and the FDA has stated that infectivity studies may be required to demonstrate that the source of the activity is not an infectious retrovirus.[7]

8.0 EXPERIMENTAL DESIGN

Each test article was assayed unspiked in triplicate, undilute and 100x diluted (if required), and each dilution was spiked with two amounts of AMV reverse transcriptase (in triplicate) as a test for the presence of substances in the test article that can interfere with either the reverse transcription step or the PCR amplification step of the assay. This procedure was performed both with and without activated DNA, such that each test article was assayed in 18 reactions, nine without activated DNA (three unspiked, six spiked) and nine with activated DNA (three unspiked, six spiked) in the reactions.

8.1 The test article was clarified by low-speed centrifugation to minimize the amount of cellular debris present. It was ultracentrifuged at 4°C and resuspended in a disruption buffer which contains detergent to release reverse transcriptase from any viral particles which may be present in the sample.

8.2 A specified amount (5 µl) of test article was added to a reverse transcription reaction mix containing BMV RNA template, One-step RT PCR Mix, specific oligonucleotide DNA primer, with or without sonicated DNA to suppress false-positive signals.[5,6]

8.3 The cDNA was amplified by the addition of a RT-PCR reaction mix containing *Taq* polymerase, PCR buffer, dNTPs and BMV specific primers, using ABI Prism 7500. Amplification was captured in real-time measuring fluorescence produced by a FAM probe.

[redacted]

9.0 TEST ARTICLE PREPARATION

On January 29, 2013 WuXi AppTec, Inc. received 1 vial containing 1 mL of "WA14 GMP testing samples, cells and medium suspension", frozen on dry ice and designated for use in this assay. The test article was placed on hold pending client clarification and stored at ≤-60°C. On January 31, 2013 the test article was transferred to the Molecular Biology Laboratory and continued to be stored at ≤-60°C until the assay was initiated.

10.0 POSITIVE CONTROLS

Purified AMV-RT at 10^{-5} and 10^{-6} units per reaction was used as a positive control for each reaction condition (with and without activated DNA).

11.0 NEGATIVE CONTROLS

11.1 Reaction tubes containing only disruption buffer (with and without activated DNA) served as negative controls.

11.2 Reaction tubes containing only cell culture grade water (with and without activated DNA) served as negative controls.

11.3 Reaction tubes containing only 1X PBS (with and without activated DNA) served as negative controls.

12.0 ASSAY VALIDITY

The following validity criteria were evaluated by the fluorescent signals expressed in cycle threshold (Ct) values generated by the SDS software of the ABI 7500. Fluorescent signals were detected if at least two of the three replicates for each control sample yielded a cycle threshold (Ct) value.

12.1 Fluorescent signals are not detected in any of the negative controls.

12.2 Fluorescent signals are detected for both of the AMV-RT positive controls lacking activated DNA.

12.3 Fluorescent signals are detected for both of the AMV-RT positive controls containing activated DNA.

13.0 TEST EVALUATION

All test results are judged by the fluorescent signals expressed in cycle threshold (Ct) values generated by the SDS software of the ABI 7500. Fluorescent signals are detected for spiked test articles if at least two of the three replicates for each sample yield a cycle threshold (Ct) value. Fluorescent signals are detected for the unspiked test articles if any of the three replicates of unspiked test article generate a cycle threshold (Ct) value.

[redacted]

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Evaluation of results is performed using the undilute test article. If the undilute test article contains interfering substances, the 1:100 dilution of test article is reported.

If no fluorescent signals are detected in the unspiked test article (both with and without activated DNA) and both levels of spike of each test article condition (both with and without activated DNA) yield a fluorescent signal, then the test article is judged free of detectable reverse transcriptase activity.

If a fluorescent signal is detected in the unspiked test article (both with and without activated DNA) and both levels of spike of each test article condition (both with and without activated DNA) yield a fluorescent signal, then the test article is judged to contain reverse transcriptase activity.

If a fluorescent signal is detected in the unspiked test article without activated DNA but is not detected in the unspiked test article containing activated DNA, and both levels of spike of each test article condition (both with and without activated DNA) yield a fluorescent signal, then the test article is judged to be free of detectable reverse transcriptase activity but possesses cellular DNA polymerase activity.

If a fluorescent signal is detected in the unspiked test article containing activated DNA but is not detected in the unspiked test article without activated DNA, and both levels of spike of each test article condition (both with and without activated DNA) yield a fluorescent signal, contact the Study Director and Sponsor.

If the 10^{-5} and/or 10^{-6} units AMV-RT positive control spikes of each test article condition (both with and without activated DNA) do not yield a fluorescent signal, the test article is judged to contain interfering substances. If both the neat and 1:100 dilution of test article are judged to contain interfering substances, the client would be informed that further dilution of the test article is necessary.

14.0 RESULTS

The test was valid. Fluorescent signals were not detected in the negative controls. Fluorescent signals were detected for both of the AMV-RT positive controls lacking activated DNA. Fluorescent signals were detected for both of the AMV-RT positive controls containing activated DNA.

Fluorescent signals were not detected in the unspiked test article (with or without activated DNA) and the 10^{-5} and 10^{-6} units AMV-RT positive control of each test article condition (with and without activated DNA) yielded a fluorescent signal, therefore the test article is determined to be free of detectable reverse transcriptase activity.

[redacted]

Control Sample	Replicate Ct values without activated DNA (from ABI 7500 printout)	Replicate Ct values with activated DNA (from ABI 7500 printout)	Overall Result (+ or -)
Disruption Buffer Negative Control	Undetermined	Undetermined	-
	Undetermined	Undetermined	
	Undetermined	Undetermined	
1X PBS Negative Control	Undetermined	Undetermined	-
	Undetermined	Undetermined	
	Undetermined	Undetermined	
Cell Culture Water Negative Control	Undetermined	Undetermined	-
	Undetermined	Undetermined	
	Undetermined	Undetermined	
1x 10^{-5} U AMV-RT Positive Control	25.9131	27.7	+
	25.2196	25.4447	
	22.176	25.4938	
1x 10^{-6} U AMV-RT Positive Control	27.6428	32.0235	+
	26.7993	33.2664	
	23.2802	30.2669	

Sample	Replicate Ct values without activated DNA (from ABI 7500 printout)	Replicate Ct values with activated DNA (from ABI 7500 printout)	Overall Result (+ or -)
13-000225	Undetermined	Undetermined	-
	Undetermined	Undetermined	
	Undetermined	Undetermined	
13-000225 + 1 x 10^{-5} U AMV-RT	23.708	27.8928	+
	26.0296	28.0196	
	26.5024	28.7602	
13-000225 + 1 x 10^{-6} U AMV-RT	30.2203	33.5099	+
	29.454	32.0971	
	33.9817	38.4635	

[redacted]

Ref. FOI-QCP-028 #19177 JS 29Mar2013 ROM 09Apr2013

15.0 CONCLUSION

Evidence of retroviral reverse transcriptase activity was not detected in the test article.

16.0 STATISTICAL DATA ANALYSIS

Statistical analysis of the data was not required.

17.0 DEVIATIONS

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

No amendments to the protocol were generated.

18.0 RECORD RETENTION

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

19.0 REFERENCES

1. Coffin, JM. Retroviridae: The Viruses and Their Replication. In: Fields, B.N., Knipe, D.M., Howley, P.M., et al. *Fields Virology*, Third Edition. Lippincott-Raven Publishers, Phila. 1996, 1767 - 1847

2. Silver, J., Maudru, T., Fujita, K., and Repaske, R. (1993). An RT-PCR assay for the enzyme activity of reverse transcriptase capable of detecting single virions. *Nucleic Acids Research* 21, 3593 – 3594

3. Pyra, H., Boni, J., and Schupbach, J. (1994). Ultrasensitive retrovirus detection by a reverse transcriptase assay based on product enhancement. *Poc. Natl. Acad. Sci.* 91, 1544 – 1548

4. Heneine, W., Yamamoto, S., Switzer, W.M., Spira, T.J., and Folks, T.M. (1995). Detection of reverse transcriptase by a highly sensitive assay in sera from persons infected with Human Immunodeficiency Virus Type 1. *J. Infectious Diseases* **171**, 1210 – 1216

5. Arnold, B.A., Hepler, R.W. and Keller, P.M. (1998). One-step Fluorescent Probe Product-Enhanced Reverse Transcriptase Assay. *Biotechniques* 25:98-106

6. Lugert, R., Konig, H., Kurth, R., and Tonjes, R.R. (1996). Specific suppression of false-positive signals in the Product-Enhanced Reverse Transcriptase assay. *Biotechniques* 20, 210 - 217

7. Letter to manufacturers of viral vaccine products. (December 14, 1998). Division of Vaccines and Related Products Applications, Center for Biologics Evaluation and Research, Food and Drug Administration

[redacted]

Ref: Fol-QcP-028 #19177 JS 29Mar2013 ROM 09Apr2013

E-FR-1-13-000225.30001

FINAL STUDY REPORT

STUDY TITLE: **Mouse Antibody Production (MAP) Test with LCMV Challenge**

TEST PROTOCOL NUMBER: 30001.22

TEST ARTICLE IDENTIFICATION	WUXI APPTEC ACCESSION NUMBER
CRM-WA14-MB-001	13-000225

SPONSOR: [redacted]

PERFORMING LABORATORY: WuXi AppTec, Inc. [redacted]

SUBCONTRACTED TO: Charles River Laboratories [redacted]

WUXI APPTEC ACCESSION NUMBER	RESULTS
13-000225	The presence of 19 murine adventitious viral contaminants was not detected in the test article.

[redacted]

ATTACH TO F01-QCP-028- 19177 JS 09Apr-2013 ROM 09Apr2013

QUALITY ASSURANCE UNIT SUMMARY

STUDY: Mouse Antibody Production (MAP) Test with LCMV Challenge

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

Refer to Charles River Laboratories Final Report.

[redacted] 05 APR 13

Quality Assurance Date

GOOD LABORATORY PRACTICES STATEMENT

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

[redacted] 05 Apr 13

Study Director Date

Performed at Charles River Laboratories: Charles River Laboratories [redacted]

[redacted]

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1.0 SCHEDULING

DATE SAMPLES RECEIVED:	January 29, 2013
STUDY INITIATION DATE:	February 4, 2013
STUDY COMPLETION DATE:	See page 2 for Study Director's signature and date.

2.0 TEST ARTICLE PREPARATION

On January 29, 2013, WuXi AppTec, Inc. received 1 tube containing 5.0 mL of "WA14 GMP testing samples, cells and medium suspension," frozen in dry ice and designated for use in this assay. The test article was stored at ≤ -60°C until shipment to the subcontractor. On February 4, 2013, 1 tube containing 5.0 mL of test article was shipped in dry ice via overnight carrier to the subcontractor.

3.0 RECORD RETENTION

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

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[redacted]

charles river

FINAL REPORT

Study No.: 2013-000041

Test Article: 13-000225

Protocol No.: PR-31-9

Protocol Title: Mouse Antibody Production (MAP) Test with LCMV Challenge

Report Date: 29Mar2013

Sponsor: WuXi AppTec, Inc.

[redacted]

Prepared By:

CHARLES RIVER

[redacted]

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charles river

Final Report

Protocol No.: PR-31-9
Protocol Title: Mouse Antibody Production (MAP) Test with LCMV Challenge
Study No.: 2013-000041

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charles river

Final Report

Protocol No.: PR-31-9
Protocol Title: Mouse Antibody Production (MAP) Test with LCMV Challenge
Study No.: 2013-000041

I. PURPOSE/JUSTIFICATION

The purpose of this study was to determine whether the test article contains murine viruses that are known to infect mouse tissues.

With the growth of the biotechnology industry, biologics derived from rodent tissues and cell culture are being administered to people more frequently. To be used safely, and to comply with government regulations, these products must be tested to show that they are free of extraneous rodent viruses. Although viruses may cause disease in animals and cytopathic effects in cell culture, monitoring by these methods is not reliable, as the viruses are often inapparent or non-specific. However, when laboratory animals are the host system, serum samples from inoculated animals can be assayed for virus-specific antibodies formed in response to infection. Some murine viruses, such as lymphocytic choriomeningitis virus (LCMV), are zoonotic agents that can cause disease in people. Redundant testing for LCMV is built into this protocol by the inclusion of the intracranial challenge of mice that have been inoculated with the test article with live LCMV.

II. TEST FACILITY

Charles River Laboratories
[redacted]

III. PERSONNEL

Name	Title
[redacted]	Study Director
	Senior Technologist

IV. TEST SCHEDULE

The study was initiated on 06Feb2013.

V. QUALITY ASSURANCE

This study was conducted to comply with 21 CFR Part 58 Good Laboratory Practice for Non Clinical Laboratory Studies. This study was assessed in compliance with the protocol and the Standard Operating Procedures (SOP) of Charles River. The Quality Assurance Unit (QA), personnel independent of the staff involved in the study, periodically inspected the study and/or the testing facility. The Final Report of the study was also audited and a Quality Assurance Statement, which includes the dates and phases of the inspections, was issued and included in the Final Report.

VI. TEST MATERIAL

A. Test Article

1.	Designation:	13-000225
2.	Lot:	Not applicable
3.	Species:	Human
4.	Cell Type:	Not applicable
5.	Vial Label:	13.000225.005 30001 5mL 1x10^7c/ml 11Jan13 CRM-MB001
6.	Quantity:	5mL
7.	Received by and Date:	Dolores Welch on 05Feb2013.
8.	Condition on receipt:	Frozen on Dry Ice

B. Control Article

1.	Material Name:	Hank's Balanced Salt Solution (HBSS)
2.	Manufacturer:	Sigma
3.	Lot #:	RNBC0806
4.	Expiration Date:	10/2013

VII. TEST SYSTEM

A. Species/Strain

Mouse/*Mus musculus*/CD-1

B. Sex

Female

C. Age at Inoculation

Mice were 37 days old at the time of inoculation.

D. Total Number of Test Systems

Seventeen

E. Source

All animals were obtained from a Charles River facility on which routine health monitoring is performed. Colonies from which the animals were obtained are screened

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for the viruses included on the panel for this test.

F. Identification

Each animal was individually identified with a uniquely numbered ear tag. Cage cards were used as a redundant method of identification.

VIII. HOUSING AND HUSBANDRY PRACTICES

Charles River, Wilmington, MA facility is accredited by the American Association for Accreditation of Laboratory Animal Care.

A. Housing

Animals were housed within a limited access facility in microisolator-type cage units containing sterile bedding, per the Guide for Care and Use of Laboratory Animals. Test article inoculated animals and control article inoculated animals were housed in separate cages.

B. Acclimation

Mice were acclimated for a minimum of three days.

C. Feed and Water

1. γ-irradiated rodent diet was provided *ad libitum*.
2. Chlorinated, filtered water was supplied by bottle with sipper tube *ad libitum*.

D. Environment

1. Temperature was maintained at 70°F ± 8°F (21.1°C ± 4.4°C)
2. Relative humidity was maintained between 30%-70%.
3. Light cycle was maintained at 12 hours light/12 hours dark.

E. Animal Welfare

This protocol and its associated procedures have been reviewed and approved by the Institutional Animal Care and Use Committee. The veterinary care of the animals will be in accordance with this protocol and the Guide for Care and Use of Laboratory Animals.

IX. EXPERIMENTAL DESIGN

The Test Article, at two different dilutions, was inoculated into mice by multiple routes to increase the likelihood of infection with viruses which may be present in the test article. The serum from one subset of mice was assayed for the presence of Lactose Dehyrogenase Elevating Virus (LDV). A second subset of mice was intracranially challenged with LCMV. The remaining mice were monitored for at least 28 days post-inoculation for clinical signs. At the

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end of the observation period, the animals were euthanized and blood samples were collected from each animal. Sera from the inoculated mice were assayed.

X. PROCEDURE

All study procedures, including husbandry, were performed in a biological safety cabinet.

A. Test Article Receipt and Processing

1. Upon receipt, the Test Article was maintained in a dedicated storage unit at the appropriate temperature specified by the Sponsor.

2. Prior to inoculation, a sample of the Test Article was diluted 1/10 in HBSS. The undiluted Test Article was designated "Inoc I". The diluted Test Article was designated "Inoc II".

B. Animal Inoculation

Test Article Inoculation- Day 0, 11Feb2013

1. The appropriate test material was administered to the mice.

Inoculum (Test Material)	Dose/Route of Inoculation	Animal ID #	Typical Assay Designation
Inoc I (TA)	0.5mL IP, 0.05mL IN, 0.05ml PO	1, 2, 3, 4	Serology
		13	LDH
		17	LCMV Challenge
Inoc II (TA)	0.5mL IP, 0.05mL IN, 0.05ml PO	7, 8, 9, 10	Serology
		14	LDH
		18	LCMV Challenge
Control Article	0.5mL IP, 0.05mL IN, 0.05ml PO	5, 6	Serology
		15, 16	LDH
		19	LCMV Challenge

Key: IP: Intraperitoneal IN: Intranasal PO: per os

Note: ID # 11 and 12 was reserved for positive control serum used for the LDH assay and were not used to identify animals on this test.

2. Test Article inoculated mice were housed together to facilitate the spread of potential low-titered virus from Inoculum I mice to Inoculum II mice. Control article inoculated mice were housed separately from test article inoculated mice.

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C. Clinical Observations

All animals were observed daily. Any animal displaying abnormalities, morbidities, or mortalities were reported. No abnormal observations were noted on this study.

D. Euthanasia and Blood Collection

1. Because enzyme levels are optimal between days three and twenty-one post inoculation, two test article inoculated animals, one each from INOC I and INOC II, and two control article inoculated animals were euthanized using CO_2 asphyxiation on day 9 (± 2 days) post inoculation. A blood sample was collected from each mouse and serum samples were prepared for LDH analysis. The serum was split between two tubes; one was retained for passage into new mice if necessary.

2. 28 days post inoculation, the remaining mice were euthanized using CO_2 asphyxiation. A blood sample was collected from each mouse and serum samples were prepared for serologic testing.

E. Serological Assay

1. Lactate Dehydrogenase (LDH) Assay to Detect LDH Elevating Virus (LDV)

   Serum from euthanized mice in Section X.D.1. was individually tested for LDH activity.

2. LCMV Challenge

   a) 23 days post inoculation, a mouse from each of the two test-article inoculated groups (INOC I and II) and a control article-inoculated mouse was anesthetized. They are then injected intracranially (IC) with an amount of neurotropic lymphocytic choriomeningitis virus (LCMV strain E 350 prepared from ATCC stock VR 1271) sufficient to cause 100% mortality within six to nine days after the IC injection.

   b) Animals were monitored daily through day 5 post challenge and twice daily thereafter. The day the animal dies was recorded.

   c) Any animal that exhibited severe clinical signs were euthanized; the day euthanasia was performed was recorded as the day of death.

