# cGMP Certificate of Analysis

## Thaw and Culture Details

| Cell Line Name | **WA01** | |
|---|---|---|
| Lot Number | **PACT-ESC-WA01-MB-001** | |
| Parent Material | WA01-DL-11 | |
| Provider/Client | WiCell | |
| Banked By | Waisman Biomanufacturing | |
| Thaw and Culture Recommendations | WiCell recommends thawing 1 vial into 6 wells of a 6 well plate using mTeSR™1 and Matrigel®. | |
| Protocol | WiCell Feeder Independent Pluripotent Stem Cell Protocol | |
| Culture Platform Prior to Freeze | Medium: mTeSR™1 | Matrix: Matrigel® |
| Passage Number | p29<br>Cells were cultured for 28 passages prior to freeze, 6 of them in mTeSR/Matrigel. Plated cells at thaw should be labeled passage 29. | |
| Date Vialed | 19-May-2010 | |
| Vial Label | Waisman Clinical Manufacturing Facility<br>WA01 MASTER CELL BANK<br>Lot #: PACT-ESC-WA01-MB-001<br>Vialed: 19May2010<br>Store in LN2 | |
| 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. | |
| Compliance Statement | The lot listed above has been manufactured and tested in compliance with current Good Manufacturing Practices for phase 1 and clinical trial materials. | |

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.

# cGMP Certificate of Analysis

## Lot Release Testing Results

| Test Description | Test Provider | Test Method | Test Specification | Result |
|---|---|---|---|---|
| Viability | Waisman Biomanufacturing | Post Thaw % Viability and Post Thaw Viable Cell Count | Report Results | 92% viable recovery $1.77 \times 10^6$ viable cells |
| Identity by STR | University of Wisconsin Molecular Diagnostics Laboratory | PowerPlex 1.2 Systems by Promega | Matches STR Profile of Parental Lot | Pass |
| Karyotype | WiCell | Karyotype by G Band (20 Metaphase Spreads) | Normal with No Recurrent Nonclonals; No clonal Abnormalities | Pass |
| Bacteriastasis & Fungistatsis | WuXi Apptec | Sterility Method Suitability (Bacteriastasis/Fungistasis) Immersion | No growth | Pass |
| Sterility – Direct Transfer Method | WuXi Apptec | Direct Inoculation Method Sterility Testing | No inhibition detected | Pass |
| ESC Identity by Flow Cytometry | WiCell | Flow Cytometry for ESC Marker Expression | >80% OCT4+/SSEA4+ Cells | Pass |
| Cultivatable and Non-cultivatable Mycoplasma in compliance with USP/EP/PTC/JP | Charles River | Protocol #GP-V611.22 | No Inhibition Detected No Mycoplasma Detected | Pass |

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.

# cGMP Certificate of Analysis

## Lot Release Testing Results

| Test Description | Test Provider | Test Method | Test Specification | Result |
|---|---|---|---|---|
| *In Vitro* Adventitious Agents | Charles River | Protocol #GP-V602.22 | Not Detected | Pass |
| *In Vivo* Adventitious Agents | Charles River | Protocol #CRL-PR-424 | Not Detected | Pass |
| Bovine Adventitious Agents (modified 9CFR) | Charles River | Protocol #GP-V615.3 | Not Detected | Pass |
| Porcine Adventitious Agents (modified 9CFR) | Charles River | Protocol #GP-V727.2 | Not Detected | Pass |
| Mouse Antibody Production (MAP) Test | Charles River | Protocol #CRL-PR-8 | Not Detected | Pass |
| Lymphocytic Choriomeningitis Virus (LCMV) RNA by QF-RT-PCR Assay | Charles River | Protocol #GP-V1173 | Not Detected | Pass |
| Extended, Focus Induction Assay for Murine Leukemia Virus with MiCL1 (S+L-) Detection | Charles River | Protocol #GP-V600.1 | Not Detected | Pass |

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.

# cGMP Certificate of Analysis

## Lot Release Testing Results

| Test Description | Test Provider | Test Method | Test Specification | Result |
|---|---|---|---|---|
| Retrovirus by PCR-Based Reverse Transcriptase (PBRT) Assay | Charles River | Protocol #GP-V692 | Not Detected | Pass |
| Ultrastructural TEM Evaluation of Cell Cultures for Retrovirus-like and Viral Particles | Charles River | Protocol #ERK-ATM-00443/2 | Not Detected | Pass |

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.

# cGMP Certificate of Analysis

## Cell Line Testing Results

**The following tests were performed on the cell line.**
**The tests, except gene expression, were performed on a master cell bank, WA01-DDL-13.**
**Gene expression was performed on WA01-MCB-01.**
**Please see the individual test reports for results of each test.**

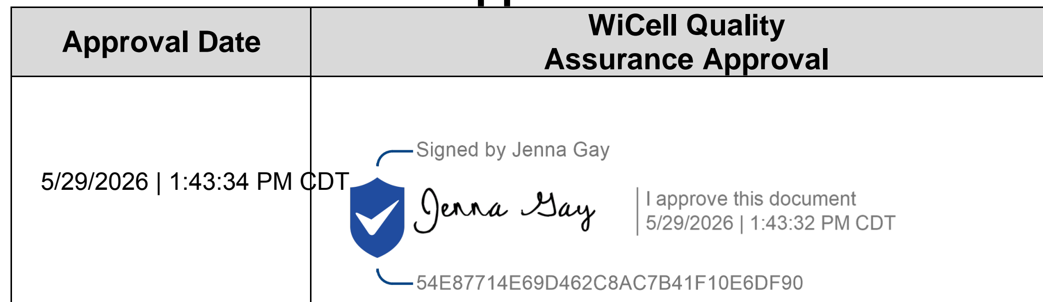
| Test Description | Test Provider | Test Method | Test Specification | Result |
|---|---|---|---|---|
| HLA profile | UW Molecular Diagnostics Laboratory | AlleleSEQR Kits by Abbott | Consistent with known profile | Pass |
| Co-cultivation with Mus Dunni Cells and PG4 S+L- assay | WuXi Apptec | 30201 | No contamination detected | Pass |
| HIV 1&2 by PCR | BioReliance | 105010 | Negative | Pass |
| HTLV 1&2 by PCR | BioReliance | 105013 | Negative | Pass |
| HBV by PCR | BioReliance | 105042 | Negative | Pass |
| HCV by PCR | BioReliance | 107207 | Negative | Pass |
| CMV by PCR | BioReliance | 105012 | Negative | Pass |
| EBV by PCR | BioReliance | 105011 | Negative | Pass |
| HHV-6 by PCR | BioReliance | 105020 | Negative | Pass |
| HHV-7 by PCR | BioReliance | 105029 | Negative | Pass |
| HHV-8 by PCR | BioReliance | 105056 | Negative | Pass |
| HP B19 by PCR | BioReliance | 105037 | Negative | Pass |

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.

# cGMP Certificate of Analysis

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

## Approval

| Approval Date | WiCell Quality Assurance Approval |
|---|---|
| 5/29/2026 \| 1:43:34 PM CDT |  |



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.

UWHealth

University of Wisconsin
Hospital and Clinics

# Short Tandem Repeat Analysis*

**Molecular Diagnostics Laboratory**
**600 Highland Avenue**
**Clinical Sciences Center (D4/211)**
**Madison, WI 53792-2472**

**Samples Report:**
**(1) 11454-STR 248.95 ng/uL (260/280=1.90)**

**DNA Extracted by WiCell Research Institute**

**Requestor:**
WiCell Research Institute
Phone: 608-316-4719
qa@wicell.org
Contact: Jenna Gay

**Sample Date(s):** 10/28/15
**Receive Date(s):** 10/28/15
**Assay Date(s):** 10/28/15
**File Name(s):** 151029 KJL
**Report Date(s):** 10/29/15

| STR Locus | STR Genotype Repeat # | (1) |
|---|---|---|
| FGA | 16–18, 18.2, 19, 19.2, 20, 20.2, 21, 21.2, 22, 22.2, 23, 23.2, 24, 24.2, 25, 25.2, 26–30, 31.2, 43.2, 44.2, 45.2, 46.2 | 20,24 |
| TPOX | 6-13 | 8,11 |
| D8S1179 | 7-18 | 12,13 |
| vWA | 10-22 | 15,17 |
| Amelogenin | X,Y | X,Y |
| Penta_D | 2.2, 3.2, 5, 7-17 | 10,13 |
| CSF1PO | 6-15 | 12,13 |
| D16S539 | 5, 8-15 | 9,13 |
| D7S820 | 6-14 | 8,12 |
| D13S317 | 7-15 | 8,11 |
| D5S818 | 7-16 | 9,11 |
| Penta_E | 5-24 | 10,12 |
| D18S51 | 8-10, 10.2, 11-13, 13.2, 14-27 | 17,18 |
| D21S11 | 24,24.2,25,25.2,26-28,28.2,29,29.2, 30, 30.2,31, 31.2,32,32.2,33,33.2, 34,34.2,35,35.2,36-38 | 28,32.2 |
| TH01 | 4-9,9.3,10-11,13.3 | 9.3,9.3 |
| D3S1358 | 12-20 | 15,15 |

**Comments: Based on the 11454-STR DNA dated and received on 10/28/15 from WI Cell Research Institute, this sample (Label on Tube: 11454-STR) exactly matches the STR profile of the human stem cell line WA01 (H1) comprising 28 allelic polymorphisms across the 15 STR loci analyzed. No STR polymorphisms other than those corresponding to the human WA01 (H1) 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 11454-STR DNA sample submitted corresponds to the WA01 (H1) stem cell line and 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%.**

10/29/15
Keith Challoner, Manager — Date
Molecular Diagnostics Laboratory

10/29/15
William M. Rehrauer, PhD, Director — Date
Molecular Diagnostics Laboratory

* Testing 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

WiCell Research Institute

# Chromosome Analysis Report: 007078

**Report Date: January 20, 2012**

**Cell Line:** PACT-ESC-WA01-MB-001 10369

**Passage #:** 31

**Date of Sample:** 1/13/2012

**Date Completed:** 1/19/2012

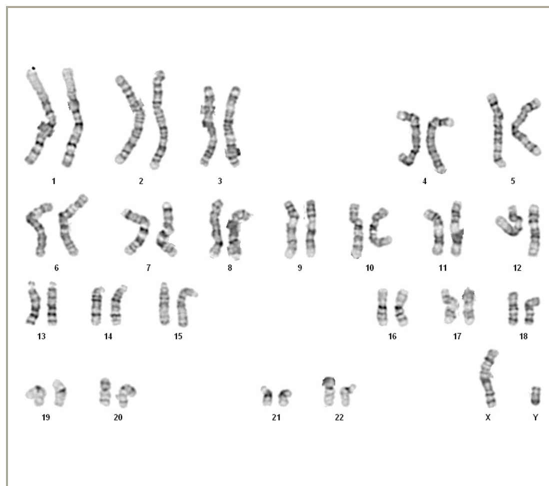
**Specimen:** hESC on Matrigel

**Cell Line Gender:** Male

**Reason for Testing:** Contract testing

**Investigator:** Dan Felkner, Wisconsin International Stem Cell Bank

**Results:** 46,XY




**Cell:** S01-16

**Slide:** 1-R1 (8) KARYOTYPE

**Slide Type:** Karyotyping

**# of Cells Counted:** 20

**# of Cells Karyotyped:** 4

**# of Cells Analyzed:** 8

**Band Level:** 450-525

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

**Completed by Erik McIntire, CG(ASCP), on 1/19/2012**
**Reviewed and interpreted by Karen Dyer Montgomery, PhD, FACMG, on 1/19/2012**

A signed copy of this report is available upon request.

**Date:**___________________________ **Sent To:**_________________________

**Sent By:**______________________________ **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".*

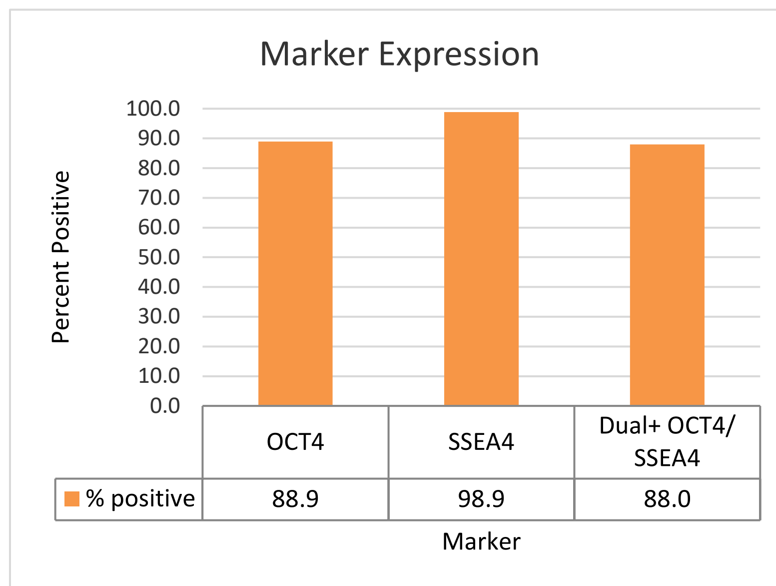
*This assay was conducted solely for listed investigator/institution. The results may not be relied upon by any other party without the prior written consent of the Director of the WiCell Cytogenetics Laboratory. The results of this assay are for research use only. If the results of this assay are to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

**Assessment of Undifferentiation Status Report**

FORM SOP-65.06
Version 11.0

| Sample Information | |
|---|---|
| Sample/Cell Line Name | PACT-ESC-WA01-MB-001 |
| WiCell Sample ID/CTR Number | 104023 |
| Passage Number at Assessment | 29 |
| Assessment Date | 23-Sep-24 |

## Results




## Interpretation

The cell line is 88.0% undifferentiated based on the dual expression of Oct4/SSEA4.

## Approvals

| 9/23/2024 | 9/25/2024 | 9/26/2024 |
|---|---|---|
| X James Johnson | X Aimee Pankonin | X Hunter Hefti |
| Tech #1<br>Characterization<br>Signed by: Johnson, James | Tech #2<br>Characterization<br>Signed by: Pankonin, Aimee | QA Review<br>Quality Assurance<br>Signed by: Hefti, Hunter |

*Unless otherwise mutually agreed in writing, the services provided to you hereunder by WiCell Research Institute, Inc. ("WiCell") are governed solely by WiCell's Terms and Conditions of Service, found at http://www.wicell.org/privacyandterms. Any terms you may attach to a purchase order or other document that are inconsistent, add to, or conflict with WiCell's Terms and Conditions of Service are null and void and of no legal force or effect.*

Test Facility:
1265 Kennestone Circle
Marietta, GA 30066

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

Report Number
846059


Waisman Clinical Biomanufacturing Facility
University of Wisconsin-Madison
1500 Highland Avenue / T480 Waisman Center
Madison, WI 53705

September 08, 2010
P.O. #: B349576

Attn: Lisa Marie Byrne

# GENERAL MICROBIOLOGY TEST REPORT

**Sample Information:** WA01 Master Cell Bank, Lot Number PACT-ESC-WA01-MB-001

**Date Received:** August 31, 2010
**Date in Test:** September 01, 2010
**Date Completed:** September 05, 2010

**Test Information:** Test Code: 30736
Sterility Method Suitability (Bacteriostasis / Fungistasis)
Immersion, USP / 21 CFR 610.12
Procedure #: BS210WSM.204
Media Volume: 200 mL
Volume Tested: 1.0 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) | 12 | 21 | 27 |
| RESULTS | PASS | PASS | PASS |

| FTM | *S. aureus* ATCC 6538 | *K. rhizophila* ATCC 9341 | *C. sporogenes* ATCC 11437 |
|---|---|---|---|
| Test Sample | Positive | Positive | Positive |
| Inoculated Control | Positive | Positive | Positive |
| Inoculum Level (CFU) | 42 | 55 | 9 |
| RESULTS | PASS | PASS | PASS |

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

Note: Reference Sterility Test Report(s): 846062

ATTACH TO F01-QCP-028-13106 LAB 10/4/10

09-08-10
QA Reviewer Date

09-08-10
Technical Reviewer Date

*Testing conducted in accordance with current Good Manufacturing Practices.*

Test Facility:
1265 Kennestone Circle
Marietta, GA 30066

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.