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3. Serological Assay

a) Serum from each mouse in Section X.D.2. was assayed for antibodies to the viruses listed in the following table:

Name	Family/Genus	Report Abbrev.
Sendai virus	Paramyxoviridae	SEND
Pneumonia virus of mice	Paramyxoviridae	PVM
Mouse hepatitis virus	Coronaviridae	MHV
Minute virus of mice	Parvoviridae	MVM
Mouse parvovirus, Non-Structural Protein 1	Parvoviridae	PARV NS1
Mouse Parvovirus 1 & 2	Parvoviridae	MPV
Theiler's murine encephalomyelitis virus	Picornaviridae	GDVII
Reovirus Type 3	Reoviridae	REO
Epizootic diarrhea of infant mice	Rotaviridae	EDIM
Mouse pneumonitis virus	Papovaviridae	K
Ectromelia	Poxviridae	ECTRO
Polyoma virus	Papovaviridae	POLY
Mouse adenovirus	Adenoviridae	MAV 1 & 2
Lymphocytic choriomeningitis virus	Arenaviridae	LCMV
Mouse cytomegalovirus	Herpesviridae	MCMV
Mouse thymic virus	Herpesviridae	MTLV
Hantaan virus	Bunyaviridae	HANT
Prospect Hill Virus	Bunyaviridae	PHV

XI. EVALUATION OF THE TEST RESULTS

A. Evaluation of LDH Analysis

1. In order for the test to be evaluated:

a) Positive control sera, samples 11 and 12, must yield results greater than or equal to 1476 IU/L

b) Negative control sera, from control article inoculated mice, must yield results lower than 1476 IU/L.

2. An LDH level of 1476 IU/L or greater in serum from a test-article inoculated mouse suggests that the test-article is contaminated with LDV.

a) Significantly elevated serum LDH activity is not specific for LDV infection.

b) To confirm that LDV is the cause of elevated LDH activity in the serum sample from a test article inoculated animal, the retention samples created in X.D.1. are prepared and passaged into additional mice as outlined in section XI.D.

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B. Evaluation of LCMV Challenge

1. The test is valid if the **control** article inoculated mouse dies between six to nine days post IC inoculation with LCMV. This is indicative that the challenge dose was lethal for a non-immune mouse.

2. Death of the **test article** inoculated mice between six and nine days post inoculation indicates that the mice were not previously infected with LCMV and, therefore, the test article is free of this virus.

C. Evaluation of Serological Assay Results

1. In order for the results of the assay to be evaluated:

   a) Standard positive and negative control sera run with the assay yield acceptable serologic test results.

   b) At least four test article-inoculated mice (INOC I or INOC II) must survive at least 28 days post inoculation with serum available for the serological assay.

      (1) A sample yielding multiple non-specific or TC results, may be omitted from the evaluation process if there are four samples from test article inoculated mice remaining for evaluation.

   c) Serum from at least one of the control article inoculated mice must be available for serology testing.

      (1) Control Article inoculated mice must be seronegative for all viruses.

2. If the test does not meet the above criteria, it is not valid and therefore, the test article results cannot be evaluated.

3. Serology results for all animals assigned to this study are reported.

D. Interpretation of the Mouse Antibody Production Test Results

Once each assay has been deemed valid, the results are interpreted as follows:

1. Serology Panel

   Results are reported individually for each virus on the serology panel. A positive result will be reported if:

   a) Sera from two or more mice have a positive response on original and confirmatory testing against a specific virus.

   b) If serum from only one mouse has a positive response on confirmatory testing, the serology test will be declared indeterminate for that virus and may be repeated.

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2. LDH Analysis

   If LDH levels greater than 1476 IU/L are obtained in the original and subsequent confirmatory test, the sample is considered to be positive for LDV.

3. LCMV Challenge

   Survival of the mice beyond the prescribed observation period following the LCMV injection indicates that the mice developed immunity in response to LCMV in the test article.

E. According to the results of enzymatic assays for LDH, the LCMV challenge, and serologic testing for the virus-specific antibodies, murine viruses were not detected in the test article, 13-000225.

XII. STORAGE AND ARCHIVING

A. The following will be maintained by Charles River in archives for a period of one year after the completion of the study.

1. A copy of the Final Report
2. The Secondary Signature page signed by the Study Director
3. All raw data and pathology specimens generated during the study
4. The correspondence with the Sponsor concerning the study

B. The Sponsor will be responsible for the long term disposition of the above items per the appropriate compliance guidelines.

1. Arrangements may be made for the materials listed above to be shipped to the Sponsor for archiving.
2. Arrangements may be made to archive the materials listed above at a Charles River facility, at an additional cost to the Sponsor.

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XIII. APPROVALS

This study was conducted in compliance with the Food and Drug Administration Good Laboratory Practice Regulations (GLP) as set forth in Title 21 of the U.S. Code of Federal Regulations, Part 58. There were no amendments to this protocol and no deviations that affected the quality or integrity of the study or the interpretation of the results in the report. The Sponsor did not supply stability data or compliance information for test article characterization; the absence of this information did not impact the conduct of the study.

[redacted] 29 Mar 2013

CHARLES RIVER — Date

Study Director

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Charles River In Vivo Biosafety

[redacted]

In Vivo Biosafety Serology Results Report

Sponsor: WuXi AppTec Inc. **Accession #: 2013-000041 (GLP)**

MAP Test Serology Profile

Sample #: / Code :	1 I	2 I	3 I	4 I	5 CON	6 CON	7 II	8 II	9 II	10 II
ELISA SEND	-	-	-	-	-	-	-	-	-	-
ELISA PVM	-	-	-	-	-	-	-	-	-	-
EIA MHV	-	-	-	-	-	-	-	-	-	-
ELISA MVM	-	-	-	-	-	-	-	-	-	-
ELISA GDVII	-	-	-	-	-	-	-	-	-	-
ELISA REO	-	-	-	-	-	-	-	-	-	-
ELISA LCMV	-	-	-	-	-	-	-	-	-	-
ELISA ECTRO	-	-	-	-	-	-	-	-	-	-
ELISA K	-	-	-	-	-	-	-	-	-	-
ELISA POLY	-	-	-	-	-	-	-	-	-	-
ELISA EDIM	-	-	-	-	-	-	-	-	-	-
ELISA MCMV	-	-	-	-	-	-	-	-	-	-
ELISA HANT	-	-	-	-	-	-	-	-	-	-
IFA MTLV	-	-	-	-	-	-	-	-	-	-
IFA PHV	-	-	-	-	-	-	-	-	-	-
ELISA MAV 1 & 2	-	-	-	-	-	-	-	-	-	-
ELISA MPV-1/2	-	-	-	-	-	-	-	-	-	-
ELISA PARV NS1	-	-	-	-	-	-	-	-	-	-

Assays

Sample #: / Code :	11 LDV	12 LDV	13 I	14 II	15 CON	16
Enzyme LDV	**3010**	**2975**	**552**	**330**	**259**	**340**

Remarks: ELISA/IFA Results: - = Negative; +/- = Equivocal; + = Moderate to strong positive; TC = Non-specific reaction with tissue control. HAI Results: number = Titer; AGG = Hemagglutination by specimen. Enzyme LDV: Level of lactate dehydrogenase in IU/L. These test results are valid according to the following criteria:

1. Standard positive and negative control serum reactions were within acceptable limits in the antibody immunoassays.

2. Negative control (CON) mice 5 and 6 were free of antibodies to the infectious agents listed in this report.

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Attachment 1

Charles River In Vivo Biosafety

[redacted]

In Vivo Biosafety Results Report

Sponsor: WuXi AppTec Inc. **Accession #: 2013-000041 (GLP)**

Assays

Sample #: / Code :	17	18	19
LCMV Challenge	7	8	7

Remarks: Challenge LCMV: Number indicated represents days post intracranial (IC) inoculation with LCMV that mouse was found dead or euthanized due to severity of clinical signs.

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Attachment 2

QUALITY ASSURANCE STATEMENT

Protocol: 2013-000041, PR-31-9

This Study has been audited by the Quality Assurance Unit in accordance with the applicable Good Laboratory Practice regulations. Reports were submitted in accordance with SOPs as follows:

QA INSPECTION DATES

		Dates Findings Submitted to:	
Date(s) of Audit	**Phase(s) Audited**	**Study Director**	**Study Director Management**
08-Feb-2013	Final Protocol	08-Feb-2013	08-Feb-2013
11-Mar-2013	Sample Collection	12-Mar-2013	12-Mar-2013
28-Mar-2013	Final Report - IVB	28-Mar-2013	28-Mar-2013

In addition to the above-mentioned audits, process-based and/or routine facility inspections were also conducted during the course of this study. Inspection findings, if any, specific to this study were reported by the Quality Assurance Unit to the Study Director and Management and listed as a Phase Audit on this Quality Assurance Statement.

The Quality Assurance Statements for the work conducted at the Test Sites were reviewed and are included in the appropriate section of this report.

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

[redacted]

Charles River Laboratories

28 Mar 2013

Date

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UW Health

University of Wisconsin
Hospital and Clinics

Histocompatibility/Molecular Diagnostics Laboratory

Date: 01/18/2008 12:35:12

To: Cytogenetics, WiCell Research Institute

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, WA14 3617-HLA H14	DQB SSP		0301	0702	0702/27	0101				
57761 /	A,B,C Seq	01/11/2008	2501	1801/17N	1203/11	0701				
01/11/2008	DRB Seq	01/11/2008								

HLA/Molecular Diagnostics Laboratory

1-18-08

Date

HLA/Molecular Diagnostics Laboratory

1/22/08

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.

PROTOCOL AMENDMENT NUMBER 1

QA Reviewed
JP 15-May-08
Init. Date

BIORELIANCE STUDY NO: AC13RD.032901.BSV

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

SECTION TO BE AMENDED: 3.2.3.1 Negative Control Media

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

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

APPROVAL:

_____ _____ 20Mar08

STUDY DIRECTOR DATE

_____ _____

SPONSOR REPRESENTATIVE DATE

BioReliance®

Final Report

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

Study Number: AC13RD.032901.BSV

Test Article ID: H14-MCB-1

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

Bovine viruses were not detected when the test article, H14-MCB-1, was examined for the presence of nine specific bovine viruses using IFA. In addition, hemadsorption and CPE were not observed in the test article inoculated cultures.

BioReliance®

STUDY INFORMATION

Test Article: H14-MCB-1 was received by BioReliance on 18-Mar-2008 and 02-Apr-2008. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor. Retention of reserve sample from each batch of test article is the responsibility of the sponsor.

Testing Facility: BioReliance

Schedule:

Study Initiation: 20-Mar-2008

Lab Initiation: 04-Apr-2008

Lab Completion: 28-Apr-2008

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

Study Director:

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

BioReliance

Positive Controls:

Bovine viral diarrhea virus (BVDV), ATCC VR-534
Source: American Type Culture Collection (ATCC)
Manassas, VA

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

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

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

Bovine respiratory syncytial virus (BRSV), ATCC VR-1339
Source: ATCC

Reovirus type 3 (REO-3), Strain Abney, ATCC VR-232
Source: ATCC

Infectious bovine rhinotracheitis virus (IBR)
(Bovine herpesvirus 1), ATCC VR-188
Source: ATCC

Bovine parainfluenza virus type 3 (PI3)
(Shipping fever virus), ATCC VR-281
Source: ATCC

Rabies virus (positive control slides)
Source: NVSL

Negative Control:

Dulbecco's Modified Eagle's Medium + 110 mg/mL Sodium Pyruvate + 15% foal serum, 1% L-Glutamine, 0.1% Amphotericin B, and 0.1% Gentamicin (BT)

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

Source: BioReliance

Test System:

Bovine turbinate (BT) cells, ATCC CRL 1390 or equivalent
Source: ATCC
Indicator cells for BVDV, BAV5, BPV, BTV, BRSV, IBR, PI3 and Rabies

Vero (African green monkey kidney) cells, ATCC CCL 81
Source: ATCC
Indicator cells for REO-3, PI3 and Rabies

Erythrocytes:
Chicken erythrocytes
Source: Cambrex Biosciences Walkersville
Walkersville, MD

Guinea-pig erythrocytes
Source: BioReliance

Antibodies:
FITC-conjugated virus-specific immunoglobulins
Source: VMRD Inc. Pullman, Washington
NVSL Ames, Iowa

OBJECTIVE

The study objective is to determine if the test article contains bovine viruses that can be detected by culture with BT and Vero cells. The detection of these viruses is based upon microscopic observation of viral cytopathology in indicator cells, immunofluorescent staining with virus-specific antibodies, a hemadsorption assay, and a cytological staining procedure.

PROCEDURES

Sample Preparation

The test article provided by the sponsor on 02-Apr-2008 was frozen and thawed three times and the resulting lysate was clarified by low speed centrifugation prior to inoculation onto indicator cells.

Methods

The assay was performed according to SOP OPBT0834. The test article was prepared as described above and was used to inoculate subconfluent monolayers of BT and Vero indicator cells seeded at an appropriate passage level for each cell line. After adsorption for 90 ± 9 minutes at 36 ± 2° C, the test article was aspirated and the cells were refed with negative control medium. The cells were observed for viral cytopathology throughout the assay. Negative control and test article cells

control and test article cells were subcultured into 75cm^2 flasks and 6-well plates.

Prior to the second subculture, negative control cells from each indicator line were subcultured to 25-cm^2 flasks and 6-well plates for the positive control inoculation. At the time of the second subculture, flasks of Vero cells were inoculated with REO-3 and flasks of BT cells inoculated with BVDV, BAV5, BPV, BTV, IBR, PI3 and BRSV at 100-300 $FAID_{50}$. The cells were fixed for immunofluorescent staining when the monolayers exhibited ≥ 10% CPE or on day 21 and slides were stored at ≤-60°C. One flask each of BT and Vero negative control and test article cells were harvested the same day their respective positive control flasks were harvested and fixed for IFA testing. Additional flasks of test article and negative control cells were maintained in the lab until assay completion, at which time they were fixed for IFA testing. All fixed cells were stained for IFA at the completion of the assay.

Also at the second subculture, 6-well plates seeded with BT cells were inoculated with PI3 and BVDV, positive controls for hemadsorption and cytological staining, respectively. The Vero cell 6-well plates were inoculated with PI3 for both hemadsorption and cytological staining. The hemadsorption assay and the cytological staining procedure were performed on all conditions at the completion of the assay or when CPE became apparent.

Immunofluorescent Staining

Fixed indicator cells were evaluated for the presence of BVDV, BAV5, BPV, BTV, BRSV, REO-3, IBR, PI3, and Rabies by immunofluorescent staining according to SOP BPBT0829. FITC-conjugated antibodies were incubated with the fixed cells for approximately 60 minutes at 36 ± 2°C. Following incubation, cells were washed with PBS, counterstained with Evans Blue, washed with PBS, and examined by fluorescent microscopy.

Hemadsorption Assay

The negative control, test article and positive control inoculated cultures in 6-well plates were tested by hemadsorption according to SOP OPBT0608. Guinea pig and chicken erythrocytes were inoculated onto the plates and incubated at 2 - 8°C for approximately 30 minutes. The 6-well plates were then incubated at 20 - 25°C for an additional 30 minutes. Cultures were examined microscopically for areas of adherent erythrocytes after each incubation.

Cytological Staining

The negative control, test article and positive control inoculated cultures in 6-well plates were observed for CPE according to SOP OPBT1223. The plates were fixed with a methanol solution and stained with Giemsa, washed with PBS and then examined for CPE.

RESULTS

Bovine viruses were not detected in the test article, H14-MCB-1. Cytopathic effects were not observed in the test article-inoculated BT or Vero cells cultured for 21 days (Table 1). Additionally, CPE was not observed in the test article inoculated BT or Vero cells using cytological staining (Table 2). The test article-inoculated cultures did not hemadsorb with either erythrocyte at either temperature (Table 3). The acetone-fixed indicator cell suspensions did not exhibit fluorescence when reacted with antisera specific for the nine bovine viruses used in this assay (Table 4). All assay controls met the criteria for a valid assay.

APPROVAL

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

16 May 08
Date

Study Director

TABLE 1

Observations for Cytopathic Effects in Cultures of BT and Vero Cells Inoculated with H14-MCB-1

Sample	Results
Negative control[a]	-
Test article[a]	-
Negative control[b]	-
Test article[b]	-
Positive Control Cultures:	
BAV5[a]	+
BPV[a]	+
BRSV[a]	+
BTV[a]	+
BVDV[a]	+
REO-3[b]	+
IBR[a]	+
PI3[a]	+

[a] Inoculated onto BT cells
[b] Inoculated onto Vero cells
- CPE not observed
+ CPE observed

TABLE 2

Observations for CPE using Cytological Staining on BT and Vero Cultures Inoculated with H14-MCB-1

Cytological Staining Results	
Day 21 BT Cells	
Negative Control	-
Test Article	-
Positive Control BVDV [a]	+
Day 21 Vero Cells	
Negative Control	-
Test Article	-
Positive Control PI3 [b]	+

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

TABLE 3

Observations for Hemadsorption in BT and Vero Cultures Inoculated with H14-MCB-1

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

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

TABLE 4

Immunofluorescent Staining Results for BT and Vero Cultures Inoculated with H14-MCB-1

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

[a] Tested in BT indicator cells
[b] Tested in Vero indicator cells
[c] Tested on Rabies infected Vero positive control slide
- Immunofluorescence not observed
+ Immunofluorescence observed
[d] Data reflects results of positive control slides that were prepared on day 17
NA = Not Applicable

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Quality Assurance Statement

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

Study Number: AC13RD.032901.BSV

Study Director:

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

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

**	Inspect On	15-May-08 - 15-May-08 To Study Dir 15-May-08 To Mgmt 16-May-08
	Phase	Final Report and data audit
*	Inspect On	05-Mar-08 - 06-Mar-08 To Study Dir 06-Mar-08 To Mgmt 06-Mar-08
	Phase	Systems Inspection - Test System Preparation
*	Inspect On	05-Mar-08 - 06-Mar-08 To Study Dir 06-Mar-08 To Mgmt 06-Mar-08
	Phase	Systems Inspection - Administration of Test Substance to Test System
*	Inspect On	05-Mar-08 - 06-Mar-08 To Study Dir 06-Mar-08 To Mgmt 06-Mar-08
	Phase	Systems Inspection - Manipulation of Test System
*	Inspect On	07-Mar-08 - 17-Mar-08 To Study Dir 17-Mar-08 To Mgmt 17-Mar-08
	Phase	Systems Inspection - Observation of Test System/Data Collection and/or Analysis

** Inspection specific for this study
* Systems Inspection

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

May 16, 2008
DATE

QUALITY ASSURANCE

PROTOCOL AMENDMENT NUMBER 1

QA Reviewed
JP 15-May-08
Init. Date

BIORELIANCE STUDY NO: AC13RD.032901.BSV

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

SECTION TO BE AMENDED: 3.2.3.1 Negative Control Media

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

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

APPROVAL:

STUDY DIRECTOR _____ 20Mar08 DATE

SPONSOR REPRESENTATIVE _____ DATE

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Final Report

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

Study Number: AC13RD.033901.BSV

Test Article ID: H14-MCB-1

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

Porcine viruses, BVDV, reovirus and Rabies were not detected when the test article H14-MCB-1 was examined for the presence of porcine viruses using IFA. In addition, hemadsorption and CPE were not observed in the test article inoculated cultures.