Waisman Clinical Biomanufacturing Facility
University of Wisconsin-Madison
1500 Highland Avenue / T480 Waisman Center
Madison, WI 53705

September 20, 2010
P.O. #: B349576

Attn: Lisa Marie Byrne

# STERILITY TEST REPORT

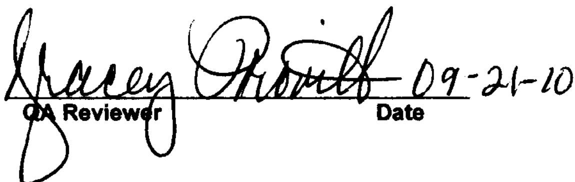
**Sample Information:** WA01 Master Cell Bank, Lot Number PACT-ESC-WA01-MB-001

**Date Received:** August 31, 2010
**Date in Test:** September 02, 2010
**Date Completed:** September 16, 2010

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

| TEST PARAMETERS | PRODUCT | |
|---|---|---|
| Approximate Volume Tested | 1.0 mL | 1.0 mL |
| Number Tested | 1 | 1 |
| 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 | 1 NEGATIVE | 1 NEGATIVE |

ATTACH TO
F01-QCP-028-13106
LMB
10/14/10



09-21-10
QA Reviewer Date

09-21-10
Technical Reviewer Date

*Testing conducted in accordance with current Good Manufacturing Practices.*

charles river

## Report of Analysis

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

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

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

**Sample Identification:** **PACT-ESC-WA01-MB-001**

**Sample Number:** **554770**

**Protocol Number:** GP-V611.22

**Protocol Effective Date:** 29 Dec 2021

**Date(s) of Testing:** 03 Feb 2025 to 04 Mar 2025

| Results | |
|---|---|
| Mycoplasma | **Not Detected** |
| Mycoplasmastasis | **No Inhibition** |

**Report Comments:**
None

**Exception Document(s):**
None

**Certification:**
This report summarizes testing performed at the Charles River Laboratories, Inc., PA-Biologics facility, which is operated in compliance with the applicable requirements of the U.S. Food and Drug Administration's and European Union's Good Manufacturing Practice regulations as found in Title 21 CFR Parts 210 and 211, and EudraLex Volume 4, respectively. There were no deviations from the test method(s) that impacted the quality or integrity of the test result(s).

| | | | |
|---|---|---|---|
| **Technical Approval:** | Ashley Morgan | **Date:** | 04 Mar 2025 14:31 |
| **Quality Assurance:** | Jebadiah Hickman | **Date:** | 07 Mar 2025 13:42 |

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



Attach to F01-QCP-028 - 44061 JS 04-01-25 CBR 04/30/25

Attach to F01-QCP-028 - 4906135 04-01-25 CGC 06/30/25

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

**TABLE 1**

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

| Inoculum | Broth (0 HR) | Initial Plates (0 HR) | Passage 1 (3 Days) | Passage 2 (7 Days) | Passage 3 (14 Days) | Passage 4 (21 Days) |
|---|---|---|---|---|---|---|
| | MIC[a] | MIC | MIC | MIC | MIC | MIC |
| 554770 | – | – | – | – | – | – |
| Neg. Control | – | – | – | – | – | – |
| 554770 + *M. pneumoniae* | + | + | + | + | + | + |
| 554770 + *M. orale* | + | + | + | + | + | –[b] |
| *M. pneumoniae* | + | + | + | + | + | + |
| *M. orale* | + | + | + | + | –[b] | –[b] |

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

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

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

| TABLE 2 – Inhibition<br><br>COLONY COUNTS FOR SPIKED MATERIAL<br>DAY 14 (18FEB2025) | | |
|---|---|---|
| **Sample** | **Microaerophilic** | |
| | **CFU/0.2mL** | **Average CFU/0.2mL** |
| **554770 + *M. pneumoniae*** | 23, 18, 19, 25 | 21 |
| **554770 + *M. orale*** | 35, 32, 39, 29 | 34 |
| ***M. pneumoniae*** | 36, 41, 32, 37 | 37 |
| ***M. orale*** | 32, 36, 37, 39 | 36 |

| TABLE 3<br><br>MYCOPLASMA TEST RESULTS<br>AGAR NON-CULTIVABLE | |
|---|---|
| **Inoculum** | **Indicator Cell Culture** |
| | **Cell Substrate** |
| **554770** | – |
| **Negative Control** | – |
| ***M. orale*** | + |
| ***M. hyorhinis*** | + |

Cell Substrate = Vero76
Stain Method = Hoechst



Attach to F01-QCP-028 – 44061 JS 04-01-25 CCR 04/30/25

charles river

## REPORT OF ANALYSIS

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

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

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

**Protocol Number:** GP-V602.22

**Protocol Effective Date:** 16DEC2024

**On-Test Date:** 05FEB2025

**Results:**

| Sample Identification | Sample Number | Assay Result Adventitious Viral Agents |
|---|---|---|
| Lot: PACT-ESC-WA01-MB-001 | 554761 | Not Detected |

**Comments:**

None

**Exception Document(s):**

PR ID: 2011091

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



Attach to F01-QCP-028 -44061 JS 04-11-25 CB 06/30/25

**Sample Preparation:** Prior to test initiation, client sample material was subjected to a lysis procedure (three freeze/thaws and a centrifugation step) as described in GP-V985 to ensure that no intact cells were present in the lysate. Refer to PR ID: 2011091.

| TISSUE CULTURE SAFETY TESTING (*In vitro*) | | | | |
|---|---|---|---|---|
| **Inoculum** | **Test** | **MRC-5** | **Vero 76** | **HeLa** |
| **Negative** | **CPE[a]** | –[b] | – | – |
| **554761** | **CPE** | – | – | – |
| **Positive[c]** | **CPE** | +[d] | + | + |
| **Negative** | **HAD[e]** | – | – | – |
| **554761** | **HAD** | – | – | – |
| **Positive[c]** | **HAD** | + | + | + |
| **Negative** | **HA[f]** | – | – | – |
| **554761** | **HA** | – | – | – |
| **Positive[c]** | **HA** | + | + | + |

[a] Observations for cytopathogenic effect

[b] Negative result obtained

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

[d] Positive result obtained

[e] Hemadsorption Test

[f] Hemagglutination Test



Attach to F01-QCP-028 – 44061 JS 04-11-25 CER 04/30/25

charles river

**Certification:**
This report summarizes testing performed at the Charles River Laboratories, Inc., PA-Biologics facility, which is operated in compliance with the applicable requirements of the U.S. Food and Drug Administration's and European Union's Good Manufacturing Practice regulations as found in Title 21 CFR Parts 210 and 211, and EudraLex Volume 4, respectively. There were no deviations from the test method(s) that impacted the quality or integrity of the test result(s).

| | | | |
|---|---|---|---|
| **Study Lead Signer Name:** | Erica Rice | **Date:** | 07 Mar 2025 12:46 |
| **Quality Assurance Signer Name:** | Gbemisola Olaoye | **Date:** | 11 Apr 2025 13:48 |

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



Attach ~~to F01 QCP-028~~ to F01-QCP-028 - 44061 JS 04-11-25 CER 07/07/25
① addressed overwrite error. JS 07-07-25

charles river

**Final Report/Certificate of Analysis, Version I**

**Title: Detection of Inapparent Viruses in a Biological Sample per EU and US Regulations**

**Document Number: WLM-PRT-00014(1)**

**Legacy Number: PR-424-6**

**Protocol Effective Date: 28-Jun-2024**

---

**1.0 Client**

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

**2.0 Test Article Identity:** Lot: PACT-ESC-WA01-MB-001

**CR ID #:** 554756

**3.0 Test Facility**

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

**4.0 Lab Initiation date:** 18-Feb-2025

**5.0 Lab Completion date:** 10-Apr-2025

**6.0 References**

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

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

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

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

International Conference on Harmonization, Guidance for Industry Q5A (R2): Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin (2023)

**7.0 Procedure**

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





Attach to F01-QCP-028 – 44061 JJ 04-23-25 CCR 04/30/25

## 8.0 Test Article Preparation

A low-speed centrifugation (1500 x g for 10 minutes) was performed on the test article prior to inoculation for the guinea pig, post weaning mice, allantoic fluid and yolk sac phases due to the sample aliquots being turbid with visible debris.

The test article was observed to be thick, turbid, and with visible debris present; it is standard practice to perform centrifugation on test article with visible debris present. To mitigate the potential of a high number of mortalities during the suckling mice phase, IVB requested that the test article undergoes a low speed centrifugation and then the supernatant is passed through at 0.45µ filter prior to inoculations. The Client approved. Prior to inoculation of the suckling mice, the test article underwent a low-speed centrifugation (1500 x g for 10 minutes) and the supernatant was then passed through a 0.45µ filter.

## 9.0 Unexpected Results

There was a high number of embryo mortalities observed in the yolk sac passage phase of testing, a Phase I Laboratory Investigation was performed (PR ID 2032003). Two test group eggs were observed to be non-viable on day post inoculation (DPI) 3, one test group egg was observed to be non-viable on DPI 7, and one test group egg was observed to be non-viable on DPI 9. At 60% survivability for the test group eggs, the yolk sac passage phase did not meet the required ≥80% survival and needed to be repeated. Bioburden testing was performed for the non-viable eggs, and hemagglutination (HA) testing was performed on fluids from the surviving eggs.

Bioburden testing was performed on the non-viable eggs and completed with negative results for microbial growth. There was no bacterial contamination present.

HA testing was performed on fluids from the surviving eggs and completed with negative results for viral contamination.

The health of the host system was assessed, and no abnormalities were noted. However, review of the egg receipt's health report shows that the flock from which we receive eggs from is of an older age, which could have an impact on the overall health of the eggs. Previously, IVB observed an increase in unexplained embryo mortalities when the flocks age was older and have successfully reduced the occurrence of mortalities by changing to an alternate/younger flock. Starting the week of 09-Mar-2025, the IVB department will receive eggs from a younger flock.

The in vivo testing is on "live" test systems, of which the embryonic chicken eggs are the most sensitive due to their extremely young age, and it is not uncommon for a small percentage to die on test for unknown reasons. Mortalities for unknown reasons may be expected and can sometimes result in a phase failure. The root cause of the mortalities has not been definitively identified, however, the unexpected mortalities could be indicative of a flock issue, such as an older age.

The yolk sac passage phase was repeated using eggs from a younger flock and with retained homogenate; the retained homogenate was passed through at 0.45µ filter prior to inoculation to remove any potential bacterial contaminants.

## 10.0 Deviations

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

## 11.0 Results

### 11.1 Survival Summary

| | Number of hosts inoculation | Number of hosts to survive 24 hours* | Number of hosts to survive observation period | % survival * |
|---|---|---|---|---|
| Guinea Pigs | 6 | 6 | 6 | 100 |
| Post weaning Mice | 20 | 15 | 15 | 100 |
| Suckling Mice | 20 | 19 | 19 | 100 |
| Suckling Mice Passage | 20 | 20 | 20 | 100 |
| Embryonated Eggs via Allantoic Fluid Route | 10 | 10 | 10 | 100 |
| Embryonated Eggs via Allantoic Fluid Route - Passage | 10 | 10 | 10 | 100 |
| Embryonated Eggs via Yolk Sac Route | 10 | 9 | 9 | 100 |
| Embryonated Eggs via Yolk Sac Route - Passage | 10 | 10 | 6 | 60** |
| Embryonated Eggs via Yolk Sac Route - Passage **Repeat** | 10 | 10 | 10 | 100 |

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

**See Section 9.0 Unexpected results

### 11.2 Hemagglutination Assay

| Hemagglutination Assay (HA) | No agglutination observed - Negative |
|---|---|

### 11.3 Conclusion:

In all cases, the survival rates for test article-inoculated animals and embryonated eggs were 80% or higher. There were no gross abnormalities indicative of viral infection in the guinea pigs that were submitted for necropsy. Animals showed no signs of viral infection or transmissible disease. Embryos in eggs inoculated with the test article appeared healthy at the conclusion of the observation period and hemagglutinating activity was not detected in fluid from test article-inoculated eggs. Therefore, infectious virus was not detected in the test article, Lot: PACT-ESC-WA01-MB-001.



Attach to F01-QCP-028 - 44061 JS 04-23-25 CER 04/30/25

charles river

**Certification:**

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

| | | | |
|---|---|---|---|
| **Study Lead Signer Name:** | Leslie Scola | **Date:** | 16 Apr 2025 11:45 |
| **Quality Assurance Signer Name:** | Alexander Cameron | **Date:** | 16 Apr 2025 15:16 |

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



Attach to F01-QCP-028 – 44061 JS 04-23-25 CCR 04/30/25

charles river

## REPORT OF ANALYSIS

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

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

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

**Protocol Number:** GP-V615.3

**Protocol Effective Date:** 04APR2022

**On-Test Date:** 05FEB2025

**Results:**

| Sample Identification | Sample Number | Assay Result Bovine Adventitious Viral Agents |
|---|---|---|
| Lot: PACT-ESC-WA01-MB-001 | 554766 | Not Detected |

**Comments:**

None

**Exception Document(s):**

PR ID: 2011091

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





Attach to F01-QCP-028 - 44061 JS 04-14-25 CSR 04/30/25

**Sample Preparation:** Prior to inoculation, three freeze/thaws and a centrifugation step were performed to ensure that no intact cells were present in the lysate.

| TABLE 1<br>OBSERVATIONS FOR IMMUNOFLUORESCENCE IN INDICATOR CELLS ON DAYS 7, 14, AND 21 | |
|---|---|
| **Inoculum** | **Indicator Cell Line** |
| | **Bovine Turbinate** |
| **554766** | -[a] |
| **Negative Control** | - |
| **Bovine Adenovirus** | +[b] |
| **Bovine Parvovirus** | + |
| **Bovine Respiratory Syncytial Virus** | + |
| **Bovine Viral Diarrhea Virus, non-cytopathic NY-1 Strain** | + |
| **Rabies Virus** | + |
| **Reovirus** | + |
| **Inoculum** | **Vero 76** |
| **554766** | - |
| **Negative Control** | - |
| **Bovine Respiratory Syncytial Virus** | + |
| **Bluetongue Virus** | + |
| **Rabies Virus** | + |
| **Reovirus** | + |

[a] Negative result obtained

[b] Positive result obtained



Attach to F01-QCP-028 - 44061 JS 04-14-25 CER 06/30/25

| TABLE 2<br>OBSERVATIONS FOR HEMADSORPTION | | |
|---|---|---|
| Inoculum | Indicator Cells | |
| | BT | Vero |
| 554766 | -[a] | - |
| Positive Control PI3[b] (Day 0) | +[c] | + |
| Negative Control | - | - |

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

| TABLE 3<br>OBSERVATIONS FOR CYTOPATHOLOGY | | |
|---|---|---|
| Inoculum | Indicator Cells | |
| | BT | Vero |
| 554766 | -[a] | - |
| **Positive Controls:** | | |
| Parainfluenza 3 | +[b] | + |
| Bovine Viral Diarrhea Virus, non-cytopathic NY-1 Strain | - | NT[c] |
| Bovine Parvovirus | + | NT |
| Bovine Adenovirus | + | NT |
| Bovine Respiratory Syncytial Virus | + | + |
| Bluetongue Virus | NT | + |
| Reovirus | - | + |
| Rabies Virus | - | - |
| **Negative Controls** | - | - |

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



Attach to F01-QCP-028 - 44061 JJ 04-14-25 CCR 04/30/25

charles river

**Certification:**

This report summarizes testing performed at the Charles River Laboratories, Inc., PA-Biologics facility, which is operated in compliance with the applicable requirements of the U.S. Food and Drug Administration's and European Union's Good Manufacturing Practice regulations as found in Title 21 CFR Parts 210 and 211, and EudraLex Volume 4, respectively. There were no deviations from the test method(s) that impacted the quality or integrity of the test result(s).

| | | | |
|---|---|---|---|
| **Study Lead Signer Name:** | Lindsey Allebach | **Date:** | 07 Mar 2025 14:40 |
| **Quality Assurance Signer Name:** | Gbemisola Olaoye | **Date:** | 14 Apr 2025 09:31 |

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



Attach to F01-QCP-028 - 44061 JS 04-14-25 CtR 04/30/25

charles river

## REPORT OF ANALYSIS

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

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

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

**Protocol Number:** GP-V727.2

**Protocol Effective Date:** 19JUL2021

**On-Test Date:** 12FEB2025

**Results:**

| Sample Identification | Sample Number | Assay Result Porcine Adventitious Viral Agents |
|---|---|---|
| Lot: PACT-ESC-WA01-MB-001 | 554763 | Not Detected |

**Comments:**

None

**Exception Document(s):**

None

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



Attach to F01-QCP-028 – 4406155 03-13-25 CCL 04/30/25

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

| TABLE 1<br>OBSERVATIONS FOR IMMUNOFLUORESCENCE IN INDICATOR CELLS ON DAYS 7, 14, AND 21 | |
|---|---|
| Inoculum | Indicator Cell Line |
| | Bovine Turbinate |
| 554763 | -[a] |
| Negative Control | - |
| Bovine Viral Diarrhea Virus | +[b] |
| Pseudorabies Virus | + |
| Rabies Virus | + |
| Inoculum | Vero 76 |
| 554763 | - |
| Negative Control | - |
| Reovirus | + |
| Pseudorabies Virus | + |
| Rabies Virus | + |
| Inoculum | MA104 |
| 554763 | - |
| Negative Control | - |
| Porcine Reproductive and Respiratory Syndrome Virus | + |
| Inoculum | St Neb |
| 554763 | - |
| Negative Control | - |
| Porcine Parvovirus | + |
| Transmissible Gastroenteritis Virus | + |
| Inoculum | PK-15 |
| 554763 | - |
| Negative Control | - |
| Porcine Adenovirus | + |
| Inoculum | HCT-8 |
| 554763 | - |
| Negative Control | - |
| Porcine Hemagglutinating Encephalomyelitis Virus | + |

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



Attach to F01-QCP-028 – 44061 JS 03-13-25 CCR 04/30/25

| TABLE 2<br>OBSERVATIONS FOR HEMADSORPTION | | |
|---|---|---|
| Inoculum | Indicator Cell Line | |
| | Bovine Turbinate | Vero 76 |
| 554763 | -[a] | - |
| Positive Control PI3[b] Day 0 | +[c] | + |
| Positive Control PI3 Day 14 | + | + |
| Negative Control | - | - |

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

| TABLE 3<br>OBSERVATIONS FOR CYTOPATHOGENIC EFFECT IN INDICATOR CELLS FOR SAMPLE AND NEGATIVE CONTROLS | | |
|---|---|---|
| Indicator Cell Line | Inoculum | |
| | 554763 | Negative Control |
| Bovine Turbinate | -[a] | - |
| Vero 76 | - | - |
| MA104 | - | - |
| ST Neb | - | - |
| PK-15 | - | - |
| HCT-8 | - | - |

[a] Negative result obtained



Attach to F01-QCP-028 -44061 JS 03-13-25 CSR 06/30/25

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

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



Attach to F01-QCP-028 – 4406 / JS 03-13-25 CGR 04/30/25

charles river

**Certification:**

This report summarizes testing performed at the Charles River Laboratories, Inc., PA-Biologics facility, which is operated in compliance with the applicable requirements of the U.S. Food and Drug Administration's and European Union's Good Manufacturing Practice regulations as found in Title 21 CFR Parts 210 and 211, and EudraLex Volume 4, respectively. There were no deviations from the test method(s) that impacted the quality or integrity of the test result(s).

| | | | |
|---|---|---|---|
| **Study Lead Signer Name:** | Timothy Starosta | **Date:** | 13 Mar 2025 13:18 |
| **Quality Assurance Signer Name:** | Gbemisola Olaoye | **Date:** | 13 Mar 2025 14:08 |

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

Attach to F01-QCP-028 - 440C1 JS 03-13-25 CER 09/30/25

charles river

**Final Report/Certificate of Analysis, Version I**
**Title: Mouse Antibody Production (MAP)Test**
**Document Number: WLM-PRT-00008-1**
**Legacy Number: PR-8-13**
**Protocol Effective Date: 28-Jun-2024**

---

**1.0 Client**

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

**2.0 Test Article Identity:** PACT-ESC-WA01-MB-001

**CR ID #:** 554765

**3.0 Test Facility**

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

**4.0 Lab Initiation date:** 10-Feb-2025

**5.0 Lab Completion date:** 18-Mar-2025

**6.0 References**

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

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

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

International Conference on Harmonisation, Guidance for Industry Q5A (R2): Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin (2023)

**7.0 Procedure**

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

**8.0 Unexpected Results**

None

**9.0 Deviations**

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



Attach to F01-QCP-028 - 44061 JS 03-27-25 CERC 06/30/25

## 10.0 Acceptance Criteria

In order for the assay to be considered valid:

At least four test article inoculated mice must survive at least 28 days post inoculation with serum available for the serological assay.

Samples from saline control inoculated mice must be seronegative for all viruses.

Standard positive and negative control sera run with the assay yield acceptable serological test results.

## 11.0 Results

11.1 Serology

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

## 12.0 Conclusion:

The acceptance criteria outlined in section 10.0 was met, therefore the assay is valid.
Viral contamination was not detected in the test article, PACT-ESC-WA01-MB-001.



Attach to F01-QCP-028 -44061 JS 03-27-25 CGR 06/30/25

charles river

**Certification:**

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

| | | | |
|---|---|---|---|
| **Study Lead Signer Name:** | Eduardo De Los Santos | **Date:** | 24 Mar 2025 09:13 |
| **Quality Assurance Signer Name:** | Alexander Cameron | **Date:** | 26 Mar 2025 09:02 |

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

Attach to F01-QCP-028 - 44061 JS 03-27-25 CGR 06/30/25

charles river

# REPORT OF ANALYSIS

## Detection and Quantitation of Lymphocytic Choriomeningitis Virus (LCMV) RNA by One Step QF-RT-PCR using a DNA Copy Number Standard

| | |
|---|---|
| **Client:** | University Of Wisconsin<br>1500 Highland Avenue<br>Madison, WI 53705-2274 USA |
| **Testing Facility:** | Charles River Laboratories<br>466 Devon Park Drive<br>Wayne, PA 19087 |
| **Protocol Number:** | GP-V1173 |
| **Protocol Effective Date:** | 23FEB2024 |
| **On-Test Date:** | 05FEB2025 |

**Results:**

| Sample Identification | Sample Number | Result |
|---|---|---|
| Lot: PACT-ESC-WA01-MB-001 | 554762 | Not Detected |

**Comments:**

None

**Exception Documents:**

None

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

Attach to F01-QCP-028 -4 Y061 JS 02-19-25 CCR 04/30/25

| TABLE 1<br>CALCULATION OF APPROXIMATE COPIES OF LCMV RNA | | | | |
|---|---|---|---|---|
| **LCMV-S424 Copy Number Standard DNA** | **CT (AVERAGE)** | **CV (%)** | | |
| 156,250 | 19.70 | 0.71 | | |
| 31,250 | 22.05 | 0.91 | | |
| 6,250 | 24.26 | 0.16 | | |
| 1,250 | 26.13 | 0.08 | | |
| 250 | 28.45 | 0.35 | | |
| 50 | 31.43 | 1.37 | | |
| 10 | 35.49 | 1.78 | | |
| **Sample[a]** | **CT (AVERAGE)** | **CV (%)** | **Calculated copy/reaction[b]** | **Calculated copies/mL[c]** |
| **554762** | --[d] | -- | -- | -- |
| **554762** + LCMV 250 copies | 28.42 | 0.74 | 440 | 79,153 |
| **Positive Control** | **CT (AVERAGE)** | **CV (%)** | **Calculated copy/reaction[b]** | |
| PEC (DPBS + 100,000 copies Xeno RNA) + LCMV 250 copies | 28.47 | 0.81 | 426 | |
| **Negative Controls** | **CT (AVERAGE)** | **CV (%)** | **Calculated copy/reaction[b]** | |
| NEC (DPBS) | --[d] | -- | -- | |
| NCR (CHO RNA) | --[d] | -- | -- | |
| NRC (Water) | --[d] | -- | -- | |

[a] Original client sample volume tested = 0.100 mL of neat sample.

[b] Calculations of copies were generated by linear regression analysis of the LCMV-S424 standard curve. Serial five-fold dilutions from 156250 to 10 copies per reaction. All $C_T$ values are arithmetic means of triplicate reactions. Coefficient of Determination ($R^2$ = 0.9810).

[c] The copies/mL calculated value represents the concentration of copy number DNA in the original sample prior to processing.

[d] No detectable fluorescence.

NEC= Negative Extraction Control (100 µL DPBS). PEC= Positive Extraction Control (100 µL DPBS plus 100,000 copies Xeno RNA (10 µL of Xeno RNA at 10,000 copies/µL)).

PEC= Positive Extraction Control (100 µL DPBS plus 100,000 copies Xeno RNA (10 µL of Xeno RNA at 10,000 copies/µL)).

| TABLE 2<br>CALCULATION OF APPROXIMATE COPIES OF XENO RNA SPIKE | | | | |
|---|---|---|---|---|
| **Calibrator Point (copies)** | **CT (AVERAGE)** | | | |
| Xeno RNA 100,000 copies Calibrator Point (EIC) | 25.97 | | | |
| **Sample**[a] | **CT (AVERAGE)** | **CV (%)** | **Calculated copies/reaction**[b] | **% Recovery**[c] |
| **554762** + Xeno RNA 100,000 copies | 30.24 | 0.17 | 5183 | 93 |
| **Positive Control** | **CT (AVERAGE)** | **CV (%)** | **Calculated copies/reaction**[b] | **% Recovery**[c] |
| PEC (DPBS + Xeno RNA 100,000 copies) | 30.57 | 0.20 | 4123 | 74 |
| **Negative Controls** | **CT (AVERAGE)** | **CV (%)** | **Calculated copies/reaction**[b] | **% Recovery**[c] |
| NEC (DPBS) | --[d] | -- | -- | |
| NCR (CHO RNA) | --[d] | -- | -- | |
| NRC (Water) | --[d] | -- | -- | |

[a] Original client sample volume tested = 0.100 mL of neat sample.
[b] Calculated copies per reaction is determined by dividing the EIC copies (100,000) by $2^{\Delta Ct}$ where ΔCt is the difference between the unknown Ct value (sample or controls) and EIC Ct value.
[c] Percent recovery of the samples and controls is determined by what percentage of the EIC (100,000 copies) is the calculated copies per extraction relative to the Xeno RNA calibrator value of the sample or control.
[d] No detectable fluorescence.

NEC= Negative Extraction Control (100 µL DPBS).
PEC= Positive Extraction Control (100 µL DPBS plus 100,000 copies Xeno RNA (10 µL of Xeno RNA at 10,000 copies/µL)).

Attach to F01-QCP-028 – 44061 JS 02-19-25 CSR 06/30/25

charles river

**Certification:**

This report summarizes testing performed at the Charles River Laboratories, Inc., PA-Biologics facility, which is operated in compliance with the applicable requirements of the U.S. Food and Drug Administration's and European Union's Good Manufacturing Practice regulations as found in Title 21 CFR Parts 210 and 211, and EudraLex Volume 4, respectively. There were no deviations from the test method(s) that impacted the quality or integrity of the test result(s).

| | | | |
|---|---|---|---|
| **Study Lead Signer Name:** | Ashley Callaghan | **Date:** | 11 Feb 2025 13:40 |
| **Quality Assurance Signer Name:** | Philip Bell | **Date:** | 19 Feb 2025 15:43 |

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

Attach to F01-QCP-028 - 44061 JS 02-19-25 CR 04/30/25

charles river

## REPORT OF ANALYSIS

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

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

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

**Protocol Number:** GP-V600.1

**Protocol Effective Date:** 30AUG2021

**On-Test Date:** 11FEB2025

**Results:**

| Sample Identification | Sample Number | Assay Result |
| --- | --- | --- |
| Lot: PACT-ESC-WA01-MB-001 | 554767 | Not Detected |

**Comments:**

None

**Exception Document(s):**

None

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



Attach to F01-QCP-028 – 44061 JS 03-12-25 CCR 07/07/25

| S+L- FOCUS ASSAY | |
|---|---|
| **Sample (0.1 mL/well)** | **Mean FFU[a]/mL** |
| **DIRECT ASSAY** | |
| **Negative Control** | ND[b] |
| **554767** | ND |
| **Positive Control[c]** | $9.83 \times 10^{5}$[d] |
| **EXTENDED ASSAY** | |
| **Negative Control** | ND |
| **Extended Negative Control** | ND |
| **554767** | ND |
| **Positive Control[c]** | $8.69 \times 10^{4}$[d] |
| **Extended Positive Control[e]** | $8.33 \times 10^{5}$[d] |

[a] Focus Forming Units
[b] Not Detected
[c] Established range of virus stock titer: $3.30 \times 10^{4}$ – $3.94 \times 10^{6}$ FFU/mL
[d] Calculated Average Titer
[e] Average titer of the virus stock: $3.61 \times 10^{5}$ FFU/mL
Virus control in Extended Assay was inoculated with 1.0 mL of 1:$1.09 \times 10^{4}$ virus dilution (approximately 33 FFU/mL)





Attach to F01-QCP-028 – 44061 JS 03-~~17~~ 12(1) -25 [initials] 04/04/25

(1) Addressed overwrite error. JS 07-07-25

charles river

**Certification:**

This report summarizes testing performed at the Charles River Laboratories, Inc., PA-Biologics facility, which is operated in compliance with the applicable requirements of the U.S. Food and Drug Administration's and European Union's Good Manufacturing Practice regulations as found in Title 21 CFR Parts 210 and 211, and EudraLex Volume 4, respectively. There were no deviations from the test method(s) that impacted the quality or integrity of the test result(s).

| | | | |
|---|---|---|---|
| **Study Lead Signer Name:** | Timothy Starosta | **Date:** | 11 Mar 2025 16:42 |
| **Quality Assurance Signer Name:** | Gbemisola Olaoye | **Date:** | 11 Mar 2025 18:27 |

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

Attach to F01-QCP-028 – 44061 JS 03-11-25 CBR 06/30/25

charles river

## REPORT OF ANALYSIS

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

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

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

**Protocol Number:** GP-V692

**Protocol Effective Date:** 12JAN2022

**On-Test Date:** 05FEB2025

**Results:**

| Sample Identification | Sample Number | Assay Result 1:100 Dilution | Assay Result 1:1,000 Dilution |
|---|---|---|---|
| Lot: PACT-ESC-WA01-MB-001 | 554768 | Not Detected | Not Detected |

**Comments:** None

**Exception Document(s):** None

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

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

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



Attach to F01-QCP-028 - 44061 JS 02-12-25 CCR 06/30/25

| TABLE 1<br>DETERMINATION OF REVERSE TRANSCRIPTASE ACTIVITY IN SAMPLE | | | |
|---|---|---|---|
| **Standard pUnits[a]** | **Average $C_T$ VALUE [b]** | **Correlation Coefficient (R)** | |
| $4.0 \times 10^9$<br>$8.0 \times 10^8$<br>$1.6 \times 10^8$<br>$3.2 \times 10^7$<br>$6.4 \times 10^6$<br>$1.28 \times 10^6$ | 14.84<br>16.91<br>18.59<br>22.83<br>25.15<br>26.78 | R = 0.9869<br>*The correlation coefficient (R value) must be ≥ 0.9800.* | |
| **Negative Control** | **Average $C_T$ VALUE [b]** | **Expected Result** | **Result** |
| Mv1Lu | Undetermined | Background | Background |
| **Sample Spikes** | **Average $C_T$ VALUE [b]** | **Expected Result** | **Result** |
| 554786 (1:100) + SMRV | 26.40 | Positive | Positive |
| 554786 (1:1000) + SMRV | 27.74 | Positive | Positive |
| 554786 (1:100) + TEK-1 | 31.27 | Positive | Positive |
| 554786 (1:1000) + TEK-1 | 32.12 | Positive | Positive |