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STUDY INFORMATION

Test Article: H14-MCB-1 was received by BioReliance on 18-Mar-2008. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor. Retention of reserve sample from each batch of test article is the responsibility of the sponsor.

Testing Facility: BioReliance

Schedule:

Study Initiation: 20-Mar-2008

Lab Initiation: 21-Mar-2008

Lab Completion: 14-Apr-2008

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

Study Director:

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

BioReliance,

Positive Controls:

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

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

Porcine Adenovirus (PAV)
Source: NVSL

Bovine parainfluenza virus type 3 (PI3)
(Shipping fever virus), ATCC VR-281
Source: ATCC

Rabies virus (positive control slide)
Source: NVSL

Negative Control:

Eagle's Minimum Essential Medium + 10% fetal bovine serum, 1% L-Glutamine, 0.1% Amphotericin B, 0.1% Gentamicin
Source: BioReliance

Test System:

Porcine testicle (PT-1) cells
Source: American BioResearch,
Seymour, Tennessee
Indicator cells for PAV, PPV and TGE

Erythrocytes:
Chicken erythrocytes
Source: Cambrex Bioscience Walkersville
Walkersville, MD

Guinea-pig erythrocytes
Source: BioReliance

Antibodies:
FITC-conjugated virus-specific immunoglobulins
Source: VMRD Inc.
Pullman, Washington

OBJECTIVE

The study objective is to determine if the test article contains porcine viruses that can be detected by culture with PT-1 cells. The detection of these viruses is based upon microscopic observation of viral cytopathology in indicator cells, immunofluorescent staining with virus-specific antibodies and a hemadsorption assay.

PROCEDURES

Sample Preparation

The test article provided by the sponsor was frozen and thawed three times and the resulting lysate was clarified by low speed centrifugation prior to inoculation onto PT-1 indicator cells.

Methods

The assay was performed according to SOP OPBT0874. The test article was prepared as described above and was used to inoculate subconfluent monolayers of PT-1 indicator cells. After adsorption for 90 ± 9 minutes at 36± 2°C, the test article was aspirated and cells were refed with negative control medium. The cultures were observed for viral cytopathology throughout the assay. Negative control and test article cells were first subcultured on day 7 post-inoculation. At the time of the second subculture, negative control and test article cells were subcultured into 75 cm^2 flasks and 6-well plates.

One day prior to the second subculture, negative control PT-1 cells were subcultured to 25 cm^2 flasks and a 6-well plate for the positive control inoculation. At the time of the second subculture, flasks of PT-1 cells were inoculated with PAV, PPV and TGE. The cells were fixed for immunofluorescent staining when the monolayers exhibited ≥10% CPE. The fixed cells were stained for IFA at the completion of the assay.

Also at the second subculture, 6-well plates seeded with PT-1 cells were inoculated with PI3 as positive controls for hemadsorption. The hemadsorption assay was performed on all conditions at the completion of the assay or when CPE became apparent.

Immunofluorescent Staining

Fixed indicator cells were evaluated for the presence of PAV, PPV, TGE, BVDV, REO-3 and Rabies by immunofluorescent staining according to SOP BPBT0829. FITC-conjugated antibodies were incubated with the fixed cells for approximately 60 minutes at 36 ± 2°C. Following incubation, cells were washed with PBS, counterstained with Evans Blue, washed with PBS, and examined by fluorescent microscopy.

Hemadsorption Assay

The negative control, test article and positive control inoculated cultures in 6-well plates were tested by hemadsorption according to SOP OPBT0608. Guinea pig and chicken erythrocytes were inoculated onto the plates and incubated at 2 - 8°C for approximately 30 minutes. The 6-well plates were then incubated at 20 - 25°C for an additional 30 minutes. Cultures were examined microscopically for areas of adherent erythrocytes after each incubation.

RESULTS

Porcine viruses were not detected in the test article H14-MCB-1. Cytopathic effects were not observed in the test article inoculated PT-1 indicator cells cultured for 21 days (Table 1). The test article-inoculated cultures did not hemadsorb with either erythrocyte at either temperature (Table 2). The acetone-fixed indicator cell suspensions did not exhibit fluorescence when reacted with antisera specific for the porcine and bovine viruses used in this assay (Table 3). All assay controls met the criteria for a valid assay.

APPROVAL

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

24Apr08
Date

Study Director

TABLE 1

Observations for Cytopathic Effects in Cultures of PT-1 Cells Inoculated with H14-MCB-1

Sample	Results
Negative control	-
Test article	-
Positive Control Cultures	
PAV	+
PPV	+
TGE	+

- CPE not observed
+ CPE observed

TABLE 2

Observations for Hemadsorption in Monolayers of PT-1 Cells Inoculated With H14-MCB-1

	Hemadsorption Results			
	2-8°C		20-25°C	
	C [a]	G	C	G
Day 21 PT-1 cells				
Negative Control	-	-	-	-
Test Article	-	-	-	-
Positive Control PI3 [b]	+	+	+	+

[a] Erythrocytes used in these assays: C = chicken, G = Guinea pig
[b] Positive control tested on day 19
- Hemadsorption not observed
+ Hemadsorption observed

TABLE 3

Immunofluorescent Staining Results for PT-1 cells Inoculated With H14-MCB-1

	PBS	αPAV	αPPV	αTGE	αBVDV	αREO-3	αRabies
Slides Prepared Day 21							
Test Article	-	-	-	-	-	-	-
Negative Control	-	-	-	-	-	-	-
	PBS	**αPAV**	**αPPV**	**αTGE**	**αBVDV [b]**	**αREO-3 [b]**	**αRabies [a]**
Positive Control	-	+	+	+	+	+	+

- = immunofluorescence not observed
+ = immunofluorescence observed
[a] Tested on Rabies infected Vero positive control slide
[b] Slides from corresponding bovine study.

BioReliance®

Quality Assurance Statement

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

Study Number: AC13RD.033901.BSV

Study Director:

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

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

**	Inspect On	24-Apr-08 - 24-Apr-08 To Study Dir 24-Apr-08 To Mgmt 25-Apr-08
	Phase	Final Report and data audit
*	Inspect On	05-Mar-08 - 06-Mar-08 To Study Dir 06-Mar-08 To Mgmt 06-Mar-08
	Phase	Systems Inspection - Test System Preparation
*	Inspect On	05-Mar-08 - 06-Mar-08 To Study Dir 06-Mar-08 To Mgmt 06-Mar-08
	Phase	Systems Inspection - Administration of Test Substance to Test System
*	Inspect On	05-Mar-08 - 06-Mar-08 To Study Dir 06-Mar-08 To Mgmt 06-Mar-08
	Phase	Systems Inspection - Manipulation of Test System
*	Inspect On	07-Mar-08 - 17-Mar-08 To Study Dir 17-Mar-08 To Mgmt 17-Mar-08
	Phase	Systems Inspection - Observation of Test System/Data Collection and/or Analysis

** Inspection specific for this study
* Systems Inspection

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

25 Apr 08

DATE

QUALITY ASSURANCE

WuXi AppTec

E-FR-1-08-001326.30610

FINAL STUDY REPORT

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

TEST PROTOCOL NUMBER: 30610.06

TEST ARTICLE IDENTIFICATION	WUXI APPTEC ACCESSION NUMBER
WA14-MCB-1	08-001326

SPONSOR: WiCell Research Institute

PERFORMING LABORATORY: WuXi AppTec, Inc.

WUXI APPTEC ACCESSION NUMBER	RESULTS
08-001326	Transmission electron microscopic examination of 200 cells revealed no identifiable virus-like particles nor did it reveal any other identifiable microbial agents.

QUALITY ASSURANCE UNIT SUMMARY

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

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

Phase Inspected	Date
Step 4.3.2 Ship the fixed cells on ice or with a cold pack to the subcontractor via overnight delivery.	July 2, 2008

Quality Assurance

14 Aug 08
Date

GOOD LABORATORY PRACTICES STATEMENT

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

Study Director

15 Aug 08
Date

Professional Personnel involved in study:

1.0 PURPOSE

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

2.0 SPONSOR: WiCell Research Institute

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

4.0 SCHEDULING

DATE SAMPLES RECEIVED: July 1, 2008
STUDY INITIATION DATE: July 2, 2008
STUDY COMPLETION DATE: See page 2 for Study Director's signature and date.

5.0 TEST ARTICLE CHARACTERIZATION

Determinations of strength, homogeneity, purity and stability of the test article are solely the responsibility of the Sponsor. The Sponsor is responsible for supplying to the testing laboratory results of these determinations and any others that may directly impact the testing performed by the testing laboratory, prior to initiation of testing.

6.0 TEST ARTICLE IDENTIFICATION: WA14-MCB-1

7.0 TEST SYSTEM DESCRIPTION

As described in the Points to Consider (May 1993), the morphological and growth characteristics of cell lines used for the production of biologics need to be monitored. Cells in culture possess inherent qualities, some of which are amenable to study by transmission electron microscopy. The use of electron microscopy allows for the visualization of cellular components, which help in the identification of cell type and may aid in describing any cellular changes that could occur during biopharmaceutical production.

Preparation of thin sections of virus-infected cells and tissues is an indispensable technique for the study of those aspects of virus-cell interaction that are accessible to direct examination by electron microscopy.[1,2,3] Thin sectioning is also of value in elucidating the structure of viruses; the information obtained often complements that provided by a negative staining procedure. This protocol can be utilized to visualize a variety of viral types including retroviruses, herpesviruses, adenoviruses, picornaviruses, parvoviruses, orthomyxo- and paramyxoviruses, reoviruses, and many other common viral agents. Contamination by other microbial agents such as yeast, fungi, and bacteria may also be detected.

If retroviruses are detected they will be evaluated on the basis of A-, B-, C-, D-, and R-type retrovirus-like morphologies. *A-type* viral particles are characterized as either (1) intracytoplasmic particles, 60-90 nanometers (nm) in diameter, with an electron-dense core; (2) intracisternal particles, 60-90 nm in diameter, found within the endoplasmic reticulum, with 2 dense concentric shells surrounding an electron-lucent core. *B-type* particles are spherical, enveloped particles that arise by budding at the plasma membrane. They display an eccentric, electron-dense core surrounded by an intermediate layer, and an envelope with prominent projections. *C-type* viral particles are 90-130 nm in diameter, enveloped, and contain an internal nucleoid of variable electron density and shape. They occur either within cytoplasmic vacuoles, on the cell surface, or extracellularly. *D-type* particles are spherical, enveloped particles that bud from the plasma membrane and frequently exhibit an electron-dense bar- or tube-shaped core. *R-type* particles are enveloped, spherical particles, 70-100 nm in diameter, with a central core of variable density from which characteristic spokes extend into the envelope, and are found in the cisternae of the endoplasmic reticulum.

8.0 EXPERIMENTAL DESIGN

For most purposes, optimum preservation of fine structure in animal cells, viruses, and other microbial agents is the prime consideration, and procedures for ensuring this are now fairly well standardized. The cells submitted to WuXi AppTec Laboratories were already fixed by the Client.

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

8.2 The pellet was shipped to the subcontractor Charles River Laboratories Pathology Associates (CRLPA), where typically (if enough cells are available) one-half of the cell pellet(s) were processed and embedded for transmission electron microscopy (TEM).

8.3 Thin sections were cut and mounted on 200-mesh copper grids.

8.4 The samples were stained with 5% methanolic uranyl acetate and Reynold's lead citrate.

8.5 The cells were examined by TEM to characterize morphologically the cell type comprising the culture. Cell characteristics were documented by labeled electron micrographs.

8.6 200 cells were evaluated for the presence of any type of particle with virus-like morphology, and appropriate documentation was provided for any particles found using labeled electron micrographs.

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

8.8 200 cells were evaluated for particles with A-, B-, C-, D-, and R-type retrovirus-like morphology as described in Section 3. Electron micrographs were made to document representative examples of any virus-like particles observed. Except where noted otherwise, a bar denoting 100 nanometers was placed on each micrograph for size reference.

9.0 TEST ARTICLE PREPARATION

On July 1, 2008, WuXi AppTec, Inc. received 1 vial containing 1.0 mL of "hES Cells," cold on cold packs and designated for use in the retest of this assay. On July 2, 2008, 1 vial containing a fixed and pelleted cell culture was shipped, on ice packs, in storage conditions of 2–8°C, via overnight courier to the subcontractor.

10.0 NEGATIVE CONTROLS

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

11.0 ASSAY VALIDITY

The following validity criteria are evaluated:

11.1 The test is valid if the test article cells are well preserved and at least 200 cells are examined.

12.0 TEST EVALUATION

Detailed description of unique or distinguishing characteristics of cell ultrastructure will be included and documented by labeled electron micrographs. The general appearance or preservation of the cells will be noted.

Analysis of the photomicrograph from the thin sections will provide the opportunity to observe contaminating viruses or other microbial agents and the morphological responses of the host cell. 200 cells will be examined. The type of viral particles and percentage of cells containing the particles will be enumerated.

13.0 RESULTS

The test was valid. The test article cells were well preserved, and at least 200 cells were examined.

Cellular Ultrastructure

Cells in the section were moderate to large in size and polygonal to irregular in shape (J59091). Cells had microvilli (MV: J59093) unevenly distributed on the surface. Nuclei (N: J59091) tended to be irregular, with chromatin relatively evenly dispersed or clumped along the periphery. Nuclei often had one or more nucleoli (NS: J59091) that were variably located and nuclear pores (NP: J59089) were seen.

The cytoplasm of most cells contained varying numbers of dense mitochondria (MI: J59093). Profiles of rough endoplasmic reticulum (RER: J59094) were seen among the mitochondria. Ribosomes (RB: J59094) were abundant in the cytoplasm of most cells. Cells were observed to contain tubules (T: J59089) and glycogen (G: J59092). Myelin figures (MF: J59095), and autophagic vacuoles (AV: J59095), often filled with large amounts of an electron-dense material, were also seen.

General Viral Particle Evaluation

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

Forty percent of the cells were necrotic.

14.0 CONCLUSION

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

15.0 STATISTICAL DATA ANALYSIS

Statistical analysis of the data is not required.

16.0 DEVIATIONS / AMENDMENTS

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

No amendments to the protocol were generated.

17.0 RECORD RETENTION

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

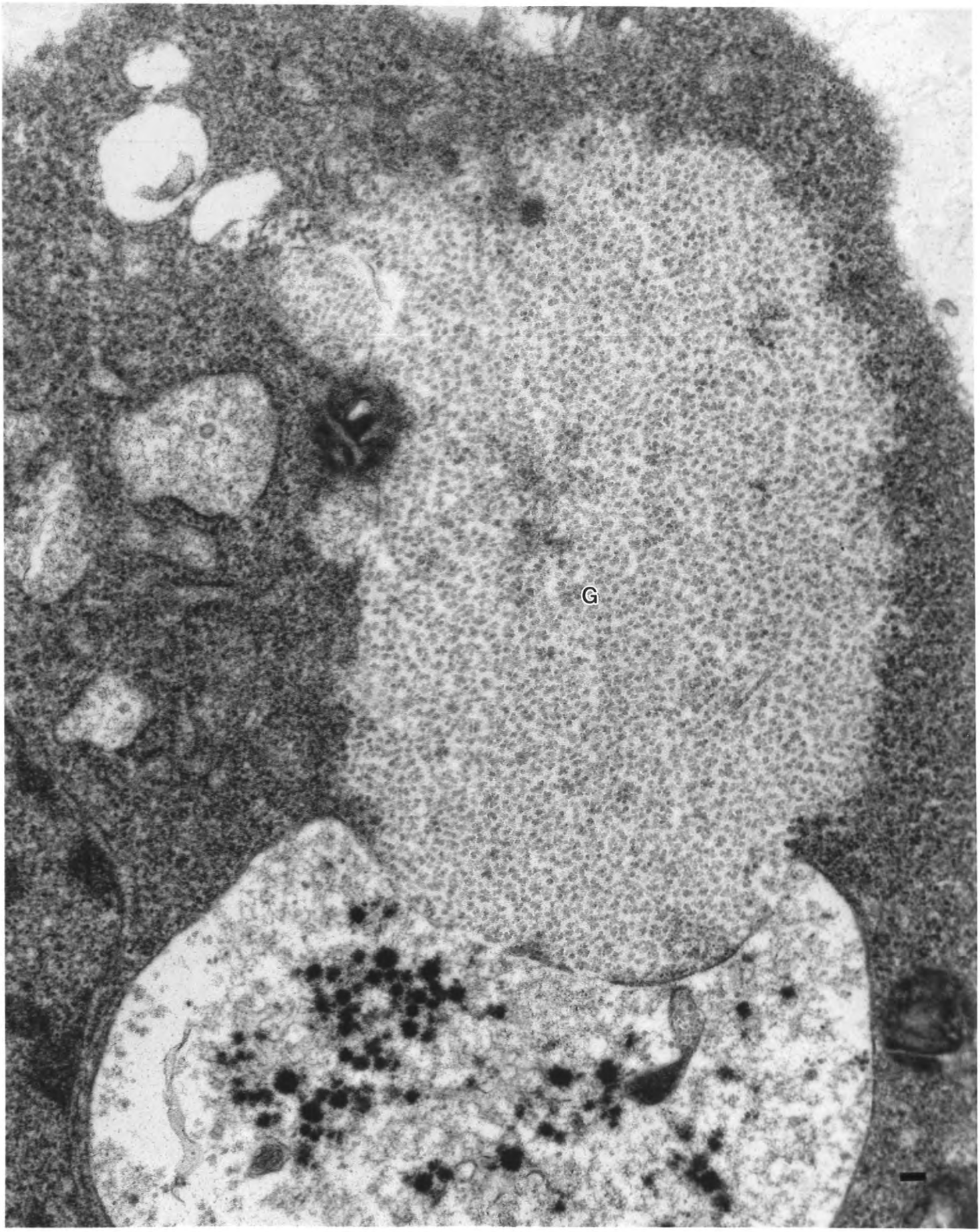
18.0 REFERENCES

1. Morgan C and Rose HM (1967). "The Application of Thin Sectioning," *Methods in Virology* Vol. **3** (Maramorosch K and Koprowski H, eds.), Academic Press, New York, NY, pp. 576-616

2. Palmer E and Martin M (1988). Retroviridae in "Electron Microscopy in Viral Diagnosis", CRC Press, Boca Raton, FL, pp. 91-103

3. Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals (1993). Office of Biologics Research and Review, Food and Drug Administration