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

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

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

| **Sample** | **$C_T$** | | | **Result: If any of 3 replicates results in RT activity, the sample is considered positive for RT activity.** |
|---|---|---|---|---|
| | **Rep 1** | **Rep 2** | **Rep 3** | |
| 554786 (1:100) | Undetermined | Undetermined | Undetermined | 3 of 3 Replicates:<br>RT Activity Not Detected |
| | Not Detected | Not Detected | Not Detected | |
| 554786 (1:1000) | Undetermined | Undetermined | Undetermined | 3 of 3 Replicates:<br>RT Activity Not Detected |
| | Not Detected | Not Detected | Not Detected | |

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



Attach to F01-QCP-028 – 44061 JS 02-12-25 CER 04/30/25

charles river

**Certification:**

This report summarizes testing performed at the Charles River Laboratories, Inc., PA-Biologics facility, which is operated in compliance with the applicable requirements of the U.S. Food and Drug Administration's and European Union's Good Manufacturing Practice regulations as found in Title 21 CFR Parts 210 and 211, and EudraLex Volume 4, respectively. There were no deviations from the test method(s) that impacted the quality or integrity of the test result(s).

| | | | |
|---|---|---|---|
| **Study Lead Signer Name:** | Nicole Hanlon | **Date:** | 12 Feb 2025 15:36 |
| **Quality Assurance Signer Name:** | Philip Bell | **Date:** | 12 Feb 2025 15:58 |

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

Attach to F01-QCP-028 - 44061 JS 02-12-25 CER 04/30/25

charles river

# Report of Analysis

**Contact:** University of Wisconsin, 1500 Highland Avenue, Room 206, Waisman Center, Madison WI 53705-2274, US

**Testing Laboratory:** Charles River Laboratories Germany GmbH, Max-Planck-Str. 15a-b, 40699 Erkrath, Germany

**Description:** V1-U/U16/25

**Product:** Human cell line

**Client Sample ID:** PACT-ESC-WA01-MB-001

**Client Lot/Charge/Batch:** PACT-ESC-WA01-MB-001

**Charles River Sample ID:** 561531

**Original Sample ID:** 559737

**Subcontractor Lab:** ATEM SD GmbH (ATEM), Büchelstrasse 54, 42855 Remscheid, Germany,

**Specification Document:** ERK-APC-00443[1]; ACF-00443.01-C42-O.01

**Test/Method:** Ultrastructural Evaluation of Cells for the Presence of Viral Particles, with Characterization and Tabulation of Retrovirus-like Particles.

**SOP/Method Ref:** ERK-ATM-00443/2

**Date(s) of Testing:** 20-May-2025 to 18-Jun-2025

| Test Parameter | Result |
|---|---|
| Detection of virus-like particles | Not Detected |
| Detection of retrovirus-like particles | Not Detected |
| Detection of other contaminations | Not Detected |

**Test Comments:** Initial glutaraldehyde fixation of the test item was performed at CRL-WAY. The results and the Report of Analysis were generated by the subcontractor ATEM SD. All documents associated with this test are archived by Charles River. The raw digital data of this test are archived at the subcontractors facility.

Although it was not the actual purpose of the TEM analysis, there was no evidence for the presence of other contaminants like bacteria, fungi or mycoplasma.



Attach to F01-QCP-028 - 44065 JS 06-26-25 CCR 06/30/25

charles river

## Report of Analysis

**Report Comments:**

There were no deviations.
A non-critical amendment (CC-V-63/25) was implemented for ERK-ATM-00443. The storage time of samples pre-fixed with glutaraldehyde was extended to 67 days and the storage temperature of pre-fixed samples was increased to ambient temperature. Based on a scientific risk assessment the change was assessed as non-critical. The system suitability criteria (SSCs) defined in ERK-ATM-00443, along with the evaluation of cell morphology and ultrastructure during data analysis, ensure that any potential negative impact resulting from the modified storage conditions is reliably detected.

The test item used in this study was fixed on 27-MAR-2025.

The suitability of the test method for its intended use was demonstrated in a generic validation according to ICH guidelines, Code V1/N01/23.

Appendices: Appendix 1 (3 pages).

**Certification:**

This is to certify that the work documented in this report was performed as stated and the results presented are accurate and truthful. The test(s) were performed in conformity with the agreed upon protocols except when clearly documented otherwise. They were executed in accordance with Charles River's Standard Operating Procedures and conform to the principles of Good Manufacturing Practice as regulated by the Principles of Good Manufacturing Practices of the European Union and United States 21 CFR Parts 210 and 211.

| | | | | |
|---|---|---|---|---|
| **Study Lead Signer Name:** | Fabian Henneberg | Approval | **Date:** | 25 Jun 2025 11:13 |
| **Quality Assurance Signer Name:** | Dr. Sarah Jahn | Approval | **Date:** | 25 Jun 2025 14:25 |

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



Attach to F01-QCP-028 – 44065 JS 06-26-25 CGR 06/30/25

## Appendix 1 Report of Analysis

charles river

**Description:** V1-U/U16/25, ERK-APC-00443[1], ACF-00443.01-C42-O.01

**Test/Method:** Ultrastructural Evaluation of Cells for the Presence of Viral Particles, with Characterization and Tabulation of Retrovirus-like Particles

**Representative images**
**1. Cells**

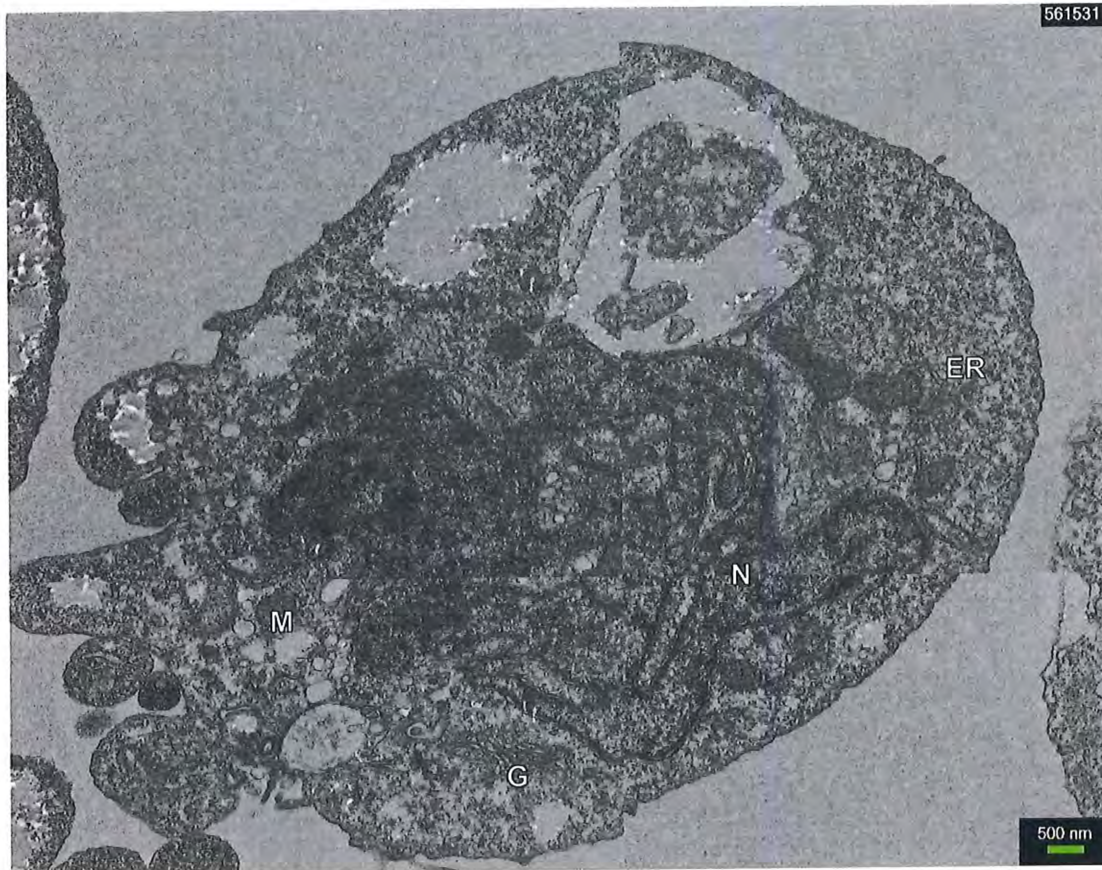



**Figure 1:** The figure shows an overview of a representative ultrathin section of a human embryonic stem cell (test item 561531). The cells in the preparation were intact and showed an intact plasma membrane. Less than estimated 10% were disrupted. The test item cells are oval-shaped. Cell organelles are embedded in an electron-dense cytoplasm. Most of the screened cell profiles contained oval or irregularly shaped nuclei (N) with dispersed or condensed chromatin along the periphery, endoplasmic reticulum (ER), and a varying number of mitochondria (M). G= Golgi Apparatus.

Original micrograph magnification: 5,300x. Image scale: bottom right. Image-ID: 561531-020



Attach to F01-QCP-028 -44065 JS 06-26-25 CQR 06/30/25

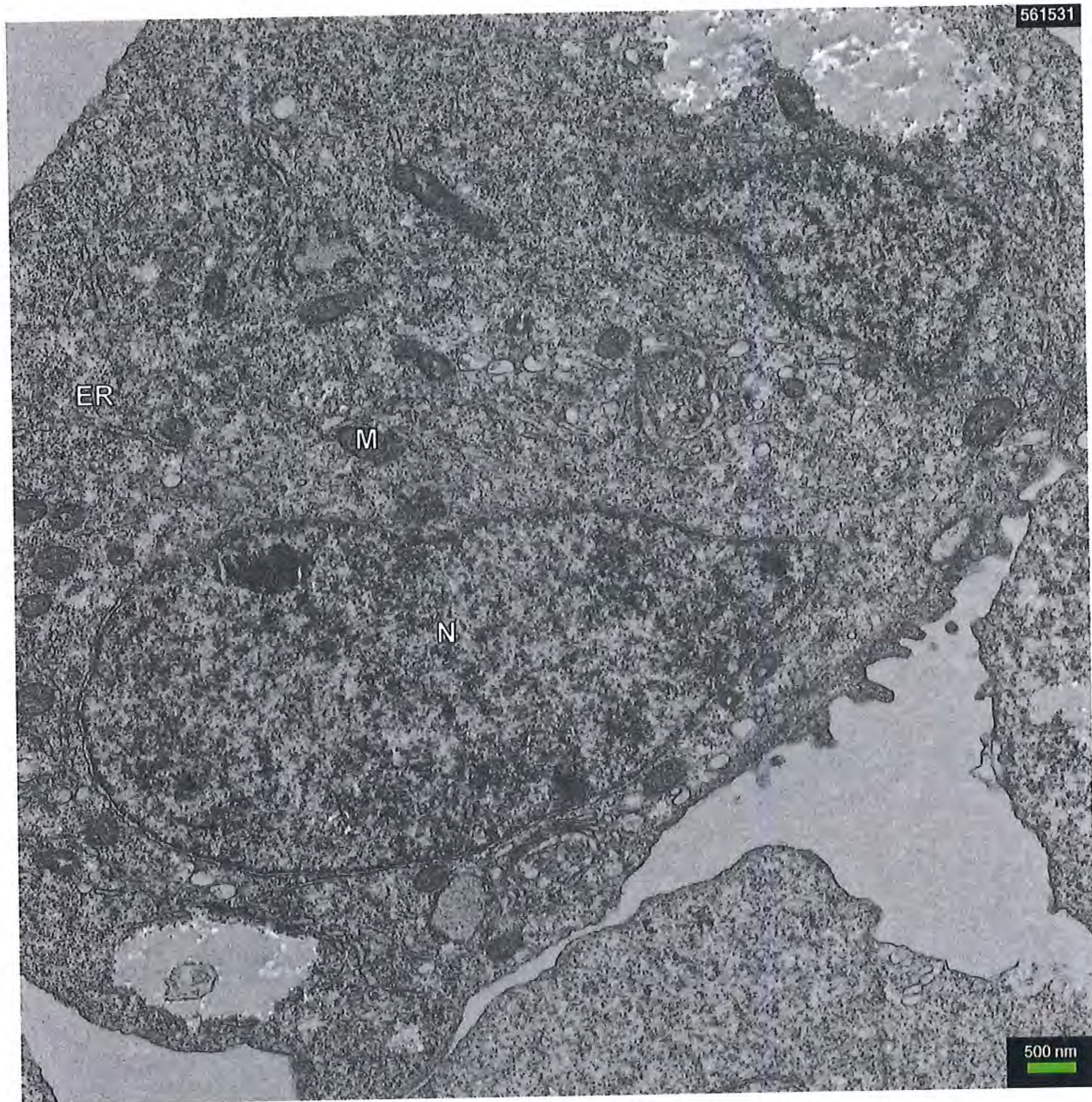



**Figure 2:** The figure shows an overview of a representative ultrathin section of a human embryonic stem cell (test item 561531). Cell organelles are embedded in an electron-dense cytoplasm. Most of the screened cell profiles contained oval or irregularly shaped nuclei (N) with dispersed or condensed chromatin along the periphery, endoplasmic reticulum (ER), and a varying number of mitochondria (M).

Original micrograph magnification: 5,300x. Image scale: Bottom right. Image-ID: 6-008-006.



Attach to F01-QCP-028 _ 44065 JS 06-26-25 (HROW 6/30/25

# Appendix 1 Report of Analysis

**Table of Results**

| | | Negative Cells | 1-5 VLPs | 6-20 VLPs | >20 VLPs | Positive Cells |
|---|---|---|---|---|---|---|
| A-Type RVLP | cytoplasmic | 200 | 0 | 0 | 0 | 0 |
| | intracisternal | 200 | 0 | 0 | 0 | 0 |
| C-Type RVLP | budding | 200 | 0 | 0 | 0 | 0 |
| | extracellular* | 200 | 0 | 0 | 0 | 0 |
| Percentage of RVLP-positive cells | | | | | | 0 % |
| Other VLPs | | 200 | 0 | 0 | 0 | 0 |

*It is not possible to relate an extracellular C-Type RVLP to a single cell, so the particle was assigned to the nearest cell.