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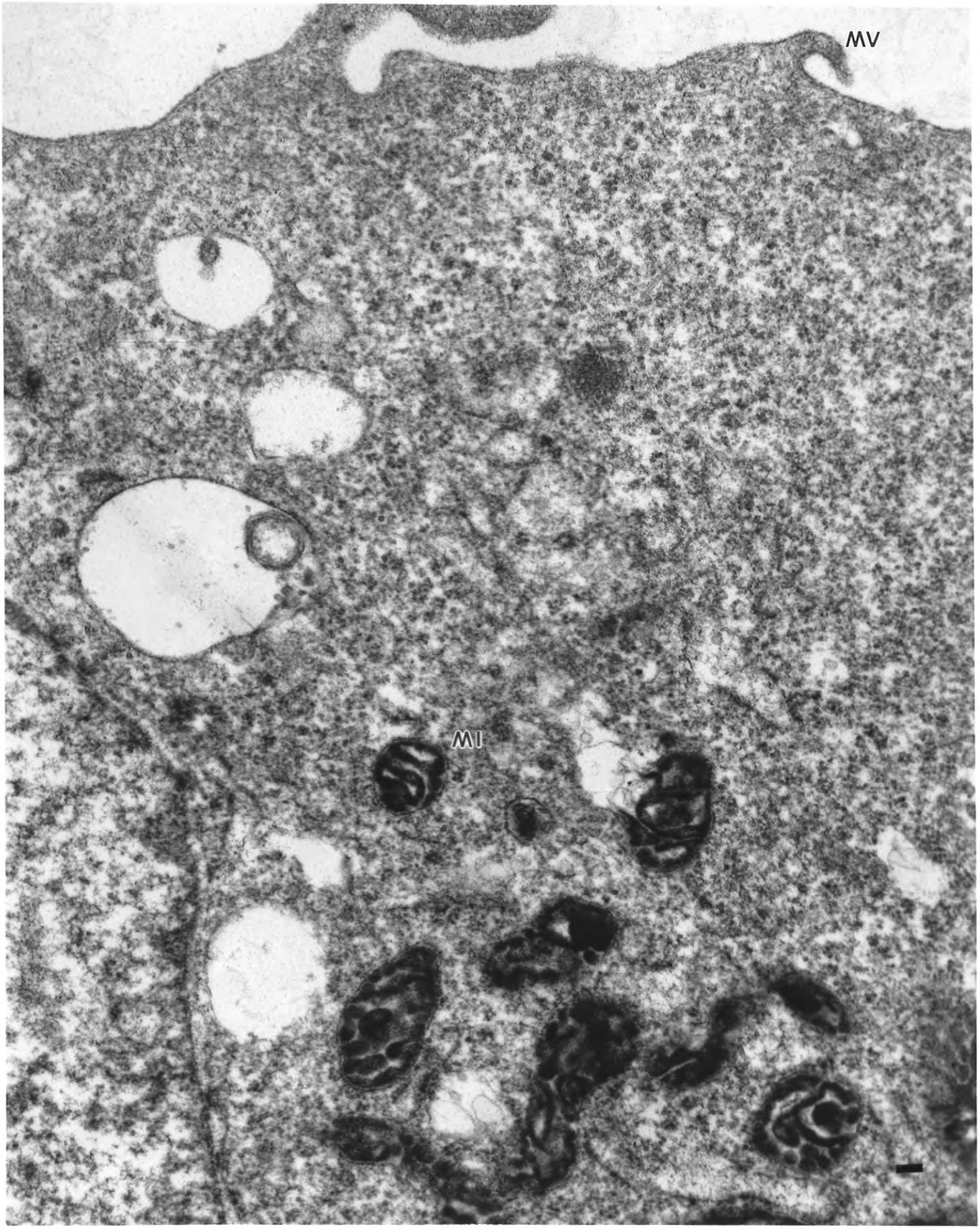
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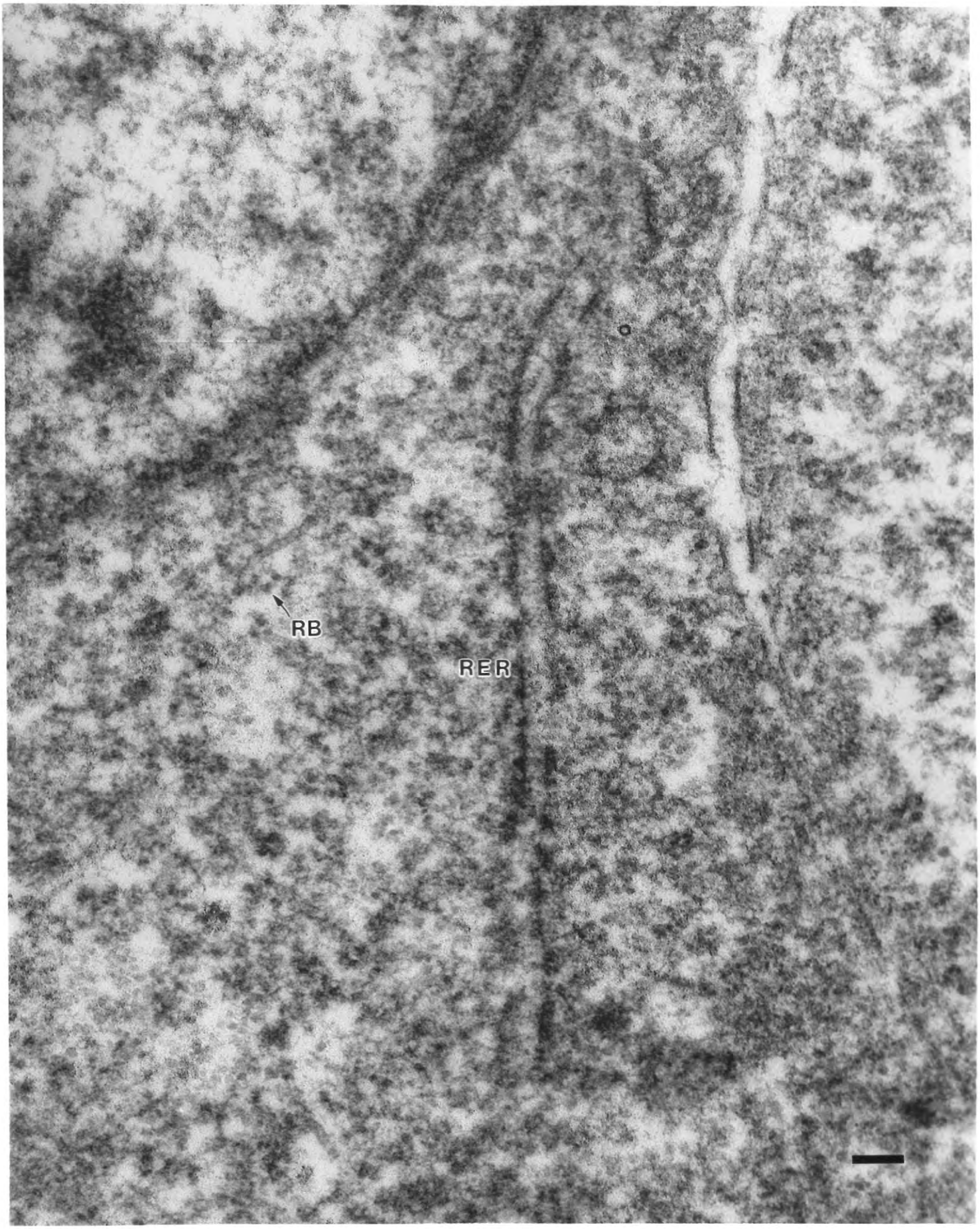
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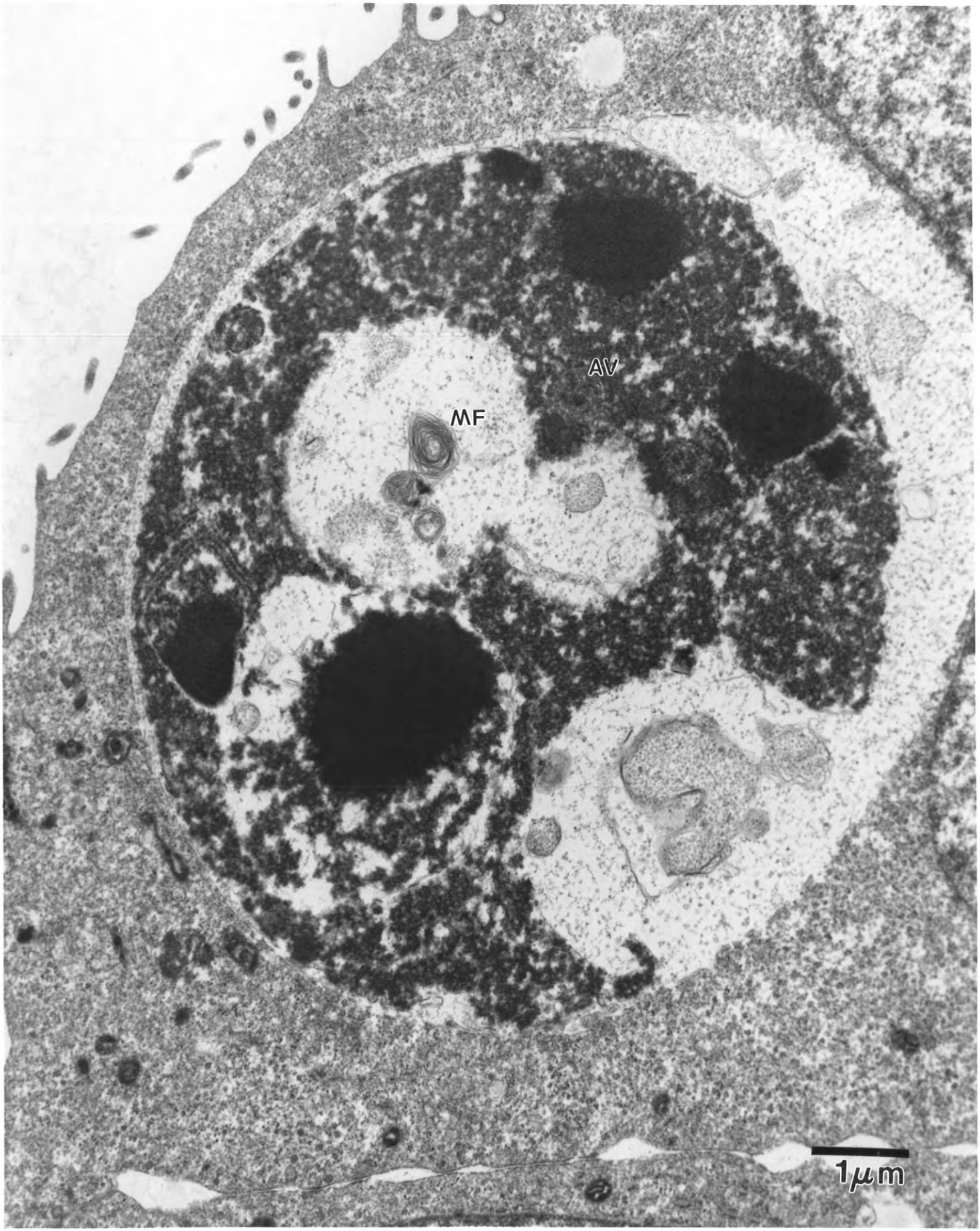
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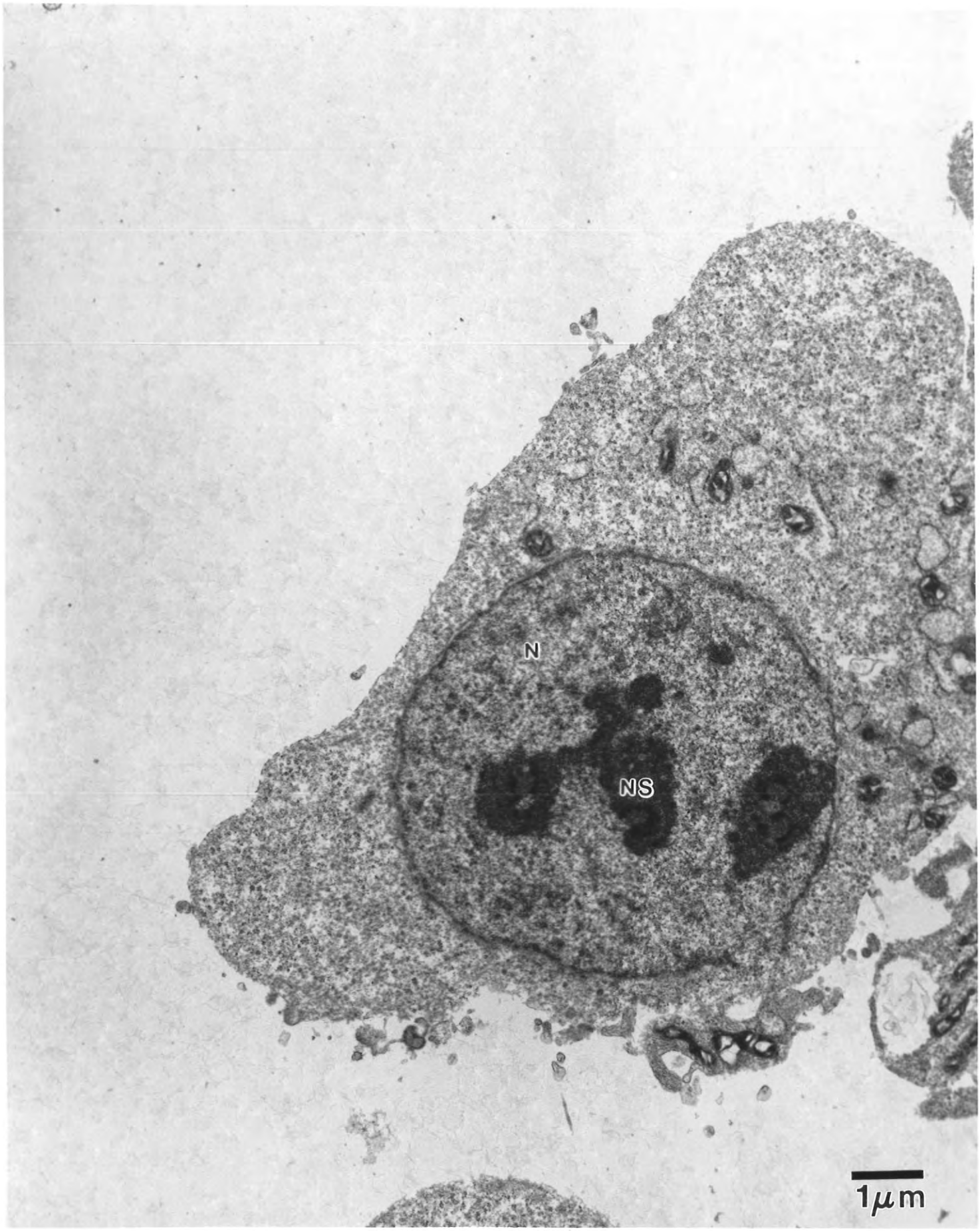
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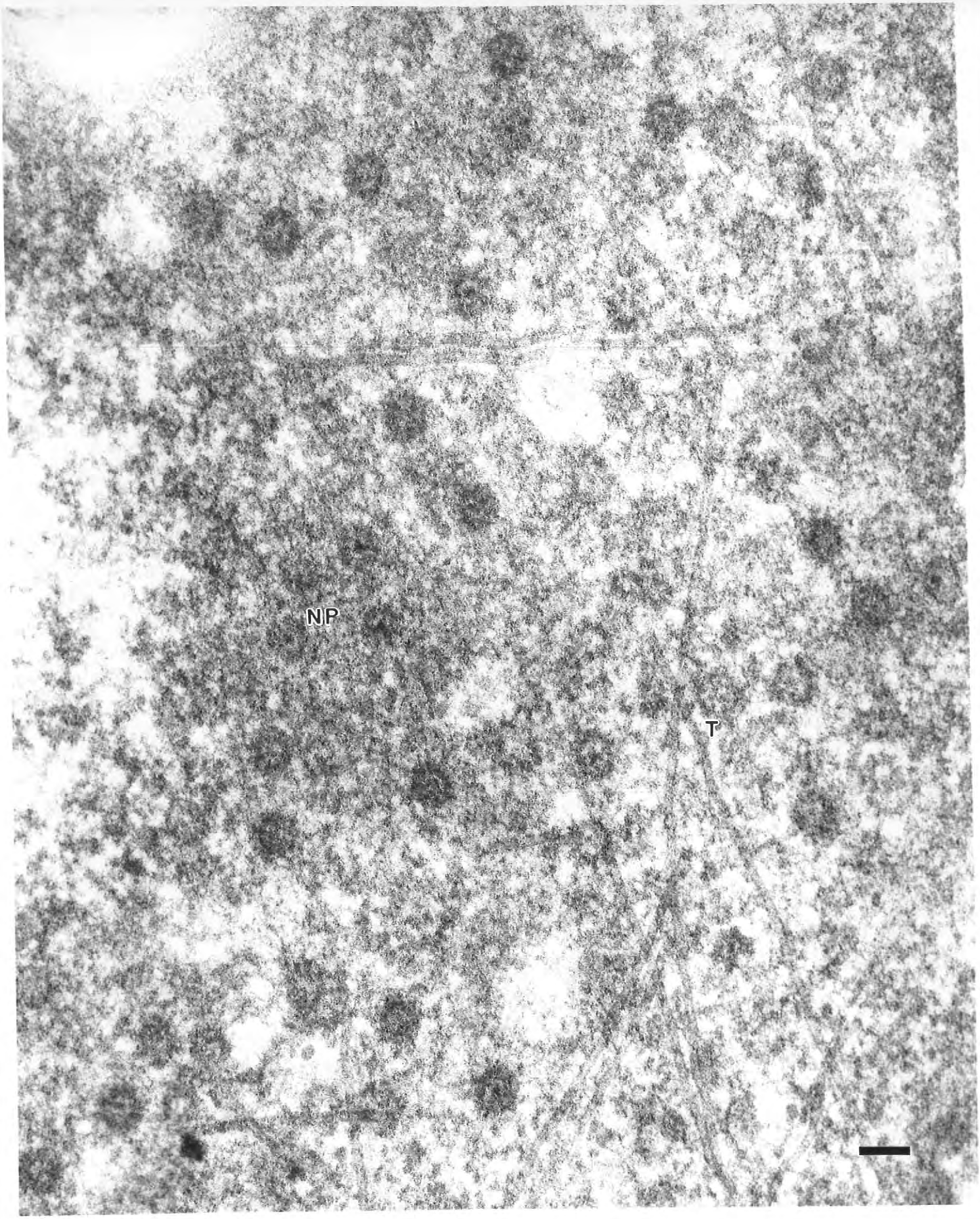
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WuXi AppTec

E-FR-1-08-000975.30201

FINAL STUDY REPORT

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

PROTOCOL: 30201.04

TEST ARTICLE IDENTIFICATION	WUXI APPTEC ACCESSION NUMBER
WA14-MCB-1	08-000975

SPONSOR: WiCell Research Institute

PERFORMING LABORATORY: WuXi AppTec, Inc.

WUXI APPTEC ACCESSION NUMBER	RESULTS
08-000975	No evidence for xenotropic, amphotropic, or MCF MuLV retroviral contamination was found in the test article. Following co-cultivation the test article demonstrated a negative response in the PG4 S^+L^- assay.

QUALITY ASSURANCE UNIT SUMMARY

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

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

Phase Inspected	Date
BR# 30201.04 Step 4.8.4 Remove the growth medium from all test article flasks.	May 19, 2008

Quality Assurance

24Jun08
Date

GOOD LABORATORY PRACTICES STATEMENT

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

Study Director

6-24-08
Date

Personnel involved in study:

1.0 PURPOSE

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

2.0 SPONSOR: WiCell Research Institute

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

4.0 SCHEDULING

DATE SAMPLES RECEIVED: May 13, 2008
STUDY INITIATION DATE: May 14, 2008
STUDY COMPLETION DATE: See page 2 for Study Director's signature and date.