Attach to F01-QCP-028 - 44065 JS 06-26-25 CR 06/30/25

**UWHealth**

University of Wisconsin
Hospital and Clinics

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

Date: 12/20/2009 14:30:58

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

Re: High-resolution HLA results

**Patient**

| Name / HLA / MR# / received | Dates | | HLA DNA-based typing* Method: PCR-SSP A* | B* | C* | Direct Sequencing DRB1* | DRB3* | DRB4* | DRB5* | PCR-SSP DQB1* |
|---|---|---|---|---|---|---|---|---|---|---|
| WICELL, 3237-HLA | DQB SSP | | 0201 | 0801/21/35/37 | 0401/09N/30 | 0101/22 | | | | |
| 62218 / | A,B,C SSP | 12/18/2009 | 0301 | 3501/04/34/42/93 | 0701/06/18 | 0301/42 | | | | |
| 12/18/2009 | DRB Seq | 12/18/2009 | | | | | | | | |

David F. Lorentzen, Manager
HLA/Molecular Diagnostics Laboratory

12-20-09 1430

Date

David I. Watkins, PhD, Director
HLA/Molecular Diagnostics Laboratory

1/8/10

Date

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

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

WuXi AppTec

E-FR-1-09-002629.30201

# 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 |
|---|---|
| WA01-DDL-13 #3915 | 09-002629 |

**SPONSOR:** WiCell Research Institute

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

| WUXI APPTEC ACCESSION NUMBER | RESULTS |
|---|---|
| 09-002629 | 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 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 Distributers, Annex 13), applicable ICH Q7 standards, and/or applicable Good Manufacturing Practices and in accordance with standard operating procedures and a test protocol. The Quality Assurance Unit maintains a copy of the test protocol and standard operating procedures and has inspected this study (as applicable) on the dates listed below. Each inspection was performed to assure the quality and integrity of the study.

| Phase Inspected | Date |
| --- | --- |
| BR #30201.04<br>Step 4.2.10<br>Initiate the co-cultivation by adding 5 mL of diluted test article cells ($3.9 \times 10^5$ cells) to each flask prepared for the test article. | November 25, 2009 |

28 Dec 09
Date

## GOOD LABORATORY PRACTICES STATEMENT

This 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 Distributers, Annex 13), applicable ICH Q7 standards. The study was inspected during at least one phase, and WuXi AppTec Quality Assurance audited the final report.

29 Dec 09
Date

Personnel involved in study:

## 1.0 PURPOSE

The purpose of this study was to detect replication-competent retroviruses from the Sponsor's test article cells by co-cultivation with *Mus dunni* cells for at least 14 days with 2 passages of the cultures. At the conclusion of the co-cultivation, the supernatants were tested in PG4 $S^+L^-$ 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:** November 24, 2009
**STUDY INITIATION DATE:** November 25, 2009
**STUDY COMPLETION DATE:** See page 2 for Study Director's signature and date.

## 5.0 TEST ARTICLE CHARACTERIZATION

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

## 6.0 TEST ARTICLE IDENTIFICATION: WA01-DDL-13 #3915

## 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.

## 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 (30201)

8.1.1 *Mus dunni* cells alone served as the negative control and were run in parallel with the test article for 14 days. Three (3x2.0 mL) aliquots of the conditioned medium were reserved as a time zero ($T_0$) time point for testing in the PG4 $S^+L^-$ assay.

8.1.2 Three (3x2.0 mL) aliquots of the test article supernatant were reserved as a time zero ($T_0$) time point for testing in the PG4 $S^+L^-$ assay.

8.1.3 *Mus dunni* cells ($5x10^5$ cells) and the test article cells ($3.9x10^5$ cells) were mixed to initiate the co-cultivation.

8.1.4 Positive controls were established last, using viral amphotropic murine leukemia retrovirus stocks (A-MuLV) inoculated between with 100 FFU.

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

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

8.1.7 Cell culture supernatants were collected post passage two from the negative control, test article, and positive control cultures on day 14. The supernatants were frozen and stored 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-cultivation samples were added, 0.5 mL per well, in triplicate starting first with the negatives and test article. The co-cultivation samples were inoculated at dilutions (in EMEM) to reduce toxic effects: negative ($T_0$ and PP2) at 1:2, Test article ($T_0$ and PP2) at 1:2. The A-MuLV co-cultivation test samples were added at three dilutions 1:10, 1:100 and 1:1,000.

8.2.3 The assay positive control (A-MuLV) was inoculated onto PG4 $S^+L^-$ cells, utilizing a few dilutions of the virus (1:1,000 and 1:10,000).

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

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

8.2.6 The plates were read on day 5, when the negative cultures were confluent. 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 November 24, 2009, WuXi AppTec, Inc. received 1 T25 flask of "WA01-DDI-#3915 hES cells," and immediately passed them onto the Virology depart. The Virology department removed the excess medium from the test article and passed it into additional T25 flasks, then stored all of the flasks at 37±2°C in a 5±2% $CO_2$ atmosphere. On November 25, 2009 the virology department utilized all the flasks to initiate the co-cultivation assay.

## 10.0 POSITIVE CONTROLS

10.1 Co-Cultivation Controls (30209)

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

10.2 Controls for PG4 $S^+L^-$ Assay (30165)

A known positive amphotropic murine leukemia retrovirus (AMuLV) was run along with the test samples as the positive control.

## 11.0 NEGATIVE CONTROLS

### 11.1 Co-Cultivation Controls (30201)

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

### 11.2 Controls for the PG4 $S^+L^-$ Assay (30165)

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

## 12.0 ASSAY VALIDITY

### 12.1 Validity Criteria for Co-Cultivation (30201)

The test was considered valid if supernatant samples derived from negative control co-cultivation cultures were negative for retroviral growth in the PG4 $S^+L^-$, 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 (30165)

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

## 13.0 TEST EVALUATION

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

## 14.0 RESULTS

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

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

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

| | Culture Inoculum | Time | FFU/mL |
|---|---|---|---|
| Co-Cultivation Samples | **Accession # 09-002629[1] (diluted 1:2)** | **$T_0$** | **ND** |
| | **Accession # 09-002629 (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 | $2.8 \times 10^5$ |

**Legend:**

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

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

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

## 15.0 CONCLUSION

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

## 16.0 STATISTICAL DATA ANALYSIS

Statistical analysis of the data was not required.

## 17.0 DEVIATIONS / AMENDMENTS

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

No amendments to the protocol were generated.

## 18.0 RECORD RETENTION

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

## 19.0 REFERENCES

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

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

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

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

# Final Report

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

**Study Number:** AC34CA.105010.BSV

**Test Article ID:** WA01-DDL-13 #3915

**Sponsor:** WiCell Research Institute

**Authorized Representative:**

### CONCLUSION

**One-half (0.5) µg of DNA (representing approximately 7.5 x $10^4$ cells) isolated from the test article was analyzed for the presence of human immunodeficiency virus types 1 and 2 (HIV-1/2) proviral DNA by the polymerase chain reaction (PCR) 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.**

## STUDY INFORMATION

**Test Article:** The test article was received by BioReliance on 11/24/2009. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor. Retention of reserve sample from each batch of test article is the responsibility of the sponsor.

**Testing Facility:** BioReliance

**Schedule:**

- **Study Initiation:** 11/30/2009
- **Lab Initiation:** 12/01/2009
- **Lab Completion:** 12/03/2009
- **Study Completion:** See Study Director's signature date in "Approval" Section.

**Study Director:**

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

BioReliance

## OBJECTIVE

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

## TEST SYSTEM

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

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

**Positive Control:**

HIV-1: Genomic DNA from MRC5 spiked with 100 copies of pCRII+HIV-1, a plasmid containing the complete genome of HIVZ6 with an interruption in the protease coding region
Source: BioReliance

HIV-2: Genomic DNA from MRC5 spiked with 100 copies of pMAHIV2, a plasmid containing a 963 bp fragment from the HIV-2 proviral genome
Source: BioReliance

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

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

HIV-1: Test article extract spiked with 100 copies of pCRII+HIV-1

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

## METHODS

### Sample Preparation

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

### DNA Amplification

Each PCR amplification was performed on 0.5 μg of test article DNA and on the assay controls, using either primers SK38 and SK39, specific for the core protein coding region of HIV-1, or primers OG63 and OG81, specific for the core protein coding region of HIV-2, employing conditions optimized to achieve detection of 100 copies of proviral DNA. Aliquots of the amplification products obtained from the test article and from the control samples were analyzed by agarose gel electrophoresis in the presence of ethidium bromide. Amplification products were visualized on a UV transilluminator and photographed using the Kodak Gel Logic 100 Imaging System (SOP OPBT0943).

## RESULTS

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

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

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

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

## DEVIATIONS

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

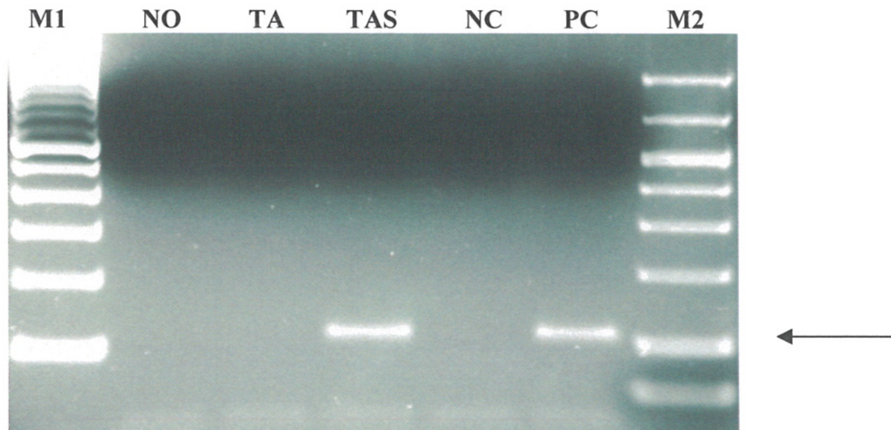
## APPROVAL

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

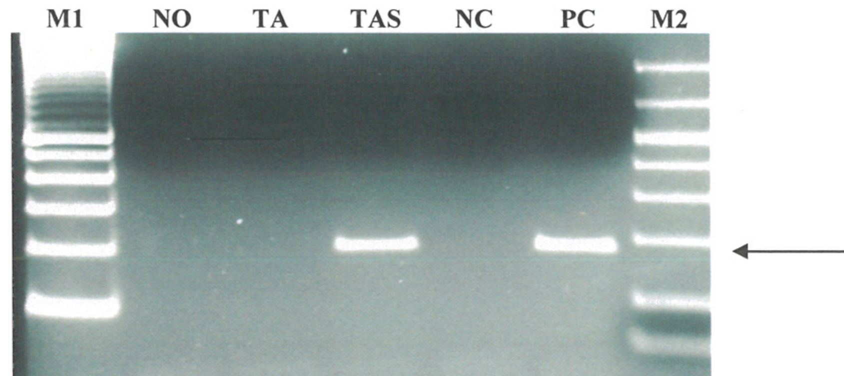
21 Dec 09
Date

## FIGURE 1

**a. HIV-1**




**b. HIV-2**




Detection of HIV-1 (**a.**) or HIV-2 (**b.**) proviral sequences in the test article utilizing agarose gel electrophoresis in the presence of ethidium bromide

**M1:** 100 bp DNA ladder
**NO:** No DNA control
**TA:** Test Article
**TAS:** Test article spiked with 100 copies of (**a.**) pCRII+HIV-1 or (**b.**) pMAHIV2
**NC:** Negative control (MRC5 genomic DNA)
**PC:** Positive control (MRC5 genomic DNA spiked with 100 copies of (**a.**) pCRII+HIV-1 or (**b.**) pMAHIV2)
**M2:** Biomarker low DNA size marker

Arrows indicate specific amplification products.

BioReliance

# Quality Assurance Statement

## Study Information

**Number:** AC34CA.105010.BSV

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

## Compliance

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

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

## Inspections

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

| Insp. Dates (From/To) | | Phase Inspected | To Study Director | To Management | |
|---|---|---|---|---|---|
| 21-Dec-2009 | 21-Dec-2009 | Data and Final Reporting | 21-Dec-2009 | 21-Dec-2009 | |
| 30-Sep-2009 | 30-Sep-2009 | Admin. Of Test Substance | 30-Sep-2009 | 30-Sep-2009 | * |
| 30-Sep-2009 | 30-Sep-2009 | Manipulation of Test System | 30-Sep-2009 | 30-Sep-2009 | * |
| 30-Sep-2009 | 30-Sep-2009 | Observation of Test System | 30-Sep-2009 | 30-Sep-2009 | * |
| 30-Sep-2009 | 30-Sep-2009 | Test System Preparation | 30-Sep-2009 | 30-Sep-2009 | * |

* Process-based Inspection

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

## E-signature

**Quality Assurance:** 21-Dec-2009 9:59 pm GMT

Reason for signature: QA Approval

**Printed by:**

**Printed on:21-Dec-09**

# Final Report

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

**Study Number:** AC34CA.105013.BSV

**Test Article ID:** WA01-DDL-13 #3915

**Sponsor:** WiCell Research Institute

**Authorized Representative:**

### CONCLUSION

**One-half (0.5) μg of DNA (representing approximately 7.5 x $10^4$ cells) isolated from the test article was analyzed for the presence of human T-cell lymphotropic virus types I and II (HTLV-I/II) proviral DNA by the polymerase chain reaction (PCR) 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.**

BioReliance®

## STUDY INFORMATION

**Test Article:** The test article was received by BioReliance on 11/24/2009. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor. Retention of reserve sample from each batch of test article is the responsibility of the sponsor.

**Testing Facility:** BioReliance

**Schedule:**

| | |
|---|---|
| **Study Initiation:** | 11/30/2009 |
| **Lab Initiation:** | 12/01/2009 |
| **Lab Completion:** | 12/08/2009 |
| **Study Completion:** | See Study Director's signature date in "Approval" Section. |

**Study Director:**

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

BioReliance

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

## METHODS

### Sample Preparation

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

**DNA Amplification**

Each PCR amplification was performed on 0.5 µg of test article DNA and on the assay controls, using primers HT-OS and HT-OA, specific for the tax/rex region of HTLV-I/II, employing conditions optimized to achieve detection of 100 copies of proviral DNA. Aliquots of the amplification products obtained from the test article and from the control samples were analyzed by agarose gel electrophoresis in the presence of ethidium bromide. Amplification products were visualized on a UV transilluminator and photographed using the Kodak Gel Logic 100 Imaging System (SOP OPBT0943).

## RESULTS

Test article DNA (0.5 µg), representing approximately $7.5 \times 10^4$ test article cells, was analyzed for the presence of HTLV-I/II proviral DNA by PCR amplification and agarose gel electrophoresis in the presence of ethidium bromide. The results are shown in Figure 1. The No DNA control (NO) and Negative control (NC) showed no bands at 158 bp. The positive controls (PC-1 and PC-2) produced a 158 bp band. The test article spiked with 100 copies of either pH750 (TAS-1) or pMAHTII (TAS-2) produced a 158 bp band, demonstrating that the test article did not inhibit the PCR reaction. The test article (TA) produced no bands at 158 bp.

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

## DEVIATIONS

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

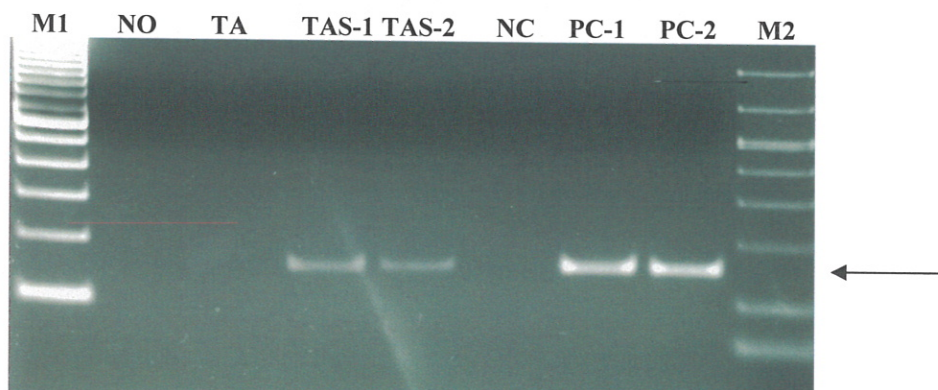
## APPROVAL

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

22 Dec 09

Date

**FIGURE 1**




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

| | |
|---|---|
| **M1:** | 100 bp DNA ladder |
| **NO:** | No DNA control |
| **TA:** | Test Article |
| **TAS-1:** | Test article spiked with 100 copies of pH750 |
| **TAS-2:** | Test article spiked with 100 copies of pMAHTII |
| **NC:** | Negative control (MRC5 genomic DNA) |
| **PC-1:** | Positive control for HTLV-I (Genomic DNA from MRC5 spiked with 100 copies pH750) |
| **PC-2:** | Positive control for HTLV-II (Genomic DNA from MRC5 spiked with 100 copies pMAHTII) |
| **M2:** | Biomarker low DNA size marker |

The arrow indicates specific amplification products.