5.0 TEST ARTICLE CHARACTERIZATION

Determinations of strength, homogeneity, purity, and stability of the test article are solely the responsibility of the Sponsor. The Sponsor is responsible for supplying to the testing laboratory results of these determinations and any others that may directly impact the testing performed by the testing laboratory, prior to initiation of testing.

6.0 TEST ARTICLE IDENTIFICATION: WA14-MCB-1

7.0 TEST SYSTEM DESCRIPTION

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

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

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

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

8.0 EXPERIMENTAL DESIGN

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

8.1 Co-Cultivation with *Mus dunni* Cells

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

8.1.2 An aliquot of the test article supernatant was saved for testing in the PG4 S^+L^- assay as a T_0 time point.

8.1.3 In order to initiate the co-cultivation, $5x10^5$ *Mus dunni* cells and $5x10^5$ of the test article cells were mixed.

8.1.4 Positive controls were established last, using viral amphotropic murine retrovirus stocks inoculated between 10 and 100 FFU.

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

8.1.6 Cultures were passaged on days 5 and 9 post-inoculation. The negative cultures were handled first, followed by the test article cultures, and finally the positive controls.

8.1.7 Cell culture supernatants were collected from the negative control, test article, and positive control cultures on day 14. The supernatants were frozen at -60°C or below until tested.

8.2 PG4 S^+L^- Assay (30165)

8.2.1 The PG4 cells were set up 1 day prior to inoculation. The cells were set up in 6-well plates using media containing polybrene to increase viral uptake.

8.2.2 On the day of inoculation, the cells were inoculated (0.5 ml per well) starting first with the assay negative controls plates, which were inoculated with Eagle's Minimum Essential Medium (EMEM). The co-cult test samples were then added, 0.5 ml per well at a 1:2 dilution, in triplicate, starting first with the negative control and followed by the test article. The co-cult positive samples were inoculated last onto the PG4 cells at 3 dilutions (1:10, 1:100 and 1:1000).

8.2.3 The assay positive control was inoculated onto PG4 S^+L^- cells, utilizing a few dilutions of the virus (1:1000 and 1:10000). The positive was an amphotropic virus.

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

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

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

9.0 TEST ARTICLE PREPARATION

On May 13, 2008, WuXi AppTec, Inc. received 1 T25 flask of "hES cells grown with mouse embryonic fibroblast feeder cells." The flask was stored at 37°C ± 2°C and placed on hold pending receipt of a sample submission form and clarification of storage conditions. On May 14, 2008, the test article was released from hold and continued to be stored at 37°C ± 2°C until the assay was initiated.

10.0 POSITIVE CONTROLS

10.1 Co-Cultivation Controls

As a positive infectious retrovirus control, *Mus dunni* cells inoculated with an amphotropic retrovirus (A-MuLV) were run in parallel with the test article cells in the co-cultivation assay for 14 days. These were assayed in the PG4 S^+L^- assay to confirm the replication of these viruses.

10.2 Controls for PG4 S^+L^- Assay

Known positive amphotropic murine leukemia virus was run along with the test samples in each assay as positive controls.

11.0 NEGATIVE CONTROLS

11.1 Co-Cultivation Controls

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

11.2 Controls for PG4 S^+L^- Assay

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

12.0 ASSAY VALIDITY

12.1 Validity Criteria for Co-Cultivation

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

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

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

13.0 TEST EVALUATION

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

14.0 RESULTS

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

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

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

	Culture Inoculum	Time	FFU/ml
Co-Cultivation Samples	**Accession # 08-000975[1] (diluted 1:2)**	**T_0**	**ND**
	Accession # 08-000975 (diluted 1:2)	**PP2**	**ND**
	Negative control[2] (diluted 1:2)	T_0	ND
	Negative control (diluted 1:2)	PP2	ND
	Positive control (A-MuLV)[3] (diluted 1:10)	PP2	TNTC
	Positive control (A-MuLV)[3] (diluted 1:100)	PP2	TNTC
	Positive control (A-MuLV)[3] (diluted 1:1000)	PP2	TNTC
PG4 S^+L^- - Assay Controls	Negative control (EMEM)	NA	ND
	High positive control (A-MuLV) (diluted 1:1000)	NA	TNTC
	Low positive control (A-MuLV) (diluted 1:10000)	NA	2.67×10^4

Legend:

T_0	-	Time 0
PP2	-	Post passage 2
NA	-	Not applicable
ND	-	None detected
TNTC	-	Too numerous to count

1 Supernatant collected from initial test article cultures used to prepare cultures for this assay.

2 Controls prepared from supernatant taken from fresh *M. dunni* cultures used to prepare cultures for assay

3 Stock virus used to initiate positive control in co-cultivation assay

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

15.0 CONCLUSION

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

16.0 STATISTICAL DATA ANALYSIS

Statistical analysis of the data was not required.

17.0 DEVIATIONS / AMENDMENTS

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

No amendments to the protocol were generated.

18.0 RECORD RETENTION

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

19.0 REFERENCES

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

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

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

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

Final Report

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

Study Number: AC13RD.105010.BSV

Test Article ID: H14-MCB-1

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

Ten (10) µl of isolated from test article H14-MCB-1 was analyzed for the presence of human immunodeficiency virus types 1 and 2 (HIV-1/2) proviral DNA by the polymerase chain reaction (PCR)[1] technique. The assay can detect 100 copies of HIV-1/2 proviral DNA in the presence of 0.5 µg of genomic DNA.

The results presented herein indicate that the test article tested negative for the presence of HIV-1/2 DNA.

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

STUDY INFORMATION

Test Article: H14-MCB-1 was received by BioReliance on 03/18/2008. Determination of the stability, purity, and concentration of the test article is the responsibility of the sponsor. Retention of reserve sample from each batch of test article is the responsibility of the sponsor.

Testing Facility: BioReliance

Schedule:

Study Initiation: 03/21/2008

Lab Initiation: 03/25/2008

Lab Completion: 03/31/2008

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

Study Director:

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

BioReliance

OBJECTIVE

The objective of the study is to detect the presence of HIV-1/2 proviral sequences in the test article by PCR amplification, an *in vitro* procedure that can generate millions of copies of a sequence from very low levels of template DNA.

TEST SYSTEM

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

Negative Control:		Genomic DNA from MRC5 human fetal lung fibroblasts Source: BioReliance
Positive Control:	HIV-1:	Negative control DNA spiked with 100 copies of pSYC1857, a plasmid containing the complete genome of HIVZ6 with an interruption in the protease coding region Source: Applied BioSystems
	HIV-2:	Negative control DNA spiked with 100 copies of pMAHIV2, a plasmid containing a 963 bp fragment from the HIV-2 proviral genome Source: BioReliance
No DNA Control:		Nuclease free water Source: USB or other commercial supplier
Spiked Control:		The spiked controls (amplification suitability controls) verify the absence of PCR inhibitors in the test article DNA.
	HIV-1:	Test article extract spiked with 100 copies of pSYC1857
	HIV-2:	Test article extract spiked with 100 copies of pMAHIV2

METHODS

Sample Preparation

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

DNA Amplification

Each PCR amplification was performed on 10 µl of test article DNA and on the assay controls, using either primers SK38 and SK39, specific for the core protein coding region of HIV-1, or primers OG63 and OG81, specific for the core protein coding region of HIV-2, employing conditions optimized to achieve detection of 100 copies of proviral DNA. Aliquots of the amplification products obtained from the test article and from the control samples were analyzed by agarose gel electrophoresis in the presence of ethidium bromide. Amplification products were visualized on a UV transilluminator and photographed using Polaroid 667 film (SOP's OPBT0922 and OPBT0933).

RESULTS

Test article DNA (10 µl) was analyzed for the presence of HIV-1/2 proviral DNA by PCR amplification and agarose gel electrophoresis in the presence of ethidium bromide. The results are shown in Figure 1a (HIV-1) and 1b (HIV-2).

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

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

These results provide evidence that the test article H14-MCB-1 tested negative for the presence of HIV-1/2 proviral DNA.

APPROVAL

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

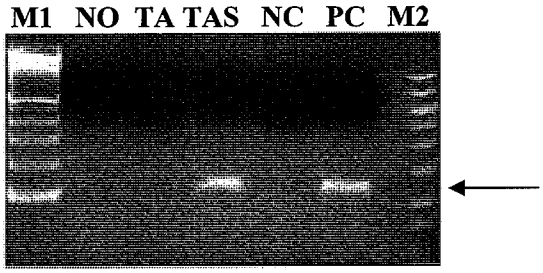
15 Apr 08

Date

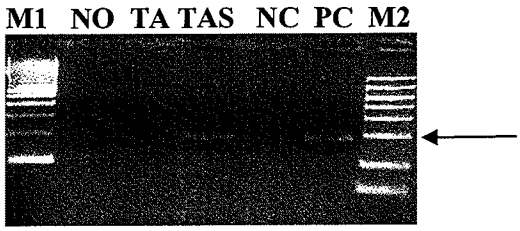
Study Director

FIGURE 1

a. HIV-1



b. HIV-2



Detection of HIV-1 (**a.**) or HIV-2 (**b.**) proviral sequences in the test article H14-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 pSYC1857 (**a.**) or pMAHIV2 (**b.**)
NC: Negative control (MRC5 genomic DNA)
PC: Positive control (negative control DNA spiked with 100 copies pSYC1857 (**a.**) or pMAHIV2 (**b.**)
M2: Biomarker low DNA size marker.

Arrows indicate specific amplification products.

Quality Assurance Statement

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

Study Number: AC13RD.105010.BSV

Study Director:

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

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

**	Inspect On	01-Apr-08 - 01-Apr-08 To Study Dir 01-Apr-08 To Mgmt 01-Apr-08
	Phase	Data Audit
**	Inspect On	15-Apr-08 - 15-Apr-08 To Study Dir 15-Apr-08 To Mgmt 15-Apr-08
	Phase	Final Report and data audit
*	Inspect On	31-Mar-08 - 31-Mar-08 To Study Dir 31-Mar-08 To Mgmt 31-Mar-08
	Phase	Systems Inspection - Administration of Test Substance to Test System
*	Inspect On	31-Mar-08 - 31-Mar-08 To Study Dir 31-Mar-08 To Mgmt 31-Mar-08
	Phase	Systems Inspection - Manipulation of Test System
*	Inspect On	31-Mar-08 - 31-Mar-08 To Study Dir 31-Mar-08 To Mgmt 31-Mar-08
	Phase	Systems Inspection - Observation of Test System/Data Collection and/or Analysis
*	Inspect On	31-Mar-08 - 31-Mar-08 To Study Dir 31-Mar-08 To Mgmt 31-Mar-08
	Phase	Systems Inspection - Test System Preparation

** Inspection specific for this study
* Systems Inspection

fm MB 15Apr'08

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

15 Apr 08

DATE

QUALITY ASSURANCE

Fm m 15 Apr 08

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: AC13RD.105013.BSV

Test Article ID: H14-MCB-1

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

Ten (10) µl of DNA isolated from test article H14-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: H14-MCB-1 was received by BioReliance on 03/18/2008. Determination of the stability, purity, and concentration of the test article is the responsibility of the sponsor. Retention of reserve sample from each batch of test article is the responsibility of the sponsor.

Testing Facility: BioReliance

Schedule:

Study Initiation: 03/21/2008

Lab Initiation: 03/25/2008

Lab Completion: 03/28/2008

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

Study Director:

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

BioReliance

OBJECTIVE

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

TEST SYSTEM

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

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

METHODS

Sample Preparation

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

DNA Amplification

Each PCR amplification was performed on 10 µl 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 (SOP OPBT0932).

RESULTS

Test article DNA (10 µl) 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 H14-MCB-1 tested negative for the presence of HTLV-I/II proviral DNA.

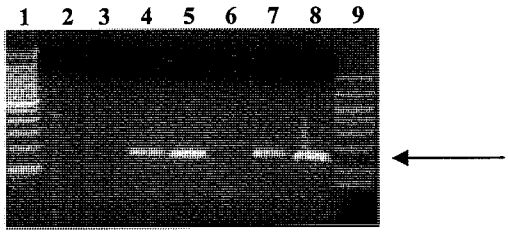
APPROVAL

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.

15 Apr 08
Date

Study Director

FIGURE 1



Detection of HTLV-I/II proviral sequences in test article H14-MCB-1 utilizing agarose gel electrophoresis in the presence of ethidium bromide

1) **M1:** 100 bp DNA ladder
2) **NO:** No DNA control
3) **TA:** Test Article
4) **TAS-I:** Test article spiked with 100 copies of pH750
5) **TAS-II:** Test article spiked with 100 copies of pMAHTII
6) **NC:** Negative control (MRC5 genomic DNA)
7) **PC-I:** Positive control for HTLV-I (negative control DNA spiked with 100 copies pH750)
8) **PC-II:** Positive control for HTLV-II (negative control DNA spiked with 100 copies pMAHTII)
9) **M2:** Biomarker low DNA size marker

The arrow indicates specific amplification products.

Quality Assurance Statement

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

Study Number: AC13RD.105013.BSV

Study Director:

Quality Assurance performed the inspections listed below for this study. Verification of the study protocol was also performed and documented by QA. Procedures, documentation, equipment records, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the 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	01-Apr-08 - 01-Apr-08 To Study Dir 01-Apr-08 To Mgmt 01-Apr-08
	Phase	Data Audit
**	Inspect On	15-Apr-08 - 15-Apr-08 To Study Dir 15-Apr-08 To Mgmt 15-Apr-08
	Phase	Final Report and data audit
*	Inspect On	31-Mar-08 - 31-Mar-08 To Study Dir 31-Mar-08 To Mgmt 31-Mar-08
	Phase	Systems Inspection - Administration of Test Substance to Test System
*	Inspect On	31-Mar-08 - 31-Mar-08 To Study Dir 31-Mar-08 To Mgmt 31-Mar-08
	Phase	Systems Inspection - Manipulation of Test System
*	Inspect On	31-Mar-08 - 31-Mar-08 To Study Dir 31-Mar-08 To Mgmt 31-Mar-08
	Phase	Systems Inspection - Observation of Test System/Data Collection and/or Analysis
*	Inspect On	31-Mar-08 - 31-Mar-08 To Study Dir 31-Mar-08 To Mgmt 31-Mar-08
	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.

15 Apr 08

DATE

QUALITY ASSURANCE

Final Report

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

Study Number: AC13RD.105042.BSV

Test Article ID: H14-MCB-1

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

Ten (10.0) µl of DNA isolated from test article H14-MCB-1 was analyzed for the presence of Hepatitis B virus (HBV) DNA by the polymerase chain reaction (PCR)[1] technique. The assay can detect 100 copies of HBV in the presence of 0.5 µg of genomic DNA.

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

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

STUDY INFORMATION

Test Article: H14-MCB-1 was received by BioReliance on 03/18/2008. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor. Retention of reserve sample from each batch of test article is the responsibility of the sponsor.

Testing Facility: BioReliance

Schedule:

Study Initiation: 03/21/2008

Lab Initiation: 03/25/2008

Lab Completion: 03/27/2008

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

Study Director:

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

BioReliance

OBJECTIVE

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

Test System:

PCR amplification is performed on test article DNA using HBV-specific primers. In the presence of HBV, these primers produce a 347 bp amplification product. The amplification products are analyzed by agarose gel electrophoresis in the presence of ethidium bromide. The following controls are included in the assay:

Negative Control:	Genomic DNA from HBV negative cells. Source: BioReliance
Positive Control:	Negative control DNA spiked with 100 copies of HBV185, a plasmid containing a 1850 bp fragment from the HBV core antigen sequence. Source: BioReliance
No DNA Control:	Nuclease-free water Source: USB or other commercial source.
Spiked Control:	Test article extract spiked with 100 copies of HBV185, to verify the absence of PCR inhibitors in the test article DNA (amplification suitability control).

METHODS

Sample Preparation

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

DNA Amplification

PCR amplification was performed on 10.0 µl of test article DNA using primers HBV-C2 and HBV-C3 specific for the HBV core antigen sequence, employing conditions optimized to achieve detection of 100 copies of HBV. Aliquots of the amplification products obtained from the test article and from the control samples were analyzed by agarose gel electrophoresis in the presence of ethidium bromide. Amplification products were visualized on a UV transilluminator and photographed using Polaroid 667 film (SOP OPBT0953).

RESULTS

Test article DNA (10.0 µl) was analyzed for the presence of HBV DNA by PCR amplification and agarose gel electrophoresis in the presence of ethidium bromide. The results are shown in Figure 1. The No DNA control (NO) and Negative control (NC) showed no bands at 347 bp. The positive control (PC) produced a 347 bp band. The test article spiked with 100 copies of HBV185 (TAS) produced a 347 bp band, demonstrating that the test article did not inhibit the PCR reaction. The test article (TA) produced no bands at 347 bp.

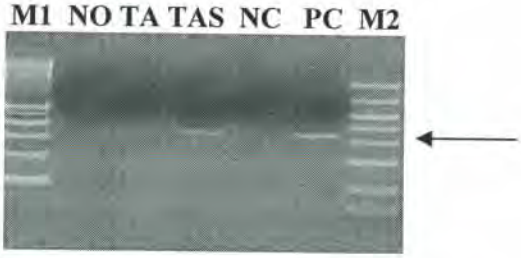
These results provide evidence that the test article H14-MCB-1 tested negative for the presence of HBV DNA.

APPROVAL

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

07 Apr 08
Date

FIGURE 1



Detection of HBV specific sequences in the test article H14-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 HBV185
NC: Negative control
PC: Positive control (NC spiked with 100 copies HBV185)
M2: Biomarker low DNA size marker

Arrow indicates the specific amplification product.

Quality Assurance Statement

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

Study Number: AC13RD.105042.BSV

Study Director:

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

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

**	Inspect On	01-Apr-08 - 01-Apr-08 To Study Dir 01-Apr-08 To Mgmt 01-Apr-08
	Phase	Data Audit
**	Inspect On	07-Apr-08 - 07-Apr-08 To Study Dir 07-Apr-08 To Mgmt 07-Apr-08
	Phase	Final Report and data audit
*	Inspect On	31-Mar-08 - 31-Mar-08 To Study Dir 31-Mar-08 To Mgmt 31-Mar-08
	Phase	Systems Inspection - Administration of Test Substance to Test System
*	Inspect On	31-Mar-08 - 31-Mar-08 To Study Dir 31-Mar-08 To Mgmt 31-Mar-08
	Phase	Systems Inspection - Manipulation of Test System
*	Inspect On	31-Mar-08 - 31-Mar-08 To Study Dir 31-Mar-08 To Mgmt 31-Mar-08
	Phase	Systems Inspection - Observation of Test System/Data Collection and/or Analysis
*	Inspect On	31-Mar-08 - 31-Mar-08 To Study Dir 31-Mar-08 To Mgmt 31-Mar-08
	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.

07 Aug 13

DATE

QUALITY ASSURANCE

Final Report

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

Study Number: AC13RD.105025.BSV

Test Article ID: H14-MCB-1

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

RNA isolated from test article H14-MCB-1 (representing approximately 5 µl of test article) was analyzed for the presence of Hepatitis C virus (HCV) RNA by the reverse-transcriptase polymerase chain reaction (RT-PCR)[1] technique. The assay can detect 100 copies of HCV in the presence of 0.5 µg of genomic RNA.

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

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

STUDY INFORMATION

Test Article: H14-MCB-1 was received by BioReliance on 03/18/2008. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor. Retention of reserve sample from each batch of test article is the responsibility of the sponsor.

Testing Facility: BioReliance

Schedule:

Study Initiation: 03/21/2008

Lab Initiation: 03/31/2008

Lab Completion: 03/31/2008

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

Study Director:

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

BioReliance

OBJECTIVE

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

Test System:

PCR amplification is performed on 5 µl of RNA extract using HCV-specific primers. In the presence of wild type HCV RNA sequences, these primers produce a 257 bp amplicon. In the presence of positive control RNA sequences, these primers produce a 144 bp amplicon. The amplification products are analyzed by agarose gel electrophoresis in the presence of ethidium bromide. The assay is performed according to SOP OPBT0956. The following controls are included in the assay:

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

METHODS

Sample Preparation

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

RT-PCR

RT-PCR amplification was performed on the test article using primers HCV-F3 and HCV-R2 specific to the highly conserved fragment of the 5' non-coding region of the HCV RNA genome, employing conditions optimized to achieve detection of 100 copies of HCV. Aliquots of the amplification products obtained from the test article and from the control samples were analyzed by agarose gel electrophoresis in the presence of ethidium bromide. Amplification products were visualized on a UV transilluminator and photographed using Polaroid 667 film (SOPs BPBT0784 and OPBT0956).

RESULTS

Test article extract, (representing approximately 5 µl of test article) was analyzed for the presence of HCV viral RNA by RT-PCR amplification and agarose gel electrophoresis in the presence of ethidium bromide. The results are shown in Figure 1. The No RNA control (NO) and Negative control (NC) showed no bands at 144 or 257 bp. The positive control (PC) produced a 144 bp band. The test article spiked with 100 copies of p19-100D (TAS) produced a 144 bp band, demonstrating that the test article did not inhibit the PCR reaction. The test article (TA) produced no bands at 144 or 257 bp.

These results provide evidence that the test article H14-MCB-1 tested negative for the presence of HCV RNA.

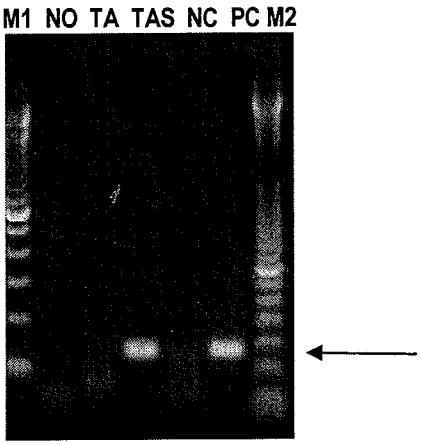
APPROVAL

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

15 Apr 08

Date

FIGURE 1



Detection of HCV RNA in the test article H14-MCB-1 utilizing agarose gel electrophoresis in the presence of ethidium bromide.

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

Arrow indicates the specific amplification product of the positive control.

Quality Assurance Statement

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

Study Number: AC13RD.105025.BSV

Study Director:

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

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

**	Inspect On	04-Apr-08 - 04-Apr-08 To Study Dir 04-Apr-08 To Mgmt 04-Apr-08
	Phase	Data Audit
**	Inspect On	15-Apr-08 - 15-Apr-08 To Study Dir 15-Apr-08 To Mgmt 15-Apr-08
	Phase	Final Report and data audit
*	Inspect On	31-Mar-08 - 31-Mar-08 To Study Dir 31-Mar-08 To Mgmt 31-Mar-08
	Phase	Systems Inspection - Administration of Test Substance to Test System
*	Inspect On	31-Mar-08 - 31-Mar-08 To Study Dir 31-Mar-08 To Mgmt 31-Mar-08
	Phase	Systems Inspection - Manipulation of Test System
*	Inspect On	31-Mar-08 - 31-Mar-08 To Study Dir 31-Mar-08 To Mgmt 31-Mar-08
	Phase	Systems Inspection - Observation of Test System/Data Collection and/or Analysis
*	Inspect On	31-Mar-08 - 31-Mar-08 To Study Dir 31-Mar-08 To Mgmt 31-Mar-08
	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.

15/Apr/08

DATE

Final Report

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

Study Number: AC13RD.105012.BSV

Test Article ID: H14-MCB-1

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

Ten (10.0) µl of DNA isolated from test article H14-MCB-1 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: H14-MCB-1 was received by BioReliance on 03/18/2008. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor. Retention of reserve sample from each batch of test article is the responsibility of the sponsor.

Testing Facility: BioReliance

Schedule:

Study Initiation: 03/21/2008

Lab Initiation: 03/25/2008

Lab Completion: 03/26/2008

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

Study Director:

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

BioReliance

OBJECTIVE

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

Test System:

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

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

METHODS

Sample Preparation

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

DNA Amplification

PCR amplification was performed on 10.0 µl of test article DNA using primers CMV100 and CMV150 specific for the polymerase region of CMV, employing conditions optimized to achieve detection of 100 copies of CMV. Aliquots of the amplification products obtained from the test article and from the control samples were analyzed by agarose gel electrophoresis in the presence of ethidium bromide. Amplification products were visualized on a UV transilluminator and photographed using Polaroid 667 film (SOP OPBT0935).

RESULTS

Test article DNA (10.0 µl) 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 H14-MCB-1 tested negative for the presence of CMV DNA.

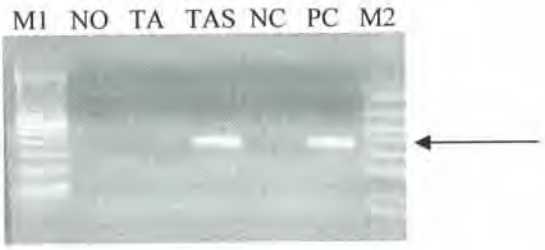
APPROVAL

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.

10 Apr 08

Date

FIGURE 1



Detection of CMV specific sequences in the test article H14-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: AC13RD.105012.BSV

Study Director:

Quality Assurance performed the inspections listed below for this study. Verification of the study protocol was also performed and documented by QA. Procedures, documentation, equipment records, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the 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	01-Apr-08 - 01-Apr-08 To Study Dir 01-Apr-08 To Mgmt 01-Apr-08
	Phase	Data Audit
**	Inspect On	09-Apr-08 - 09-Apr-08 To Study Dir 09-Apr-08 To Mgmt 10-Apr-08
	Phase	Final Report and data audit
*	Inspect On	31-Mar-08 - 31-Mar-08 To Study Dir 31-Mar-08 To Mgmt 31-Mar-08
	Phase	Systems Inspection - Administration of Test Substance to Test System
*	Inspect On	31-Mar-08 - 31-Mar-08 To Study Dir 31-Mar-08 To Mgmt 31-Mar-08
	Phase	Systems Inspection - Manipulation of Test System
*	Inspect On	31-Mar-08 - 31-Mar-08 To Study Dir 31-Mar-08 To Mgmt 31-Mar-08
	Phase	Systems Inspection - Observation of Test System/Data Collection and/or Analysis
*	Inspect On	31-Mar-08 - 31-Mar-08 To Study Dir 31-Mar-08 To Mgmt 31-Mar-08
	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.

10 Apr 08

DATE

Final Report

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

Study Number: AC13RD.105011.BSV

Test Article ID: H14-MCB-1

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

Ten (10.0) µl of DNA isolated from test article H14-MCB-1 was analyzed for the presence of Epstein Barr virus (EBV) DNA by the polymerase chain reaction (PCR)[1] technique. The assay can detect 10 copies of EBV in the presence of 0.5 µg of genomic DNA.

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

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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: H14-MCB-1 was received by BioReliance on 03/18/2008. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor. Retention of reserve sample from each batch of test article is the responsibility of the sponsor.

Testing Facility: BioReliance

Schedule:

Study Initiation: 03/21/2008

Lab Initiation: 03/25/2008

Lab Completion: 03/27/2008

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

Study Director:

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

BioReliance

OBJECTIVE

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

Test System:

PCR amplification is performed on test article DNA using EBV-specific primers. In the presence of EBV, these primers produce a 376 bp amplification product. The amplification products are analyzed by agarose gel electrophoresis in the presence of ethidium bromide. The following controls are included in the assay:

Negative Control:	Genomic DNA from MRC5 human fetal lung fibroblast line. Source: BioReliance
Positive Control:	Negative control DNA spiked with 100 copies of BamW, a plasmid containing the BamW fragment from the IR1 region of the EBV genome. Since the EBV genome includes approximately 10 tandem repeats of the IR1 region, 100 copies of BamW plasmid are approximately equivalent to 10 copies of EBV genome. Source: BioReliance
No DNA Control:	Nuclease-free water Source: USB or other commercial source.
Spiked Control:	Test article extract spiked with 100 copies of BamW, to verify the absence of PCR inhibitors in the test article DNA, (amplification suitability control).

METHODS

Sample Preparation

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

DNA Amplification

PCR amplification was performed on 10.0 µl of test article DNA using primers TC58 and TC61 specific for the BamW region of EBV, employing conditions optimized to achieve detection of 10 copies of EBV. Aliquots of the amplification products obtained from the test article and from the control samples were analyzed by agarose gel electrophoresis in the presence of ethidium bromide. Amplification products were visualized on a UV transilluminator and photographed using Polaroid 667 film (SOP OPBT0934).

RESULTS

Test article DNA (10.0 µl) was analyzed for the presence of EBV DNA by PCR amplification and agarose gel electrophoresis in the presence of ethidium bromide. The results are shown in Figure 1. The No DNA control (NO) and Negative control (NC) showed no bands at 376 bp. The positive control (PC) produced a 376 bp band. The test article spiked with 100 copies of BamW (TAS) produced a 376 bp band, demonstrating that the test article did not inhibit the PCR reaction. The test article (TA) produced no bands at 376 bp.

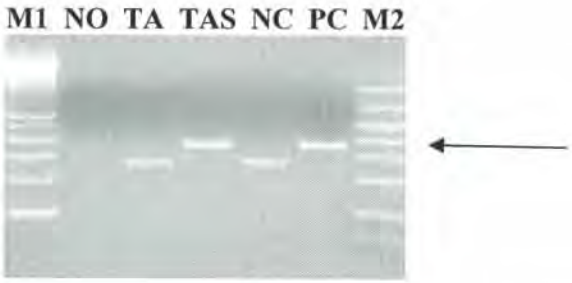
These results provide evidence that the test article H14-MCB-1 tested negative for the presence of EBV DNA.

APPROVAL

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

07 Apr 08
Date

FIGURE 1



Detection of EBV specific sequences in the test article H14-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 BamW
NC: Negative control (MRC5 DNA)
PC: Positive control (MRC5 DNA spiked with 100 copies BamW)
M2: Biomarker low DNA size marker

Arrow indicates specific amplification product.

Quality Assurance Statement

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

Study Number: AC13RD.105011.BSV

Study Director:

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

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

**	Inspect On	01-Apr-08 - 01-Apr-08 To Study Dir 01-Apr-08 To Mgmt 01-Apr-08
	Phase	Data Audit
**	Inspect On	07-Apr-08 - 07-Apr-08 To Study Dir 07-Apr-08 To Mgmt 07-Apr-08
	Phase	Final Report and data audit
*	Inspect On	31-Mar-08 - 31-Mar-08 To Study Dir 31-Mar-08 To Mgmt 31-Mar-08
	Phase	Systems Inspection - Administration of Test Substance to Test System
*	Inspect On	31-Mar-08 - 31-Mar-08 To Study Dir 31-Mar-08 To Mgmt 31-Mar-08
	Phase	Systems Inspection - Manipulation of Test System
*	Inspect On	31-Mar-08 - 31-Mar-08 To Study Dir 31-Mar-08 To Mgmt 31-Mar-08
	Phase	Systems Inspection - Observation of Test System/Data Collection and/or Analysis
*	Inspect On	31-Mar-08 - 31-Mar-08 To Study Dir 31-Mar-08 To Mgmt 31-Mar-08
	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.

07 Apr-21

DATE

Final Report

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

Study Number: AC13RD.105020.BSV

Test Article ID: H14-MCB-1

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

Ten (10) µl of DNA isolated from test article H14-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.

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

STUDY INFORMATION

Test Article: H14-MCB-1 was received by BioReliance on 03/18/2008. Determination of the stability, purity, and concentration of the test article is the responsibility of the sponsor. Retention of reserve sample from each batch of test article is the responsibility of the sponsor.

Testing Facility: BioReliance

Schedule:

Study Initiation: 03/21/2008

Lab Initiation: 03/25/2008

Lab Completion: 03/28/2008

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

Study Director:

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

BioReliance

OBJECTIVE

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

TEST SYSTEM

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

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

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

METHODS

Sample Preparation

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

DNA Amplification

PCR amplification was performed on 10 µl 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.

REPEATS

The first performance of the PCR assay (testing 10 µl of undiluted test article DNA extract) was deemed invalid, as the positive control for HHV-6B (PC-2) failed to provide sufficient amplification (results not presented). The PCR was repeated, again testing 10 µl of undiluted test article DNA extract. The repeated assay provided a valid test with a negative result (results are presented in Results section below).

RESULTS

Test article DNA (10 µl) was analyzed for the presence of HHV-6 viral DNA by PCR amplification and agarose gel electrophoresis in the presence of ethidium bromide. The results are shown in 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 H14-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.

03 Apr 08

Date

FIGURE 1

Detection of HHV-6 (variants A and B) viral sequences in test article H14-MCB-1 utilizing agarose gel electrophoresis in the presence of ethidium bromide

1) **M1:** 100 bp DNA ladder
2) **NO:** No DNA control
3) **TA:** Test Article
4) **TAS-1:** Test article spiked with 100 copies of pU1102MOD
5) **TAS-2:** Test article spiked with 100 copies of pZ29MOD
6) **NC:** Negative control (MRC5 genomic DNA)
7) **PC-1:** Positive control (negative control DNA spiked with 100 copies of pU1102MOD)
8) **PC-2:** Positive control (negative control DNA spiked with 100 copies of pZ29MOD)
9) **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: AC13RD.105020.BSV

Study Director:

Quality Assurance performed the inspections listed below for this study. Verification of the study protocol was also performed and documented by QA. Procedures, documentation, equipment records, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the 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	01-Apr-08 - 01-Apr-08 To Study Dir 01-Apr-08 To Mgmt 01-Apr-08
	Phase	Data Audit
**	Inspect On	03-Apr-08 - 03-Apr-08 To Study Dir 03-Apr-08 To Mgmt 03-Apr-08
	Phase	Final Report and data audit
*	Inspect On	31-Mar-08 - 31-Mar-08 To Study Dir 31-Mar-08 To Mgmt 31-Mar-08
	Phase	Systems Inspection - Administration of Test Substance to Test System
*	Inspect On	31-Mar-08 - 31-Mar-08 To Study Dir 31-Mar-08 To Mgmt 31-Mar-08
	Phase	Systems Inspection - Manipulation of Test System
*	Inspect On	31-Mar-08 - 31-Mar-08 To Study Dir 31-Mar-08 To Mgmt 31-Mar-08
	Phase	Systems Inspection - Observation of Test System/Data Collection and/or Analysis
*	Inspect On	31-Mar-08 - 31-Mar-08 To Study Dir 31-Mar-08 To Mgmt 31-Mar-08
	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.

03 Apr 08

DATE

Final Report

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

Study Number: AC13RD.105029.BSV

Test Article ID: H14-MCB-1

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

Ten (10) µl of DNA isolated from test article H14-MCB-1 was analyzed for the presence of Human Herpesvirus 7 (HHV-7) DNA by the polymerase chain reaction (PCR)[1] technique. The assay can detect 100 copies of HHV-7 in the presence of 0.5 µg of genomic DNA.

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

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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: H14-MCB-1 was received by BioReliance on 03/18/2008. Determination of the stability, purity, and concentration of the test article is the responsibility of the sponsor. Retention of reserve sample from each batch of test article is the responsibility of the sponsor.

Testing Facility: BioReliance

Schedule:

Study Initiation: 03/21/2008

Lab Initiation: 03/25/2008

Lab Completion: 03/26/2008

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

Study Director:

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

BioReliance

OBJECTIVE

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

TEST SYSTEM

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

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

METHODS

Sample Preparation

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

DNA Amplification

PCR amplification was performed on 10 µl of test article DNA using primers HHV7F and HHV7IR specific for sequences common to the capsid protein gene regions in the HHV-7 genome, employing conditions optimized to achieve detection of 100 copies of HHV-7 DNA. Aliquots of the amplification products obtained from the test article and from the control samples were analyzed by high resolution agarose gel electrophoresis in the presence of ethidium bromide. Amplification products were visualized on a UV transilluminator and photographed using Polaroid 667 film (SOP OPBT0929).

RESULTS

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

These results provide evidence that the test article H14-MCB-1 tested negative for the presence of HHV-7 DNA.

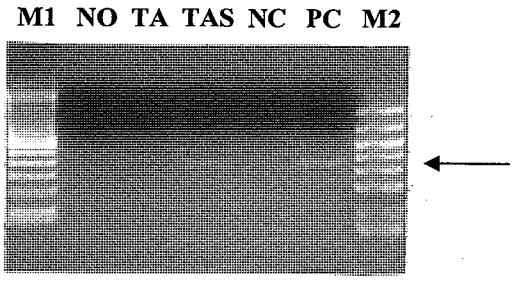
APPROVAL

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

15 Apr 08

Date

FIGURE 1



Detection of HHV-7 specific sequences in the test article H14-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 pHH7
NC: Negative control (MRC5 genomic DNA)
PC: Positive control (negative control DNA spiked with 100 copies pHH7)
M2: Biomarker low DNA size marker

Arrow indicates the 353 bp amplification product.

Quality Assurance Statement

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

Study Number: AC13RD.105029.BSV

Study Director:

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

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

**	Inspect On	01-Apr-08 - 01-Apr-08 To Study Dir 01-Apr-08 To Mgmt 01-Apr-08
	Phase	Data Audit
**	Inspect On	15-Apr-08 - 15-Apr-08 To Study Dir 15-Apr-08 To Mgmt 15-Apr-08
	Phase	Final Report and data audit
*	Inspect On	31-Mar-08 - 31-Mar-08 To Study Dir 31-Mar-08 To Mgmt 31-Mar-08
	Phase	Systems Inspection - Administration of Test Substance to Test System
*	Inspect On	31-Mar-08 - 31-Mar-08 To Study Dir 31-Mar-08 To Mgmt 31-Mar-08
	Phase	Systems Inspection - Manipulation of Test System
*	Inspect On	31-Mar-08 - 31-Mar-08 To Study Dir 31-Mar-08 To Mgmt 31-Mar-08
	Phase	Systems Inspection - Observation of Test System/Data Collection and/or Analysis
*	Inspect On	31-Mar-08 - 31-Mar-08 To Study Dir 31-Mar-08 To Mgmt 31-Mar-08
	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.