BioReliance

# Quality Assurance Statement

## Study Information

**Number:** AC34CA.105013.BSV

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

## Compliance

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

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

## Inspections

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

| Insp. Dates (From/To) | | Phase Inspected | To Study Director | To Management |
|---|---|---|---|---|
| 18-Dec-2009 | 18-Dec-2009 | Data and Final Reporting | 22-Dec-2009 | 22-Dec-2009 |
| 30-Sep-2009 | 30-Sep-2009 | Admin. Of Test Substance | 30-Sep-2009 | 30-Sep-2009 * |
| 30-Sep-2009 | 30-Sep-2009 | Manipulation of Test System | 30-Sep-2009 | 30-Sep-2009 * |
| 30-Sep-2009 | 30-Sep-2009 | Observation of Test System | 30-Sep-2009 | 30-Sep-2009 * |
| 30-Sep-2009 | 30-Sep-2009 | Test System Preparation | 30-Sep-2009 | 30-Sep-2009 * |

* Process-based Inspection

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

## E-signature

**Quality Assurance:** 22-Dec-2009 2:42 pm GMT

Reason for signature: QA Approval

**Printed by:**

**Printed on:22-Dec-09**

# Final Report

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

**Study Number:** AC34CA.105042.BSV

**Test Article ID:** WA01-DDL-13 #3915

**Sponsor:** WiCell Research Institute

**Authorized Representative:**

### CONCLUSION

**One-half (0.5) µg of DNA isolated from the test article (representing approximately 7.5 x $10^4$ cells) was analyzed for the presence of Hepatitis B virus (HBV) DNA by the polymerase chain reaction (PCR) 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.**

BioReliance®

## STUDY INFORMATION

**Test Article:** The test article was received by BioReliance on 11/24/2009. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor. Retention of reserve sample from each batch of test article is the responsibility of the sponsor.

**Testing Facility:** BioReliance

**Schedule:**

- **Study Initiation:** 11/30/2009
- **Lab Initiation:** 12/01/2009
- **Lab Completion:** 12/08/2009
- **Study Completion:** See Study Director's signature date in "Approval" Section.

**Study Director:**

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

BioReliance

## OBJECTIVE

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

## TEST SYSTEM

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

**Negative Control:** Genomic DNA from MRC5 human fetal lung fibroblast line
Source: BioReliance

**Positive Control:** Genomic DNA from MRC5 spiked with 100 copies of HBV185, a plasmid containing a 1850 bp fragment from the HBV core antigen sequence
Source: BioReliance

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

**Spiked Control:** Test article extract spiked with 100 copies of HBV185, to verify the absence of PCR inhibitors in the test article DNA (amplification suitability control)

## METHODS

### Sample Preparation

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

### DNA Amplification

PCR amplification was performed on 0.5 μg 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 the Kodak Gel Logic 100 Imaging System (SOP OPBT0943).

## RESULTS

Test article DNA (0.5 µg), representing approximately $7.5 \times 10^4$ test article cells, was analyzed for the presence of HBV DNA by PCR amplification and agarose gel electrophoresis in the presence of ethidium bromide. The results are shown in Figure 1. The No DNA control (NO) and Negative control (NC) showed no bands at 347 bp. The positive control (PC) produced a 347 bp band. The test article spiked with 100 copies of HBV185 (TAS) produced a 347 bp band, demonstrating that the test article did not inhibit the PCR reaction. The test article (TA) produced no bands at 347 bp.

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

## DEVIATIONS

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

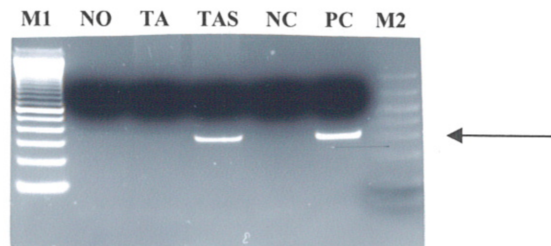
## APPROVAL

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

18Dec09

Date

**FIGURE 1**




Detection of HBV specific sequences in the test article utilizing agarose gel electrophoresis in the presence of ethidium bromide

**M1:** 100 bp DNA ladder
**NO:** No DNA control
**TA:** Test Article
**TAS:** Test article spiked with 100 copies HBV185
**NC:** Negative control (Genomic DNA from MRC5)
**PC:** Positive control (Genomic DNA from MRC5 spiked with 100 copies HBV185)
**M2:** Biomarker low DNA size marker

Arrow indicates the specific amplification product.

BioReliance®

# Quality Assurance Statement

## Study Information

**Number:** AC34CA.105042.BSV

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

## Compliance

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

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

## Inspections

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

| Insp. Dates (From/To) | | Phase Inspected | To Study Director | To Management | |
|---|---|---|---|---|---|
| 18-Dec-2009 | 18-Dec-2009 | Data and Final Reporting | 18-Dec-2009 | 18-Dec-2009 | |
| 30-Sep-2009 | 30-Sep-2009 | Admin. Of Test Substance | 30-Sep-2009 | 30-Sep-2009 | * |
| 30-Sep-2009 | 30-Sep-2009 | Manipulation of Test System | 30-Sep-2009 | 30-Sep-2009 | * |
| 30-Sep-2009 | 30-Sep-2009 | Observation of Test System | 30-Sep-2009 | 30-Sep-2009 | * |
| 30-Sep-2009 | 30-Sep-2009 | Test System Preparation | 30-Sep-2009 | 30-Sep-2009 | * |

* Process-based Inspection

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

## E-signature

**Quality Assurance:** 18-Dec-2009 7:18 pm GMT

Reason for signature: QA Approval

**Printed by:**

**Printed on:18-Dec-09**

# Final Report

## REAL TIME POLYMERASE CHAIN REACTION ASSAY FOR THE DETECTION OF *HEPATITIS C VIRUS* (HCV) IN BIOLOGICAL SAMPLES

**Study Number:** AC34CA.107207.BUK

**Test Article Designation:** WA01-DDL-13 #3915

**Sponsor:** WiCell Research Institute

**Authorised Representative:**

**CONCLUSION**

**Test article AC34CA was considered to be negative for the detection of HCV specific sequences to a sensitivity of $10^2$ copies/reaction.**

BioReliance

## STUDY INFORMATION

**Test Article:** The test article was received in BioReliance (Rockville) on 02 February 2010 and assigned site identifier AC34CA. Samples were submitted to BioReliance (Glasgow) for testing.

On 04 February 2010, two 2 ml aliquots of test article were shipped from Rockville and received at Glasgow on 08 February 2010 for testing.

Determination of the purity, strength, composition and stability of the test article is the responsibility of the Sponsor. The retention of a reserve sample from each batch of test article is the responsibility of the Sponsor.

**Testing Facility:** BioReliance

**Study Plan:** Protocol # 02.107207.R00 and associated Study Specific Protocol Supplement (SSPS).

**Test Schedule:**

**Study Initiation:** 09 February 2010

**Experimental Start:** 10 February 2010

**Experimental Completion:** 10 February 2010

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

**QA Activities:** See appended Quality Assurance Statement.

**Study Director:**

**Report Author:**

**Archives:** All raw data, records, the protocol and a copy of the Final Report are maintained for 3 years in archive facilities approved by:

BioReliance

## TEST SYSTEM

The test system used in this study employs real time polymerase chain reaction (PCR) technology and TaqMan® chemistry to detect the presence of viral contaminants within a biological sample. Real time PCR detection utilises the 5' exonuclease activity of *Taq* polymerase to hydrolyse an internal TaqMan® probe labelled with a 5' fluorescent reporter dye and a 3' quencher molecule. As amplification of the target molecule proceeds, the reporter dye is released from the 5' end of the probe and an increase in fluorescence proportional to the increase in PCR product is observed. The ABI 7900HT Sequence Detection System (SDS) software processes the raw fluorescence data to produce threshold cycle ($C_T$) values for each sample.

### Positive control(s)

The positive controls consisted of triplicate reactions containing negative control nucleic acid spiked with a known amount of appropriate positive control material containing the relevant viral contaminant target sequence. The positive control was used at concentrations equivalent to and above the pre-determined detection limit (DL).

**Table 1: Assay target positive controls**

| Target | Equivalent to DL | Above DL |
|---|---|---|
| HCV | $10^2$ copies | $10^3$ copies |

### Post-extraction spike control(s)

The test article post-extraction spike control consisted of triplicate reactions containing test article nucleic acid spiked with a known amount of appropriate positive control material containing the relevant viral contaminant target sequence. The test article post-extraction spike controls were used at concentrations equivalent to and above the pre-determined DL, and were included during testing to further assess PCR inhibition.

**Internal positive control(s)**

The internal positive controls consisted of negative control nucleic acid spiked with internal control nucleic acid target sequences at concentrations equivalent to and above the pre-determined detection limit.

**Test article internal control(s)**

The test article internal control consisted of PCR reaction mix to which an appropriate volume of test article nucleic acid (spiked prior to extraction with internal control nucleic acid) was added. An internal extraction recovery control containing the equivalent amount of control nucleic acid (unrelated to the target) in the absence of test material was also prepared and an appropriate volume added to the internal control PCR reaction mix.

**Exogenous internal positive control(s)**

TaqMan® exogenous internal positive control (IPC) reagents were included in all PCR master mixes. The IPC reagents were included in the target reaction mix to establish that all negative PCR results were truly negative and not due to failed amplification.

**No template control(s)**

Triplicate no template control (NTC) reactions were prepared and consisted of the appropriate PCR reaction mix only. NTC reactions were included to assess possible reagent or aerosol contamination prior to manipulation of the test article.

**Negative control(s)**

Triplicate assay negative controls were prepared and consisted of the appropriate PCR reaction mix to which negative control nucleic acid was added. The negative control reactions were closed after preparation to assess possible reagent contamination.

**Sentinel extraction control(s)**

Triplicate sentinel extraction control reactions were prepared and consisted of the appropriate PCR reaction mix to which sentinel extraction material was added. Sentinel extraction control reactions were included alongside the test article during extraction to assess possible airborne sample to sample cross-contamination.

**Specificity control(s)**

Since the internal control RNA was added directly to the test sample, a PCR specificity control was performed to assess the possibility of test article contamination by the internal control RNA. The specificity control consisted of target PCR reaction mix to which $10^3$ copies/µl of internal control RNA was added.

## OBJECTIVE

The purpose of this study was to detect nucleic acid sequences specific to HCV in the test article.

## METHOD

**Table 2: Sample Preparation**

| | |
|---|---|
| **Volume of sample extracted** | 280 µl (total elution volume = 240 µl) |
| **Extraction method used** | QIAamp®viral RNA mini kit |
| **Amount tested (per reaction)** | 3 µl |

The test article was extracted in a clean air cabinet where no known HCV positive materials had been handled. The PCR reactions were prepared in a buffered system with the necessary reagents for amplification. Test article samples were analysed in triplicate in conjunction with NTC, negative, sentinel extraction, specificity, internal and positive controls. A one-way system was in place to prevent contamination of the PCR reactions.

## RESULTS SUMMARY

Test article AC34CA was considered to be negative for the detection of HCV specific sequences to a sensitivity of $10^2$ copies/reaction.

## VALIDITY

The HCV test was valid.

No amplification signals were detected in the NTC, negative, sentinel extraction or specificity controls. Amplification signals were detected in the positive controls within the expected range.

The exogenous IPC results were positive in all negative viral target reactions.

The *Cowpea mosaic virus* (CPMV) internal control tests were valid.

The CPMV RNA internal control amplification was positive, demonstrating that the test article was suitable for PCR.

No amplification signals were detected in the CPMV NTC, negative or sentinel extraction controls. Amplification signals were detected in the positive internal standard controls within the expected range.

The exogenous IPC results were positive in all negative internal control reactions.

## EVENTS

No study specific deviations or other Event Records were reported.

## APPROVAL

I accept responsibility for the conduct of this study which was performed in accordance with the OECD Principles of Good Laboratory Practice as incorporated into, and in compliance with the United Kingdom Department of Health GLP regulations (The Good Laboratory Practice Regulations 1999 (Statutory Instrument 1999 No 3106) as amended) and as accepted by the Regulatory Authorities throughout the European Community, United States of America (FDA) and Japan (MHLW, MAFF and METI).

18 Feb 10

Date

**Table 3: Results**

| Sample | HCV |
|---|---|
| PCR NTC | - |
| PCR Negative control | - |
| Sentinel extraction control | - |
| Specificity control | - |
| Test article AC34CA | - |
| Test article post-spike (above DL) | +++ |
| Test article post-spike (DL) | +++ |
| Positive standard (above DL) | +++ |
| Positive standard (DL) | +++ |

**Abbreviations:**

- denotes a negative result ($C_T$ = 40.00 or 'Undetermined', i.e. no amplification following 40 cycles of PCR detected in all three replicates).

+++ denotes a positive result ($C_T$ value of ≤ 39.99 detected in all three replicates).

BioReliance

# Quality Assurance Statement

## Study Information

**Number:** AC34CA.107207.BUK

**Protocol Title:** REAL TIME POLYMERASE CHAIN REACTION ASSAY FOR THE DETECTION OF HEPATITIS C VIRUS (HCV) IN BIOLOGICAL SAMPLES

## Compliance

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

UK GLP Regulations
OECD Principles of Good Laboratory Practice

## Inspections

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

| Insp. Dates (From/To) | | Phase Inspected | To Study Director | To Management |
|---|---|---|---|---|
| 10-Feb-2010 | 10-Feb-2010 | Study Plan Review | 10-Feb-2010 | 10-Feb-2010 |
| 16-Feb-2010 | 16-Feb-2010 | Data and Final Reporting | 16-Feb-2010 | 16-Feb-2010 |
| 08-Feb-2010 | 10-Feb-2010 | Admin. Of Test Substance | 10-Feb-2010 | 10-Feb-2010 * |
| 10-Feb-2010 | 10-Feb-2010 | Manipulation of Test System | 10-Feb-2010 | 10-Feb-2010 * |
| 19-Nov-2009 | 23-Nov-2009 | Observation of Test System | 23-Nov-2009 | 23-Nov-2009 * |
| 08-Feb-2010 | 10-Feb-2010 | Test System Preparation | 10-Feb-2010 | 10-Feb-2010 * |

* Process-based Inspection

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

## E-signature

**Quality Assurance:** 18-Feb-2010 3:50 pm GMT

Reason for signature: QA Approval

**Printed by:**

**Printed on:18-Feb-10**

# Final Report

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

**Study Number:** AC34CA.105012.BSV

**Test Article ID:** WA01-DDL-13 # 3915

**Sponsor:** WiCell Research Institute

**Authorized Representative:**

### CONCLUSION

**One-half (0.5) µg of DNA isolated from the test article (representing approximately 7.5 x $10^4$ cells) was analyzed for the presence of human cytomegalovirus (CMV) DNA by the polymerase chain reaction (PCR) 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.**

BioRelliance®

## STUDY INFORMATION

**Test Article:** The test article was received by BioReliance on 11/24/2009. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor. Retention of reserve sample from each batch of test article is the responsibility of the sponsor.

**Testing Facility:** BioReliance

**Schedule:**

**Study Initiation:** 11/30/2009

**Lab Initiation:** 12/01/2009

**Lab Completion:** 12/08/2009

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

**Study Director:**

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

BioReliance

## OBJECTIVE

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

## METHODS

### Sample Preparation

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

### DNA Amplification

PCR amplification was performed on 0.5 µg of test article DNA using primers CMV100 and CMV150 specific for the polymerase region of CMV, employing conditions optimized to achieve detection of 100 copies of CMV. Aliquots of the amplification products obtained from the test article and from the control samples were analyzed by agarose gel electrophoresis in the presence of ethidium bromide. Amplification products were visualized on a UV transilluminator and photographed using the Kodak Gel Logic 100 Imaging System (SOP OPBT0943).