15 Apr 08
DATE

QUALITY ASSURANCE

Final Report

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

Study Number: AC13RD.105056.BSV

Test Article ID: H14-MCB-1

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

Ten (10) µl of DNA isolated from test article H14-MCB-1 was analyzed for the presence of Human Herpesvirus 8 (HHV-8) DNA by the polymerase chain reaction (PCR)[1] technique. The assay can detect 100 copies of HHV-8 in the presence of 0.5 µg of genomic DNA.

The results presented herein indicate that the test article tested negative for the presence of HHV-8 DNA.

---

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

STUDY INFORMATION

Test Article: H14-MCB-1 was received by BioReliance on 03/18/2008. Determination of the stability, purity, and concentration of the test article is the responsibility of the sponsor. Retention of reserve sample from each batch of test article is the responsibility of the sponsor.

Testing Facility: BioReliance

Schedule:

Study Initiation: 03/21/2008

Lab Initiation: 03/25/2008

Lab Completion: 03/31/2008

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

Study Director:

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

BioReliance

OBJECTIVE

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

TEST SYSTEM

PCR amplification is performed on test article DNA using HHV-8-specific primers. In the presence of HHV-8 DNA, these primers produce a 225 bp amplification product. The amplification products are analyzed by agarose gel electrophoresis in the presence of ethidium bromide. The following controls are included in the assay:

Negative Control:	Genomic DNA from HHV-8-negative source Source: BioReliance
Positive Control:	Negative control DNA spiked with 100 copies of pHHV-8, a plasmid containing a conserved fragment from the latency associated nuclear antigen (LANA) from the HHV-8 genome Source: BioReliance
No DNA Control:	Nuclease-free water Source: USB or other commercial source
Spiked Control:	Test article extract spiked with 100 copies of pHHV-8, to verify the absence of PCR inhibitors in the test article DNA (amplification suitability control)

METHODS

Sample Preparation

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

DNA Amplification

PCR amplification was performed on 10 µl of test article DNA using primers HHV-8F and HHV-8R specific for sequences of the latency associated nuclear antigen (LANA) in the HHV-8 genome, employing conditions optimized to achieve detection of 100 copies of HHV-8 DNA. Aliquots of the amplification products obtained from the test article and from the control samples were analyzed by agarose gel electrophoresis in the presence of ethidium bromide. Amplification products were visualized on a UV transilluminator and photographed using Polaroid 667 film (SOP OPBT0974).

RESULTS

Test article DNA (10 µl) was analyzed for the presence of HHV-8 DNA by PCR amplification and agarose gel electrophoresis in the presence of ethidium bromide. The results are shown in Figure 1. The No DNA control (NO) showed no amplification bands and Negative Control (NC) showed no band at 225 bp. The Positive Control (PC) produced a 225 bp band. The test article spiked with 100 copies of pHHV-8 (TAS) produced a 225 bp band, demonstrating that the test article did not inhibit the PCR reaction. The test article (TA) produced no band at 225 bp.

These results provide evidence that the test article H14-MCB-1 tested negative for the presence of HHV-8 DNA.

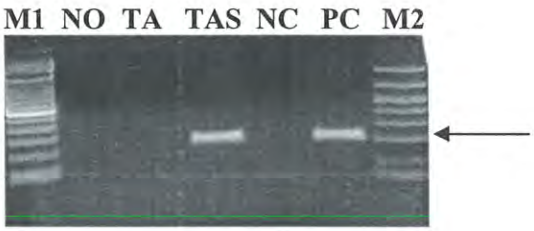
APPROVAL

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

15 Apr 08

Date

FIGURE 1



Detection of HHV-8 specific sequences in the test article H14-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 pHHV-8
NC: Negative control (genomic DNA)
PC: Positive control (negative control DNA spiked with 100 copies pHHV-8)
M2: Biomarker low DNA size marker

Arrow indicates the 225 bp amplification product.

Quality Assurance Statement

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

Study Number: AC13RD.105056.BSV

Study Director:

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

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

**	Inspect On	01-Apr-08 - 01-Apr-08 To Study Dir 01-Apr-08 To Mgmt 01-Apr-08
	Phase	Data Audit
**	Inspect On	15-Apr-08 - 15-Apr-08 To Study Dir 15-Apr-08 To Mgmt 15-Apr-08
	Phase	Final Report and data audit
*	Inspect On	31-Mar-08 - 31-Mar-08 To Study Dir 31-Mar-08 To Mgmt 31-Mar-08
	Phase	Systems Inspection - Administration of Test Substance to Test System
*	Inspect On	31-Mar-08 - 31-Mar-08 To Study Dir 31-Mar-08 To Mgmt 31-Mar-08
	Phase	Systems Inspection - Manipulation of Test System
*	Inspect On	31-Mar-08 - 31-Mar-08 To Study Dir 31-Mar-08 To Mgmt 31-Mar-08
	Phase	Systems Inspection - Observation of Test System/Data Collection and/or Analysis
*	Inspect On	31-Mar-08 - 31-Mar-08 To Study Dir 31-Mar-08 To Mgmt 31-Mar-08
	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.

15 Apr-08

DATE

Final Report

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

Study Number: AC13RD.105037.BSV

Test Article ID: H14-MCB-1

Sponsor: WiCell Research Institute

Authorized Representative:

CONCLUSION

Ten (10) µl of DNA isolated from test article H14-MCB-1 was analyzed for the presence of human parvovirus B19 DNA by the polymerase chain reaction (PCR)[1] technique. The assay can detect 100 copies of B19 in the presence of 0.5 µg of genomic DNA.

The results presented herein indicate that the test article tested negative for the presence of B19 DNA.

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

STUDY INFORMATION

Test Article: H14-MCB-1 was received by BioReliance on 03/18/2008. Determination of the stability, purity, and concentration of the test article is the responsibility of the sponsor. Retention of reserve sample from each batch of test article is the responsibility of the sponsor.

Testing Facility: BioReliance

Schedule:

Study Initiation: 03/21/2008

Lab Initiation: 03/25/2008

Lab Completion: 03/27/2008

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

Study Director:

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

BioReliance

OBJECTIVE

The objective of the study is to detect the presence of B19 sequences in the test article by PCR amplification, an *in vitro* procedure that can generate millions of copies of a sequence from very low levels of template DNA.

TEST SYSTEM:

PCR amplification is performed on test article DNA using B19-specific primers. In the presence of B19, these primers produce a 287 bp amplification product. The amplification products are analyzed by agarose gel electrophoresis in the presence of ethidium bromide. The following controls are included in the assay:

Negative Control:	Genomic DNA from MRC5 human fetal lung fibroblast line Source: BioReliance
Positive Control:	Negative control DNA spiked with 100 copies of pNPS-1, a plasmid containing a 3.6 Kb fragment from the B19 capsid gene Source: BioReliance
No DNA Control:	Nuclease free water Source: USB or other commercial supplier
Spiked Control:	Test article extract spiked with 100 copies of pNPS-1, to verify the absence of PCR inhibitors in the test article DNA (amplification suitability control)

METHODS

Sample Preparation

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

DNA Amplification

PCR amplification was performed on 10 µl of test article DNA and on the assay controls using primers B19F and B19R specific for the capsid gene of B19, employing conditions optimized to achieve detection of 100 copies of B19. Aliquots of the amplification products obtained from the test article and from the control samples were analyzed by agarose gel electrophoresis in the presence of ethidium bromide. Amplification products were visualized on a UV transilluminator and photographed using Polaroid 667 film (SOP OPBT0936).

RESULTS

Test article DNA (10 µl) was analyzed for the presence of B19 DNA by PCR amplification and agarose gel electrophoresis in the presence of ethidium bromide. The results are shown in Figure 1. The No DNA control (NO) and Negative control (NC) showed no bands at 287 bp. The positive control (PC) produced a 287 bp band. The test article spiked with 100 copies of pNPS-1 (TAS) produced a 287 bp band, demonstrating that the test article did not inhibit the PCR reaction. The test article (TA) produced no bands at 287 bp.

These results provide evidence that the test article H14-MCB-1 tested negative for the presence of B19 DNA.

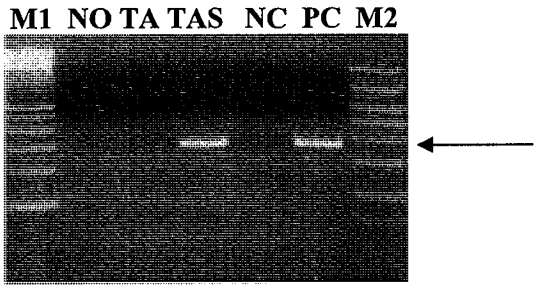
APPROVAL

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

15 Apr 08

Date

FIGURE 1



Detection of B19 specific sequences in the test article H14-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 of pNPS-1
NC: Negative control (MRC5 genomic DNA)
PC: Positive control (negative control DNA spiked with 100 copies of pNPS-1
M2: Biomarker low DNA size marker

Arrow indicates the specific amplification product.

Quality Assurance Statement

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

Study Number: AC13RD.105037.BSV

Study Director:

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

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

**	Inspect On	01-Apr-08 - 01-Apr-08 To Study Dir 01-Apr-08 To Mgmt 01-Apr-08
	Phase	Data Audit
**	Inspect On	15-Apr-08 - 15-Apr-08 To Study Dir 15-Apr-08 To Mgmt 15-Apr-08
	Phase	Final Report and data audit
*	Inspect On	31-Mar-08 - 31-Mar-08 To Study Dir 31-Mar-08 To Mgmt 31-Mar-08
	Phase	Systems Inspection - Administration of Test Substance to Test System
*	Inspect On	31-Mar-08 - 31-Mar-08 To Study Dir 31-Mar-08 To Mgmt 31-Mar-08
	Phase	Systems Inspection - Manipulation of Test System
*	Inspect On	31-Mar-08 - 31-Mar-08 To Study Dir 31-Mar-08 To Mgmt 31-Mar-08
	Phase	Systems Inspection - Observation of Test System/Data Collection and/or Analysis
*	Inspect On	31-Mar-08 - 31-Mar-08 To Study Dir 31-Mar-08 To Mgmt 31-Mar-08
	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.

15 Apr 08

DATE

WiCell Cytogenetics Report: 000091 NSCB#5246

Report Date: *April 24, 2009*

Case Details:

Cell Line: *WA14 p22 (Male)*

Reference: *WA09 p24 (Female)*

Investigator: *National Stem Cell Bank*

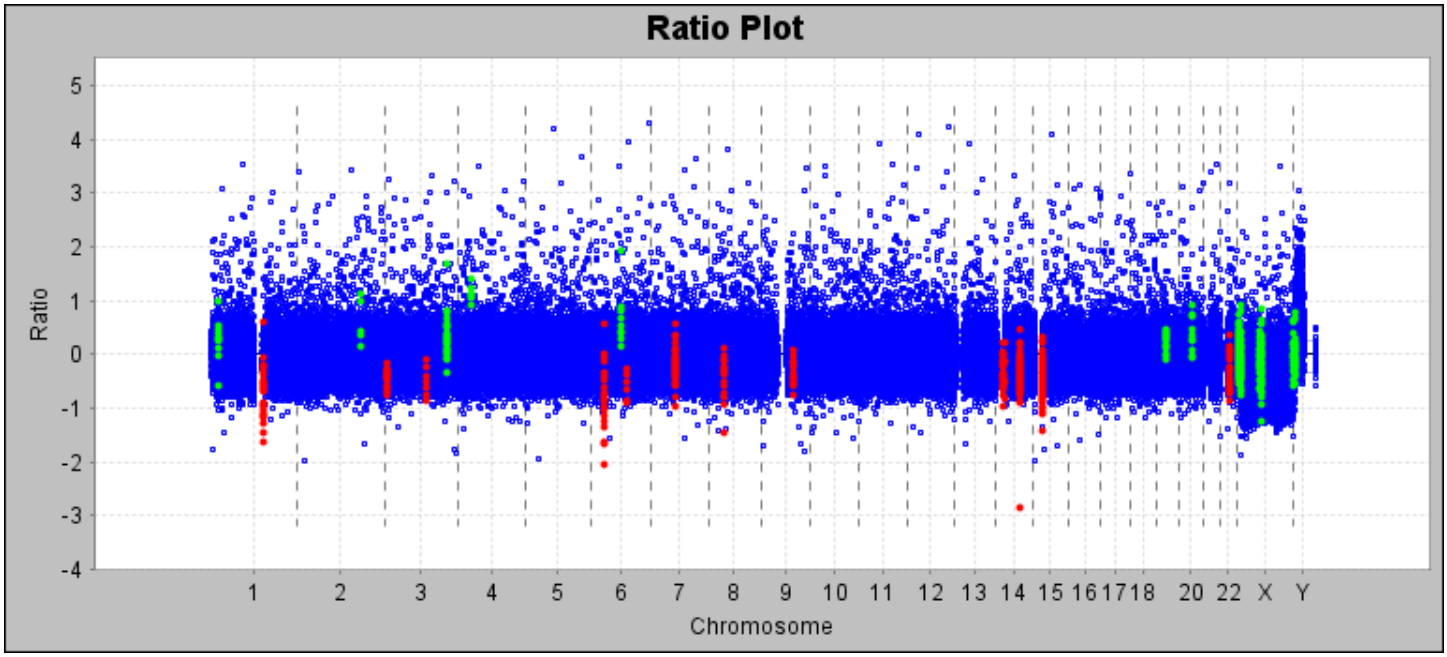
Specimen: *hES cells on MEFs*

Date of Sample: *3/17/2008*

Reason for Testing: *National Stem Cell Bank Testing*

GEO Accession #: *GSM337466*

aCGH Results:
Results are given in the attached excel spreadsheet labeled „report.' There were 34 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. Both averaged and unaveraged data was used in this analysis.



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]	*1 of 2*
Published Copy Number Changes[5,6]	*2 of 8*
Reference DNA Copy Number Changes[2]	*10 of 14*
Select Differentially Expressed Genes	*0 of 45*

These results are consistent with karyotype results [46,XY] as reported in 5246-KAR.

The quality parameters shown in the table above reflect the minimal standards for acceptance, i.e., 1 of 2 pseudoautosomal loci detected and >50% of reference CNVs detected.

Test sample gain or loss is consistent with the opposite gender reference standard. Additional analysis of this data was performed using different ratio settings and different window averaging.

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

***aCGH Specifications**:*

- *Platform: NimbleGen 385K array (HG18 CGH 385K WG Tiling v1)*
- *Relative copy number is determined by competitive differential hybridization of labeled genomic DNA to the 385,000 oligonucleotide whole genome tiling array*
- *Probe length = 50-75mers for v1 and 60mers for v2, spanning non-repetitive regions of the human genome*
- *Median probe spacing = 6270bp for v1 and 7073 for v2*
- *Analysis software: NimbleScan™ , SignalMap™, OneClickCGH™, OneClickFusion™*
- *Analysis is based on examination of unaveraged and/or 60Kbp (10X) averaged data tracks as noted. Settings for data analysis in Infoquant include an average log-ratio threshold of 0.0 and no minimum aberration length.*
- *Raw data is deposited in GEO, accession number shown above.*
- *Reported gains and losses are based on test to reference ratios within OneClickCGH™, size of aberration, 8-9 probes per gene, and coverage of at least one reported gene or overlap with the DGV.*

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

Literature Sources:

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

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

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

Sample RNA: 5246	Reference DNA:	Date of report: 10-01-2008
Sample Cell Line: WA14	Reference Cell Line: H1	Report prepared by: CY
Passage: p22	Passage:	QA Reviewed: 10/9/08 EM
Lot #: WA14-MCB-1		Date sent to Genomic Center: 081008
Sample ID:		GEO accession #:GSM325737

1. Chip design: 2007-06-15_WiCell_HG18_p14_exp.ndf
2. Sample labeling:
   Cy5: OH14-UCB 2ug;
   Cy3: Unsonicated H1 gDNA 4.5ug;

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			
GABRB3	NM_000814	2.694384449	Y
POU5F1	NM_002701	35.86154901	Y
TDGF1	NM_003212	25.79864636	Y
DNMT3B	NM_006892	26.14299424	Y
GDF3	NM_020634	3.410045662	Y
NANOG	NM_024865	11.15036045	Y
non-core ES markers			
PODXL	NM_001018111	24.74132139	Y
GRB7	NM_001030002	1.449419569	Y
CD9	NM_001769	14.35615682	Y
FGF4	NM_002007	0.322440087	Y
SOX2	NM_003106	11.0811245	Y
LEFTY2	NM_003240	19.94305436	Y
UTF1	NM_003577	0.240556199	Y
IFITM1	NM_003641	17.37625571	Y
FOXD3	NM_012183	0.55654102	Y
GAL	NM_015973	33.4879386	Y
NODAL	NM_018055	4.69193858	Y
BXDC2	NM_018321	26.5037037	Y

LEFTY1	NM_020997	19.10739857	Y
LIN28	NM_024674	10.18146214	Y
TERT	NM_198254	0.262847515	Y

5. Expression of differentiation markers:

Gene Symbol	Accession	Ratio	Expression
COL1A1	NM_000088	0.520149953	Y
IPF1	NM_000209	0.0856102	N
PAX6	NM_000280	0.147082334	N
TNNI3	NM_000363	2.484869326	Y
CGB	NM_000737	0.074753653	N
AFP	NM_001134	2.397107166	Y
CDX2	NM_001265	0.127401416	N
COL2A1	NM_001844	0.261887864	Y
FLT1	NM_002019	0.373429084	Y
GATA4	NM_002052	0.187453875	N
NEUROD1	NM_002500	0.087944664	N
SYP	NM_003179	0.083561644	N
PDHX	NM_003477	3.090271691	Y
GCM1	NM_003643	0.09623431	N
NKX2-5	NM_004387	0.107580572	N
ACTC	NM_005159	29.28993536	Y
GATA6	NM_005257	0.225694444	N
EOMES	NM_005442	0.302617801	Y
LAMA1	NM_005559	1.853333333	Y
FOXA2	NM_021784	0.230985915	N
SOX17	NM_022454	0.237762238	Y
FN1	NM_054034	0.169491525	N

American Red Cross

Blood Services
Penn-Jersey Region
Musser Blood Center

www.pleasegiveblood.org

04/21/08

SAMPLE: DNA from Cell Lines: Date received: 03/26/08
6117-ABO (TS08-0188) – WA13.C-MCB-1
1086-ABO (TS08-0189) – ES06-MCB-1
5246-ABO (TS08-0190) – WA14-MCB-1

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). *RHD* zygosity determination by hybrid box detection. 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
WA13.C	**6117-ABO:** *ABO*BO^1*; DCe/Dce	**Group B; RhD+, C+, c+, E-, e+**
ES06	**1086-ABO:** *ABO*AO^1*; ce/ce	**Group A; RhD-, C-, c+, E-, e+**
WA14	**5246-ABO:** *ABO*O^1/O^2*; DCe/DCe	**Group O; RhD+, C+, c-, E-, e+**

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

Scientific Director Molecular Biologist

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

Please Give Blood.

End of Production Testing Summary Report

End of Production (EOP) testing including karyotype, marker expression, and STR, was performed at p+10. Two independent replicates were performed. Summary results are presented here for the material described.

Product Name	WA14 Cell Bank Produced Under cGMP Conditions
Alias	H14
Lot Number	CRM-WA14-MB-001
Parent Material	WA14-WB0119
Depositor	WiCell
Banked by	Waisman Biomanufacturing
Culture Platform	Feeder Independent
	Medium: mTeSR1
	Matrix: Matrigel
Passage number at initiation of EOP study	p21 These cells were cultured for 20 passages prior to freeze, 5 of them in mTeSR1/Matrigel. One number (+1) is added to the passage number at freeze so that the number on the vial best represents the overall passage number of the cells at thaw.
Date Vialed	15-December-2012

Replicate 1

Passage at final EOP testing	Karyotype by G-banding		Identity by STR		Flow Cytometry for ESC Marker Expression	
	Test Specification	Result	Test Specification	Result	Test Specification	Result
31	Report results – no specification	Abnormal Karyotype[1]	Consistent with known profile	Pass	Report results – no specification	See report

[1]This is an abnormal karyotype, with an unbalanced structural aberration in the long arm of chromosome 20 in two of twenty-one cells examined. This abnormality, in which segments of unknown chromosome origin ("add") have been translocated to 20q, cannot be characterized by G-banded chromosome analysis. Additional testing, e.g., CGH, may be helpful in defining this abnormality. No other clonal abnormalities were found.

Replicate 2

Passage at final EOP testing	Karyotype by G-banding		Identity by STR		Flow Cytometry for ESC Marker Expression	
	Test Specification	Result	Test Specification	Result	Test Specification	Result
31	Report results – no specification	Abnormal Karyotype[2]	Consistent with known profile	Pass	Report results – no specification	See report

[2]This is an abnormal karyotype, with an unbalanced structural aberration in the long arm of chromosome 20 in five of twenty cells examined. This abnormality, in which segments of unknown chromosome origin ("add") have been translocated to 20q, cannot be characterized by G-banded chromosome analysis. Additional testing, e.g., CGH, may be helpful in defining this abnormality. No other clonal abnormalities were found.

Quality Assurance Approval

1/10/2014

X AMC

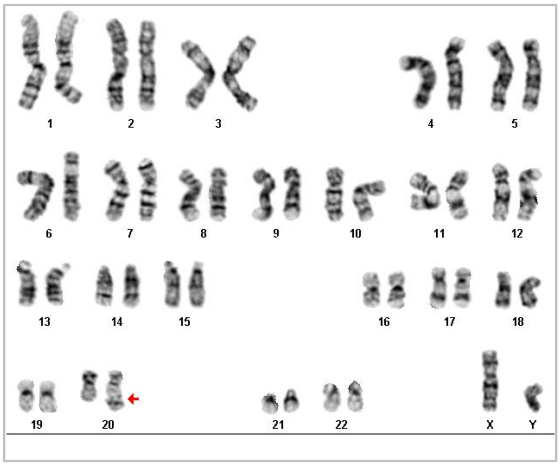
AMC
Quality Assurance
Signed by: [redacted]

©2013 WiCell Research Institute The material provided under this certificate has been subjected to the tests specified and the results and data described herein are accurate based on WiCell's reasonable knowledge and belief. Appropriate Biosafety Level practices and universal precautions should always be used with this material. For clarity, the foregoing is governed solely by WiCell's Terms and Conditions of Service, which can be found at http://www.wicell.org/privacyandterms.

Chromosome Analysis Report: 010687

Date Reported: Thursday, May 02, 2013
Cell Line: CRM-WA14-MB-001 10757
Passage#: 31
Date of Sample: 4/22/2013
Specimen: hESC
Results: 46,XY,add(20)(q11.2)[2]/46,XY[19]

Cell Line Gender: Male
Reason for Testing: end of production testing
Investigator: [redacted]



Cell: 49
Slide: 1
Slide Type: Karyotype

Total Counted: 21
Total Analyzed: 9
Total Karyotyped: 5
Band Resolution: 400 - 500

Interpretation:

This is an abnormal karyotype, with an unbalanced structural aberration in the long arm of chromosome 20 in two of twenty-one cells examined. This abnormality, in which segments of unknown chromosome origin ("add") have been translocated to 20q, cannot be characterized by G-banded chromosome analysis. Additional testing, e.g., CGH, may be helpful in defining this abnormality. No other clonal abnormalities were found.

This abnormality appears to be the same as that found as a nonclonal abnormality in WA14-RB-005 10746.

Completed by: [redacted] CG(ASCP)
Reviewed and Interpreted by: [redacted], PhD, FACMG
A signed copy of this report is available upon request.

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".

This assay was conducted solely for listed investigator/institution. The results may not be relied upon by any other party without the prior written consent of the Director of the WiCell Cytogenetics Laboratory. The results of this assay are for research use only. If the results of this assay are to be used for any other purpose, contact the Director of the WiCell Cytogenetics Laboratory.

UW Health

University of Wisconsin
Hospital and Clinics

Histocompatibility/Molecular Diagnostics Laboratory

[redacted]

Short Tandem Repeat Analysis*

Sample Report: 10757-STR Label on Tube: 10757-STR Sample Date: 04/29/13
Received Date: 04/29/13

Requestor: WiCell Research Institute
Test Date: 05/01/13 File Name: 130501 Report Date: 05/03/13

Sample Name: (label on tube)
10757-STR

Description: DNA Extracted by WiCell

265.2 ug/mL; 260/280 = 1.98

Locus	Repeat #	STR Genotype
D16S539	5, 8-15	11,13
D7S820	6-14	10,11
D13S317	7-15	11,11
D5S818	7-15	11,13
CSF1PO	6-15	11,12
TPOX	6-13	8,8
Amelogenin	NA	X,Y
TH01	5-11	6,7
vWA	11, 13-21	15,16

Comments: Based on the 10757-STR DNA dated and received on 04/29/13 from WiCell, this sample (UW HLA# Label on Tube: 10757-STR) exactly matches the STR profile of the human stem cell line WA14 (H14) comprising 14 allelic polymorphisms across the 8 STR loci analyzed. No STR polymorphisms other than those corresponding to the human WA14 (H14) stem cell line were detected and the concentration of DNA required to achieve an acceptable STR genotype (signal/noise) was equivalent to that required for the standard procedure (~1 ng/amplification reaction) from human genomic DNA. These results suggest that the 10757-STR DNA sample submitted corresponds to the WA14 (H14) stem cell line and it was not contaminated with any other human stem cells or a significant amount of mouse feeder layer cells. Sensitivity limits for detection of STR polymorphisms unique to either this or other human stem cell lines is ~5%.

[redacted] 5/6/13
Date
Molecular Diagnostics Laboratory

[redacted] 05/03/13
Date
Molecular Diagnostics Laboratory

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

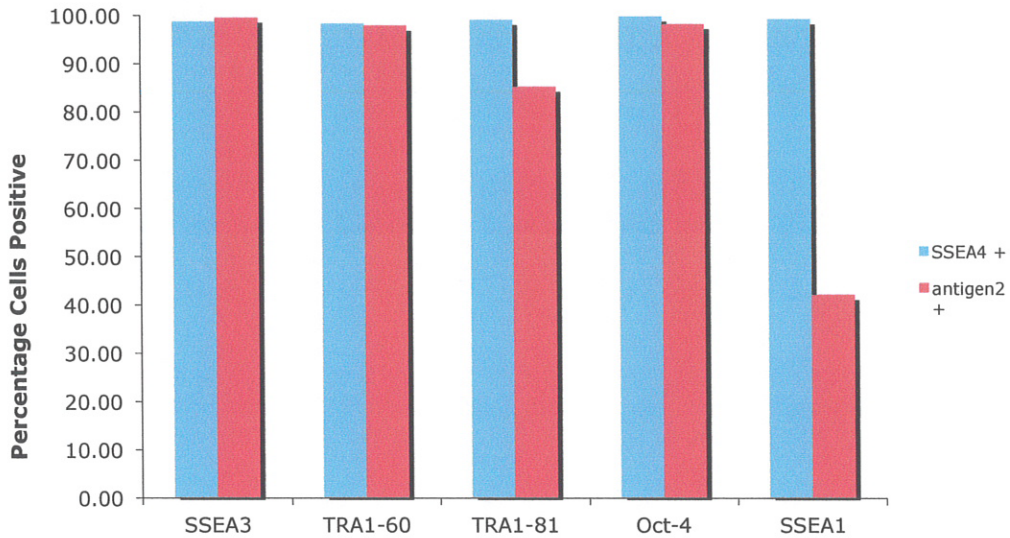
File: Final STR Report

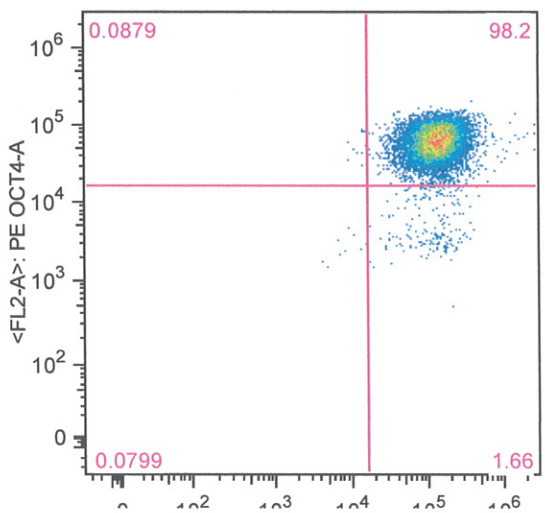
Flow Cytometry Characterization Report for Full Panel of Antibodies

Cell Line:	CRM-WA14-MB-001-K-1	Date:	4/22/13
Passage:	31① ~~40~~	Acquisition:	4/22/13
Sample ID:	10757	File Creation:	4/24/13
		File Submission:	4/24/13

antigen2:	SSEA4 - antigen2 +	SSEA4 + antigen2 +	SSEA4 + antigen2 -	SSEA4 - antigen2 -	ALL SSEA4 +	ALL antigen2 +	Verification: should be 100%
SSEA3	1.05	98.50	0.22	0.19	98.72	99.55	**99.96**
TRA1-60	1.58	96.30	2.03	0.15	98.33	97.88	**100.06**
TRA1-81	0.78	84.50	14.60	0.21	99.10	85.28	**100.08**
Oct-4	0.09	98.20	1.66	0.08	99.86	98.29	**100.03**
SSEA1	0.20	42.00	57.30	0.45	99.30	42.20	**99.95**

Percent analyzable events:	43.20%
#wells submitted:	6
Total cells recovered:	4.78 X 10^6





Report prepared By: [redacted] Date: 04/24/13

QA review By: JKG Date: 01May13

Print Date: 4/24/13

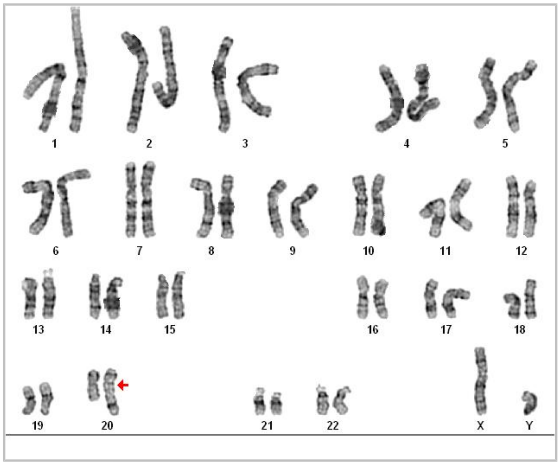
① p31. Confirmed with [redacted] Plate had "pt10" written on it indicating passages from thaw. 01May13 JKG

Chromosome Analysis Report: 011257

Date Reported: Friday, July 26, 2013
Cell Line: CRM-WA14-MB-001 10815
Passage#: 31
Date of Sample: 7/22/2013
Specimen: hESC
Results: 46,XY,add(20)(q11.2)[5]/46,XY[14]
Nonclonal finding: 47,XY,+15

Cell Line Gender: Male
Reason for Testing: End of production testing

Investigator: [redacted] WiCell CDM



Cell: 2
Slide: 3
Slide Type: Karyotype

Total Counted: 20
Total Analyzed: 8
Total Karyotyped: 4
Band Resolution: 400 - 450

Interpretation:

This is an abnormal karyotype, with an unbalanced structural aberration in the long arm of chromosome 20 in five of twenty cells examined. This abnormality, in which segments of unknown chromosome origin ("add") have been translocated to 20q, cannot be characterized by G-banded chromosome analysis. Additional testing, e.g., CGH, may be helpful in defining this abnormality. No other clonal abnormalities were found.

This abnormality appears to be the same as that found previously in WA14.

There is one nonclonal finding, listed above.
Nonclonal findings likely result from technical artifact, but may be due to a developing clonal abnormality or to low-level mosaicism.

Completed by: [redacted] CG(ASCP)
Reviewed and Interpreted by: [redacted], PhD, FACMG

A signed copy of this report is available upon request.

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".

This assay was conducted solely for listed investigator/institution. The results may not be relied upon by any other party without the prior written consent of the Director of the WiCell Cytogenetics Laboratory. The results of this assay are for research use only. If the results of this assay are to be used for any other purpose, contact the Director of the WiCell Cytogenetics Laboratory.

UW Health

University of Wisconsin
Hospital and Clinics

Histocompatibility/Molecular Diagnostics Laboratory

[redacted]

Short Tandem Repeat Analysis*

Sample Report: 10815-STR Label on Tube: 10815-STR Sample Date: 07/24/13
Received Date: 07/24/13

Requestor: WiCell Research Institute
Test Date: 07/31/13 File Name: 130731 Report Date: 08/01/13

Sample Name: (label on tube)
10815-STR

Description: DNA Extracted by WiCell

253.01 ug/mL; 260/280 = 2.00

Locus	Repeat #	STR Genotype
D16S539	5, 8-15	11,13
D7S820	6-14	10,11
D13S317	7-15	11,11
D5S818	7-15	11,13
CSF1PO	6-15	11,12
TPOX	6-13	8,8
Amelogenin	NA	X,Y
TH01	5-11	6,7
vWA	11, 13-21	15,16

Comments: Based on the 10815-STR DNA dated and received on 07/24/13 from WiCell, this sample (UW HLA# Label on Tube: 10815-STR) exactly matches the STR profile of the human stem cell line WA14 (H14) comprising 14 allelic polymorphisms across the 8 STR loci analyzed. No STR polymorphisms other than those corresponding to the human WA14 (H14) stem cell line were detected and the concentration of DNA required to achieve an acceptable STR genotype (signal/noise) was equivalent to that required for the standard procedure (~1 ng/amplification reaction) from human genomic DNA. These results suggest that the 10815-STR DNA sample submitted corresponds to the WA14 (H14) stem cell line and it was not contaminated with any other human stem cells or a significant amount of mouse feeder layer cells. Sensitivity limits for detection of STR polymorphisms unique to either this or other human stem cell lines is ~5%.

[redacted] 8/5/13
Molecular Diagnostics Laboratory Date

[redacted] 8/01/13
Molecular Diagnostics Laboratory Date

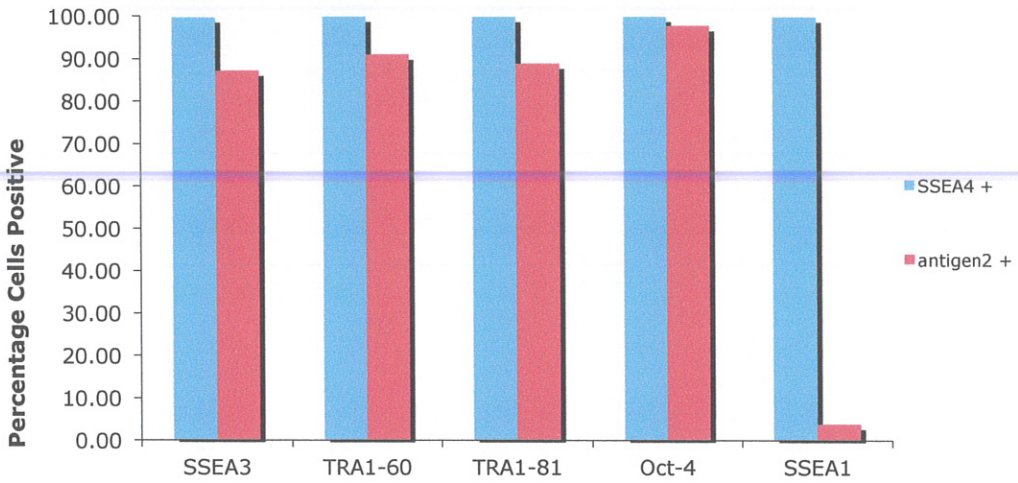
* Testing to assess engraftment following bone marrow transplantation was accomplished by analysis of human genetic polymorphisms at STR loci. This methodology has not yet been approved by the FDA and is for investigational use only.

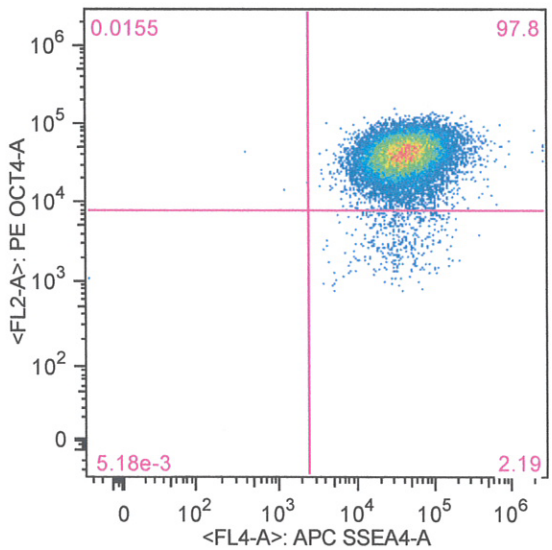
Flow Cytometry Characterization Report

Cell Line:	CRM-WA14-MB-001	Date:	7/30/13
Passage:	31	Acquisition:	7/21/13
Sample ID:	10815	File Creation:	7/21/13
		File Submission:	7/30/13

antigen2:	SSEA4 - antigen2 +	SSEA4 + antigen2 +	SSEA4 + antigen2 -	SSEA4 - antigen2 -	ALL SSEA4 +	ALL antigen2 +	Verification: should be 100%
SSEA3	0.06	87.10	12.60	0.21	99.70	87.16	**99.98**
TRA1-60	0.01	91.00	8.91	0.04	99.91	91.01	**99.96**
TRA1-81	0.01	88.90	11.00	0.04	99.90	88.91	**99.95**
Oct-4	0.02	97.80	2.19	0.01	99.99	97.82	**100.01**
SSEA1	0.01	3.80	96.10	0.08	99.90	3.81	**99.99**

Percent analyzable events: 30.80%
#wells submitted: 6
Total cells recovered: 16.4 X 10^6





Report prepared by: [redacted]
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