## RESULTS

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

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

## DEVIATIONS

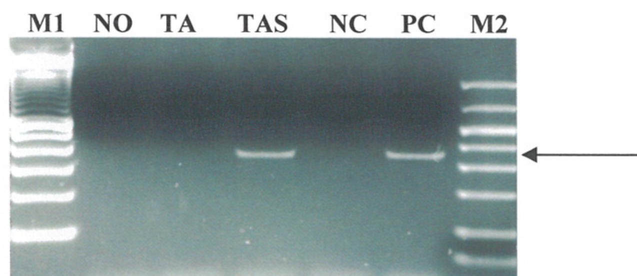
No known procedural deviations from the study protocol or pertinent assay SOPs occurred during the conduct of the study.

## APPROVAL

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

18 Dec 09
Date

**FIGURE 1**




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

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

Arrow indicates the specific amplification product.

BioReliance®

# Quality Assurance Statement

## Study Information

**Number:** AC34CA.105012.BSV

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

## Compliance

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

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

## Inspections

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

| Insp. Dates (From/To) | | Phase Inspected | To Study Director | To Management | |
|---|---|---|---|---|---|
| 18-Dec-2009 | 18-Dec-2009 | Data and Final Reporting | 18-Dec-2009 | 18-Dec-2009 | |
| 30-Sep-2009 | 30-Sep-2009 | Admin. Of Test Substance | 30-Sep-2009 | 30-Sep-2009 | * |
| 30-Sep-2009 | 30-Sep-2009 | Manipulation of Test System | 30-Sep-2009 | 30-Sep-2009 | * |
| 30-Sep-2009 | 30-Sep-2009 | Observation of Test System | 30-Sep-2009 | 30-Sep-2009 | * |
| 30-Sep-2009 | 30-Sep-2009 | Test System Preparation | 30-Sep-2009 | 30-Sep-2009 | * |

* Process-based Inspection

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

## E-signature

**Quality Assurance:** 18-Dec-2009 7:56 pm GMT

Reason for signature: QA Approval

**Printed by:**

**Printed on:18-Dec-09**

# Final Report

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

**Study Number:** AC34CA.105011.BSV

**Test Article ID:** WA01-DDL-13 #3915

**Sponsor:** WiCell Research Institute

**Authorized Representative:**

**CONCLUSION**

**One-half (0.5) μg of DNA isolated from the test article (representing approximately 7.5 x $10^4$ cells) was analyzed for the presence of Epstein Barr virus (EBV) DNA by the polymerase chain reaction (PCR) technique. The assay can detect 10 copies of EBV genome in the presence of 0.5 μg of genomic DNA.**

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

## STUDY INFORMATION

**Test Article:** The test article was received by BioReliance on 11/24/2009. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor. Retention of reserve sample from each batch of test article is the responsibility of the sponsor.

**Testing Facility:** BioReliance

**Schedule:**

**Study Initiation:** 11/30/2009

**Lab Initiation:** 12/01/2009

**Lab Completion:** 12/04/2009

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

**Study Director:**

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

BioReliance

## OBJECTIVE

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

## TEST SYSTEM

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

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

## METHODS

### Sample Preparation

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

### DNA Amplification

PCR amplification was performed on 0.5 µg of test article DNA using primers TC58 and TC61 specific for the BamW region of the EBV genome, employing conditions optimized to achieve detection of 10 copies of EBV genome. Aliquots of the amplification products obtained from the test article and from the control samples were analyzed by agarose gel electrophoresis in the presence of ethidium bromide. Amplification products were visualized on a UV transilluminator and photographed using the Kodak Gel Logic 100 Imaging System (SOP OPBT0943).

## RESULTS

Test article DNA (0.5 µg), representing approximately 7.5 x $10^4$ test article cells, was analyzed for the presence of EBV DNA by PCR amplification and agarose gel electrophoresis in the presence of ethidium bromide. The results are shown in Figure 1. The No DNA control (NO) and Negative control (NC) showed no bands at 376 bp. The positive control (PC) produced a 376 bp band. The test article spiked with 100 copies of BamW (TAS) produced a 376 bp band, demonstrating that the test article did not inhibit the PCR reaction. The test article (TA) produced no bands at 376 bp.

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

## DEVIATIONS

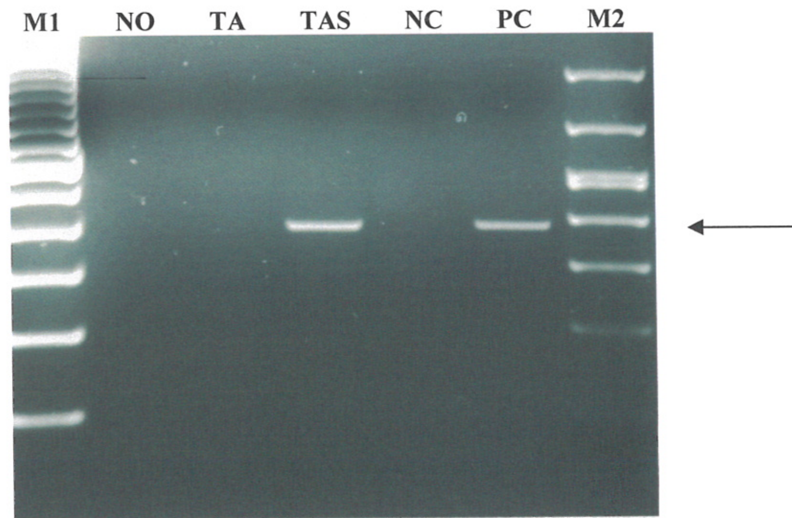
No known procedural deviations from the study protocol or pertinent assay SOPs occurred during the conduct of the study.

## APPROVAL

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

21 Dec 09
Date

FIGURE 1




Detection of EBV specific sequences in the test article utilizing agarose gel electrophoresis in the presence of ethidium bromide

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

Arrow indicates specific amplification product.

BioReliance®

# Quality Assurance Statement

## Study Information

**Number:** AC34CA.105011.BSV

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

## Compliance

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

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

## Inspections

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

| Insp. Dates (From/To) | | Phase Inspected | To Study Director | To Management | |
|---|---|---|---|---|---|
| 21-Dec-2009 | 21-Dec-2009 | Data and Final Reporting | 21-Dec-2009 | 21-Dec-2009 | |
| 30-Sep-2009 | 30-Sep-2009 | Admin. Of Test Substance | 30-Sep-2009 | 30-Sep-2009 | * |
| 30-Sep-2009 | 30-Sep-2009 | Manipulation of Test System | 30-Sep-2009 | 30-Sep-2009 | * |
| 30-Sep-2009 | 30-Sep-2009 | Observation of Test System | 30-Sep-2009 | 30-Sep-2009 | * |
| 30-Sep-2009 | 30-Sep-2009 | Test System Preparation | 30-Sep-2009 | 30-Sep-2009 | * |

* Process-based Inspection

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

## E-signature

**Quality Assurance:** 21-Dec-2009 10:06 pm GMT

Reason for signature: QA Approval

**Printed by:**

**Printed on:21-Dec-09**

# Final Report

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

**Study Number:** AC34CA.105020.BSV

**Test Article ID:** WA01-DDL-13 # 3915

**Sponsor:** WiCell Research Institute

**Authorized Representative:**

### CONCLUSION

**One-half (0.5) µg of DNA (representing approximately 7.5 x $10^4$ cells) isolated from the test article was analyzed for the presence of human herpesvirus 6 (HHV-6) viral DNA by the polymerase chain reaction (PCR) 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.**

BioReliance®

## STUDY INFORMATION

**Test Article:** The test article was received by BioReliance on 11/24/2009. Determination of the stability, purity, and concentration of the test article is the responsibility of the sponsor. Retention of reserve sample from each batch of test article is the responsibility of the sponsor.

**Testing Facility:** BioReliance

**Schedule:**

**Study Initiation:** 11/30/2009

**Lab Initiation:** 12/01/2009

**Lab Completion:** 12/09/2009

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

**Study Director:**

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

BioReliance

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

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

## METHODS

### Sample Preparation

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

### DNA Amplification

PCR amplification was performed on 0.5 µg 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 the Kodak Gel Logic 100 Imaging System (SOP OPBT0943).

## RESULTS

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

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

The test article spiked with 100 copies of pU1102MOD (TAS-1) produced a 299 bp band and the test article spiked with 100 copies of pZ29MOD (TAS-2) produced a 524 bp band, demonstrating that the test article did not inhibit the PCR reaction. The test article (TA) produced no bands at 553 or 328 bp.

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

## DEVIATIONS

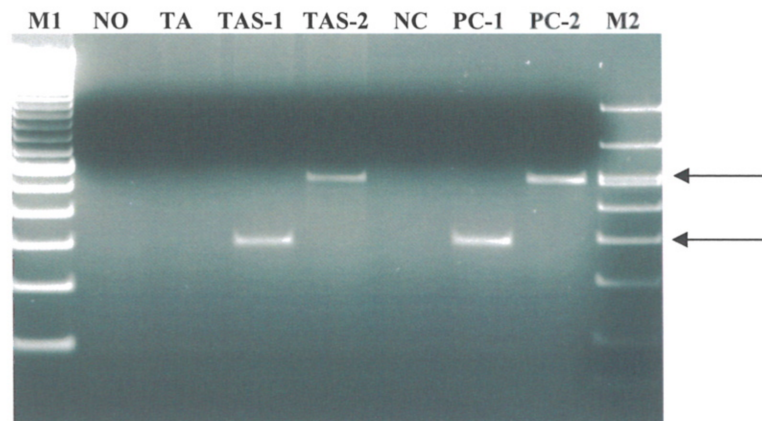
No known procedural deviations from the study protocol or pertinent assay SOPs occurred during the conduct of the study.

**APPROVAL**

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

18 Dec 09
Date

## FIGURE 1




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

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

Arrows indicate specific amplification products.

BioReliance

# Quality Assurance Statement

## Study Information

**Number:** AC34CA.105020.BSV

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

## Compliance

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

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

## Inspections

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

| Insp. Dates (From/To) | | Phase Inspected | To Study Director | To Management |
|---|---|---|---|---|
| 18-Dec-2009 | 18-Dec-2009 | Data and Final Reporting | 18-Dec-2009 | 18-Dec-2009 |
| 30-Sep-2009 | 30-Sep-2009 | Admin. Of Test Substance | 30-Sep-2009 | 30-Sep-2009 * |
| 30-Sep-2009 | 30-Sep-2009 | Manipulation of Test System | 30-Sep-2009 | 30-Sep-2009 * |
| 30-Sep-2009 | 30-Sep-2009 | Observation of Test System | 30-Sep-2009 | 30-Sep-2009 * |
| 30-Sep-2009 | 30-Sep-2009 | Test System Preparation | 30-Sep-2009 | 30-Sep-2009 * |

* Process-based Inspection

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

## E-signature

**Quality Assurance:** 18-Dec-2009 7:09 pm GMT

Reason for signature: QA Approval

**Printed by**

**Printed on:18-Dec-09**

# Final Report

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

**Study Number:** AC34CA.105029.BSV

**Test Article ID:** WA01-DDL-13 # 3915

**Sponsor:** WiCell Research Institute

**Authorized Representative:**

### CONCLUSION

**One-half (0.5) μg of DNA isolated from the test article (representing approximately 7.5 x $10^4$ cells) was analyzed for the presence of Human Herpesvirus 7 (HHV-7) DNA by the polymerase chain reaction (PCR) 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.**

## STUDY INFORMATION

**Test Article:** The test article was received by BioReliance on 11/24/2009. Determination of the stability, purity, and concentration of the test article is the responsibility of the sponsor. Retention of reserve sample from each batch of test article is the responsibility of the sponsor.

**Testing Facility:** BioReliance

**Schedule:**

**Study Initiation:** 11/30/2009

**Lab Initiation:** 12/01/2009

**Lab Completion:** 12/08/2009

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

**Study Director:**

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

BioReliance

## OBJECTIVE

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

## TEST SYSTEM

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

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

**Positive Control:** Genomic DNA from MRC5 spiked with 100 copies of pHH7, a plasmid containing a 1.2 Kb fragment of the HHV-7 genome
Source: BioReliance

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

**Spiked Control:** Test article extract spiked with 100 copies of pHH7, to verify the absence of PCR inhibitors in the test article DNA (amplification suitability control)

## METHODS

### Sample Preparation

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

### DNA Amplification

PCR amplification was performed on 0.5 μg of test article DNA using primers HHV7F and HHV7IR specific for sequences common to the capsid protein gene regions in the HHV-7 genome, employing conditions optimized to achieve detection of 100 copies of HHV-7 DNA. Aliquots of the amplification products obtained from the test article and from the control samples were analyzed by high resolution agarose gel electrophoresis in the presence of ethidium bromide. Amplification products were visualized on a UV transilluminator and photographed using the Kodak Gel Logic 100 Imaging System (SOP OPBT0943).

## RESULTS

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

These results provide evidence that the test article tested negative for the presence of HHV-7 DNA.

## DEVIATIONS

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

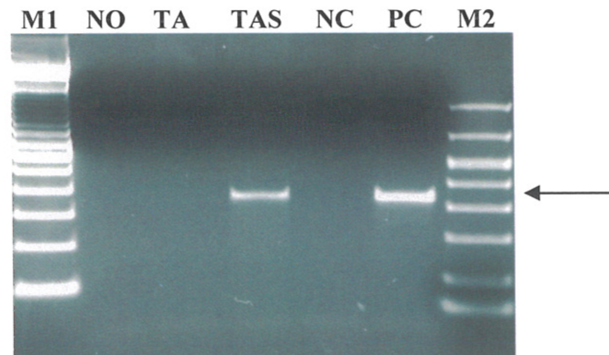
## APPROVAL

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

18 Dec 09

Date

**FIGURE 1**




Detection of HHV-7 specific sequences in the test article utilizing agarose gel electrophoresis in the presence of ethidium bromide

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

Arrow indicates the amplification product.

BioReliance

# Quality Assurance Statement

## Study Information

**Number:** AC34CA.105029.BSV

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

## Compliance

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

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

## Inspections

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

| Insp. Dates (From/To) | | Phase Inspected | To Study Director | To Management | |
|---|---|---|---|---|---|
| 18-Dec-2009 | 18-Dec-2009 | Data and Final Reporting | 18-Dec-2009 | 18-Dec-2009 | |
| 30-Sep-2009 | 30-Sep-2009 | Admin. Of Test Substance | 30-Sep-2009 | 30-Sep-2009 | * |
| 30-Sep-2009 | 30-Sep-2009 | Manipulation of Test System | 30-Sep-2009 | 30-Sep-2009 | * |
| 30-Sep-2009 | 30-Sep-2009 | Observation of Test System | 30-Sep-2009 | 30-Sep-2009 | * |
| 30-Sep-2009 | 30-Sep-2009 | Test System Preparation | 30-Sep-2009 | 30-Sep-2009 | * |

* Process-based Inspection

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

## E-signature

**Quality Assurance:** 18-Dec-2009 7:35 pm GMT

Reason for signature: QA Approval

**Printed by:**

**Printed on:18-Dec-09**

# Final Report

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

**Study Number:** AC34CA.105056.BSV

**Test Article ID:** WA01-DDL-13 # 3915

**Sponsor:** WiCell Research Institute

**Authorized Representative:**

### CONCLUSION

**One-half (0.5) µg of DNA isolated from the test article (representing approximately 7.5 x $10^4$ cells) was analyzed for the presence of Human Herpesvirus 8 (HHV-8) DNA by the polymerase chain reaction (PCR) 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.**

BioReliance®

## STUDY INFORMATION

**Test Article:** The test article was received by BioReliance on 11/24/2009. Determination of the stability, purity, and concentration of the test article is the responsibility of the sponsor. Retention of reserve sample from each batch of test article is the responsibility of the sponsor.

**Testing Facility:** BioReliance

**Schedule:**

| | |
|---|---|
| **Study Initiation:** | 11/30/2009 |
| **Lab Initiation:** | 12/01/2009 |
| **Lab Completion:** | 12/09/2009 |
| **Study Completion:** | See Study Director's signature date in "Approval" Section. |

**Study Director:**

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

BioReliance

## OBJECTIVE

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

## TEST SYSTEM

PCR amplification is performed on test article DNA using HHV-8-specific primers. In the presence of HHV-8 DNA, these primers produce a 225 bp amplification product. The amplification products are analyzed by agarose gel electrophoresis in the presence of ethidium bromide. The following controls are included in the assay:

| | |
|---|---|
| **Negative Control:** | Genomic DNA from MRC5 human fetal lung fibroblasts<br>Source: BioReliance |
| **Positive Control:** | Genomic DNA from MRC5 spiked with 100 copies of pHHV-8, a plasmid containing a conserved fragment from the latency associated nuclear antigen (LANA) from the HHV-8 genome<br>Source: BioReliance |
| **No DNA Control:** | Nuclease-free water<br>Source: USB or other commercial source |
| **Spiked Control:** | Test article extract spiked with 100 copies of pHHV-8, to verify the absence of PCR inhibitors in the test article DNA (amplification suitability control) |

## METHODS

### Sample Preparation

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

### DNA Amplification

PCR amplification was performed on 0.5 μg 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 the Kodak Gel Logic 100 Imaging System (SOP OPBT0943).

## RESULTS

Test article DNA (0.5 µg), representing approximately 7.5 x $10^4$ test article cells, was analyzed for the presence of HHV-8 DNA by PCR amplification and agarose gel electrophoresis in the presence of ethidium bromide. The results are shown in Figure 1. The No DNA control (NO) showed no amplification bands and Negative Control (NC) showed no band at 225 bp. The Positive Control (PC) produced a 225 bp band. The test article spiked with 100 copies of pHHV-8 (TAS) produced a 225 bp band, demonstrating that the test article did not inhibit the PCR reaction. The test article (TA) produced no band at 225 bp.

These results provide evidence that the test article tested negative for the presence of HHV-8 DNA.

## DEVIATIONS

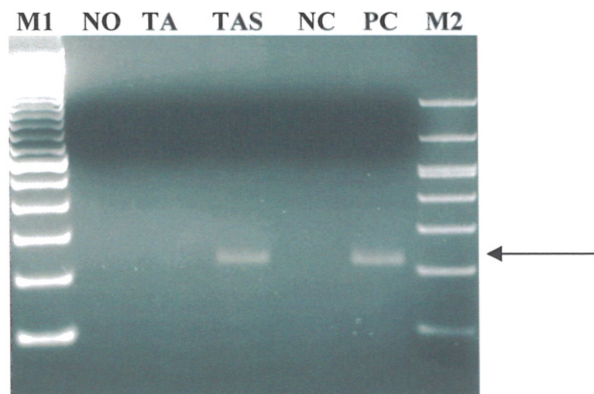
No known procedural deviations from the study protocol or pertinent assay SOPs occurred during the conduct of the study.

## APPROVAL

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

18 Dec 09
Date

**FIGURE 1**




Detection of HHV-8 specific sequences in the test article utilizing agarose gel electrophoresis in the presence of ethidium bromide

**M1:** 100 bp DNA ladder
**NO:** No DNA control
**TA:** Test Article
**TAS:** Test article spiked with 100 copies pHHV-8
**NC:** Negative control (Genomic DNA from MRC5)
**PC:** Positive control (Genomic DNA from MRC5 spiked with 100 copies pHHV-8)
**M2:** Biomarker low DNA size marker

Arrow indicates the amplification product.

BioReliance®

# Quality Assurance Statement

## Study Information

**Number:** AC34CA.105056.BSV
**Protocol Title:** PCR ASSAY FOR THE DETECTION OF HUMAN HERPESVIRUS TYPE 8 (HHV-8)

## Compliance

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

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

## Inspections

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

| Insp. Dates (From/To) | | Phase Inspected | To Study Director | To Management |
|---|---|---|---|---|
| 18-Dec-2009 | 18-Dec-2009 | Data and Final Reporting | 18-Dec-2009 | 18-Dec-2009 |
| 30-Sep-2009 | 30-Sep-2009 | Admin. Of Test Substance | 30-Sep-2009 | 30-Sep-2009 * |
| 30-Sep-2009 | 30-Sep-2009 | Manipulation of Test System | 30-Sep-2009 | 30-Sep-2009 * |
| 30-Sep-2009 | 30-Sep-2009 | Observation of Test System | 30-Sep-2009 | 30-Sep-2009 * |
| 30-Sep-2009 | 30-Sep-2009 | Test System Preparation | 30-Sep-2009 | 30-Sep-2009 * |

* Process-based Inspection

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

## E-signature

**Quality Assurance:** 18-Dec-2009 7:41 pm GMT

Reason for signature: QA Approval

**Printed by:**

**Printed on:18-Dec-09**

# Final Report

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

**Study Number:** AC34CA.105037.BSV

**Test Article ID:** WA01-DDL-13 #3915

**Sponsor:** WiCell Researh Institute

**Authorized Representative:**

### CONCLUSION

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

BioReliance®

## STUDY INFORMATION

**Test Article:** The test article was received by BioReliance on 11/24/2009. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor. Retention of reserve sample from each batch of test article is the responsibility of the sponsor.

**Testing Facility:** BioReliance

**Schedule:**

**Study Initiation:** 11/30/2009

**Lab Initiation:** 12/01/2009

**Lab Completion:** 12/09/2009

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

**Study Director:**

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

BioReliance

## 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 human parvovirus B19, these primers produce a 287 bp amplification product. The amplification products are analyzed by agarose gel electrophoresis in the presence of ethidium bromide. The following controls are included in the assay:

| | |
|---|---|
| **Negative Control:** | Genomic DNA from MRC5 human fetal lung fibroblast line<br>Source: BioReliance |
| **Positive Control:** | Genomic DNA from MRC5 spiked with 100 copies of pNPS-1, a plasmid containing a 3.6 Kb fragment from the B19 capsid gene<br>Source: BioReliance |
| **No DNA Control:** | Nuclease free water<br>Source: USB or other commercial supplier |
| **Spiked Control:** | Test article extract spiked with 100 copies of pNPS-1, to verify the absence of PCR inhibitors in the test article DNA (amplification suitability control) |

## METHODS

### Sample Preparation

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

### DNA Amplification

PCR amplification was performed on 0.5 μg of test article DNA and on the assay controls using primers B19F and B19R specific for the capsid gene of B19, employing conditions optimized to achieve detection of 100 copies of human parvovirus B19. Aliquots of the amplification products obtained from the test article and from the control samples were analyzed by agarose gel electrophoresis in the presence of ethidium bromide. Amplification products were visualized on a UV transilluminator and photographed using the Kodak Gel Logic 100 Imaging System (SOP OPBT0943).

**RESULTS**

Test article DNA (0.5 µg), representing approximately $7.5 \times 10^4$ test article cells, was analyzed for the presence of human parvovirus B19 DNA by PCR amplification and agarose gel electrophoresis in the presence of ethidium bromide. The results are shown in Figure 1. The No DNA control (NO) and Negative control (NC) showed no bands at 287 bp. The positive control (PC) produced a 287 bp band. The test article spiked with 100 copies of pNPS-1 (TAS) produced a 287 bp band, demonstrating that the test article did not inhibit the PCR reaction. The test article (TA) produced no bands at 287 bp.

These results provide evidence that the test article tested negative for the presence of human parvovirus B19 DNA.

**DEVIATIONS**

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

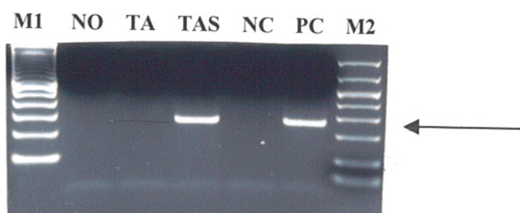
**APPROVAL**

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

22 Dec 09

Date

**FIGURE 1**




Detection of human parvovirus B19 specific sequences in the test article utilizing agarose gel electrophoresis in the presence of ethidium bromide

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

Arrow indicates the specific amplification product.

# WiCell Cytogenetics Report: 000274 NSCB3237

***Report Date:*** *3/8/2010*

***Case Details:***

***Cell Line:*** *WA01-DDL-13-E.2-p27 (Male)*

***Reference:*** *WA09-MCB-01-H.2-p23(4) (Female)*

***Investigator:*** *National Stem Cell Bank*

***Specimen:*** *hESC on MEF feeder*

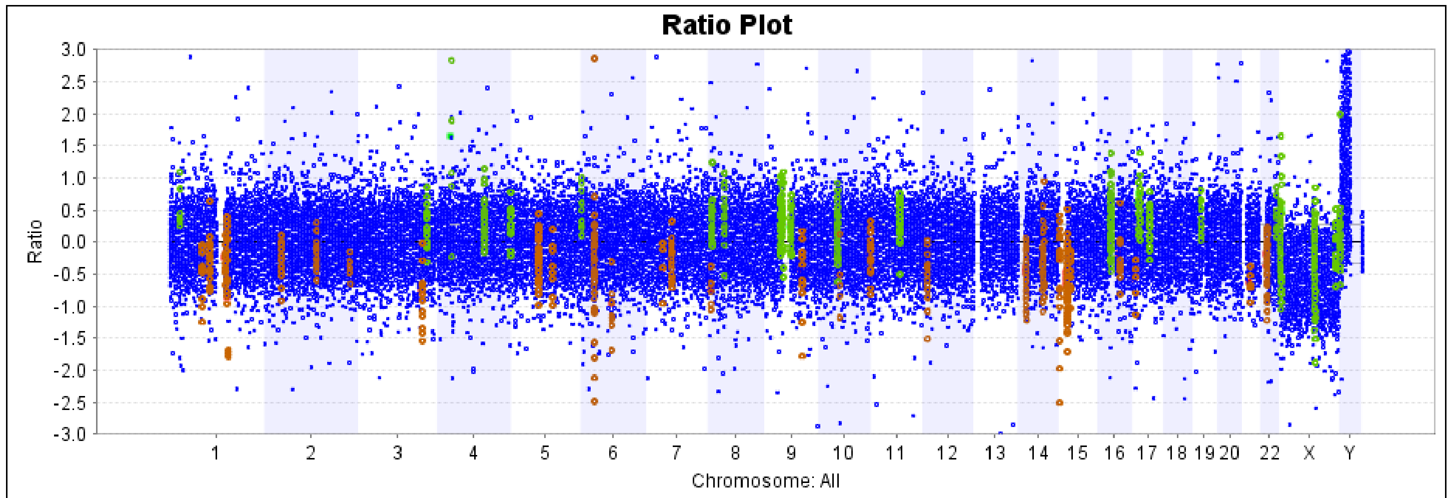
***Date of Sample:*** *2/18/2009*

***Reason for Testing:*** *NSCB FTDL*

***GEO Accession #:*** *GSM500865*

***aCGH Results:***

*Results are given in the attached Excel spreadsheet labeled "report." There were 70 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 CGH Fusion™ software.*




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

| | |
|---|---|
| *X-chromosome Gains or Losses at Pseudoautosomal Loci[3]* | *2 of 2* |
| *Published Copy Number Changes[5,6]* | *2 of 8* |
| *Reference DNA Copy Number Changes[2]* | *14 of 17* |
| *Select Differentially Expressed Genes* | *0 of 88* |

*These results are consistent with karyotype results [46,XY] as reported in 000927-021809 3237-KAR.*

Research Institute

# ***WiCell Cytogenetics Report: 000274 NSCB3237***

*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:*** *Benjamin Nisler, Seth Taapken CG(ASCP)$^{CM}$*
***Reviewed and Interpreted By:*** *Karen Dyer Montgomery, PhD, FACMG*

---

***aCGH Specifications***:

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

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

***Literature Sources:***


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


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

---

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

National Molecular Blood Group
and Platelet Testing Laboratory

**American Red Cross**

12/18/09

**SAMPLE:** **DNA on 3237-ABO** (ML09-1363)

Date received: 12/15/09
Sample date: 12/11/09

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

**HISTORY:** DNA sample from cell line.

**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. *RHCE:* PCR-RFLP for e/E in exon 5 (676G>C).
***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 |
|---|---|---|
| **3237-ABO:** | *ABO*$O^1$/$O^1$; RHD, RHC, RHe* | **Group O; RhD**+, C+c-E-e+ |

**RH COMMENTS:** The sample was negative for the *RHD*-inactivating pseudogene.

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.**

| Sample RNA: 9592 | Reference DNA: | Date of report: 10-01-2008 |
|---|---|---|
| Sample Cell Line: WA01 | Reference Cell Line: H1 | Report prepared by: CY |
| Passage: p37 | Passage: | QA Reviewed: 10/9/08 EM |
| Lot #: WA01-MCB-1 | | Date sent to Genomic Center: 081008 |
| Sample ID: | | GEO accession #:GSM325739 |

1. Chip design: 2007-06-15_WiCell_HG18_p14_exp.ndf
2. Sample labeling:
   Cy5: WA01 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 | 2.787072243 | Y |
| POU5F1 | NM_002701 | 35.44672897 | Y |
| TDGF1 | NM_003212 | 29.13366337 | Y |
| DNMT3B | NM_006892 | 29.27272727 | Y |
| GDF3 | NM_020634 | 3.454924875 | Y |
| NANOG | NM_024865 | 13.01133391 | Y |
| non-core ES markers | | | |
| PODXL | NM_001018111 | 32.21308411 | Y |
| GRB7 | NM_001030002 | 1.347118644 | Y |
| CD9 | NM_001769 | 14.65519253 | Y |
| FGF4 | NM_002007 | 0.25334608 | N |
| SOX2 | NM_003106 | 16.44760479 | Y |
| LEFTY2 | NM_003240 | 7.207194245 | Y |
| UTF1 | NM_003577 | 0.23730872 | N |
| IFITM1 | NM_003641 | 14.19598394 | Y |
| FOXD3 | NM_012183 | 0.368965517 | Y |
| GAL | NM_015973 | 40.32815534 | Y |
| NODAL | NM_018055 | 2.840858623 | Y |
| BXDC2 | NM_018321 | 26.56896552 | Y |

| | | | |
|---|---|---|---|
| LEFTY1 | NM_020997 | 8.199752628 | Y |
| LIN28 | NM_024674 | 7.882191781 | Y |
| TERT | NM_198254 | 0.20400859 | N |

5. Expression of differentiation markers:

| Gene Symbol | Accession | Ratio | Expression |
|---|---|---|---|
| COL1A1 | NM_000088 | 0.633814783 | Y |
| IPF1 | NM_000209 | 0.074313409 | N |
| PAX6 | NM_000280 | 0.479360852 | Y |
| TNNI3 | NM_000363 | 2.705882353 | Y |
| CGB | NM_000737 | 0.07079906 | N |
| AFP | NM_001134 | 1.087959343 | Y |
| CDX2 | NM_001265 | 0.116242038 | N |
| COL2A1 | NM_001844 | 0.519463087 | Y |
| FLT1 | NM_002019 | 0.285271318 | Y |
| GATA4 | NM_002052 | 0.170716113 | N |
| NEUROD1 | NM_002500 | 0.087415946 | N |
| SYP | NM_003179 | 0.094231616 | N |
| PDHX | NM_003477 | 3.564766839 | Y |
| GCM1 | NM_003643 | 0.086124402 | N |
| NKX2-5 | NM_004387 | 0.057471264 | N |
| ACTC | NM_005159 | 19.79487179 | Y |
| GATA6 | NM_005257 | 0.334590009 | Y |
| EOMES | NM_005442 | 0.38292011 | Y |
| LAMA1 | NM_005559 | 1.68872549 | Y |
| FOXA2 | NM_021784 | 0.203196347 | N |
| SOX17 | NM_022454 | 0.295302013 | Y |
| FN1 | NM_054034 | 0.165234002 | N |