Certificate of Analysis - Amended Distribution Lot

Product Description	WA09 (H9) Distribution Lot
Cell Line Provider	WiCell
MCB Lot Number	(WA09) H9-MCB-1
Distribution Lot Number	WA09 (H9)-DL-5
Date Vialed	1-April-2008
Passage Number	p25
Culture Method	SOP-CC-030B, SOP-CC-001B, SOP-CC-022B, SOP-CC-020B, SOP-CC-037A
Cryopreservation Method	SOP-CC-035D

The following testing specifications have been met for the specified product lot:

Test Description	Test Method	Test Specification	Result
Post-Thaw Viable Cell Recovery	SOP-CH-305A	Viable cells recovered	Pass
Identity by STR	SOP-CH-302A	Positive identity	Pass
Sterility - Direct transfer method	SOP-CH-304A	No contamination detected	Pass
Mycoplasma	SOP-CH-320A	No contamination detected	Pass
Karyotype by G-banding	SOP-CH-003A	Normal karyotype	Pass
Comparative Genome Hybridization	SOP-SS-010A SOP-CH-309A SOP-CH-310A SOP-SS-001A	Report copy number variants	Report available on website
Flow Cytometry for ESC Marker Expression	SOP-CH-101B SOP-CH-102B SOP-CH-103B SOP-CH-105B	Report values Oct-4 > 90%	Report available on website
Gene Expression Profile	SOP-CH-321A SOP-CH-322A SOP-CH-333A SOP-CH-311B	Report level of gene expression	Report available on website

Distribution lot cells are expanded from vials of Master Cell Bank (MCB) cells. MCB cells are thoroughly tested and known to be free of many viruses and pathogens. Cells distributed by the National Stem Cell Bank are intended for research purposes only and are not intended for use in humans. These cells have undergone extensive testing and are not known to harbor any human pathogens or adventitious agents of murine, bovine, or

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porcine origin. However, 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. The NSCB is not responsible for damages or injuries that may result from the use of these cells.

Electronic versions of the MCB and distribution lot certificates (CoAs) complete with electronic copies of individual reports, results, and procedures are available on our website, www.wicell.org. There are also archived CoAs for past cell lots.

Please visit the technical service portion of the website for assistance with your human ES Cells. The knowledgeable technical support staff can assist with embryonic stem cell culture concerns, training, and any other customer service concerns you may encounter.

Amendment(s):

Reason for Amendment	Date
CoA updated to include copyright information and electronic signature, and update to WiCell logo. Links updated.	See signature
Original CoA	15-September-2008

Date of Lot Release	Quality Assurance Approval
15-September-2008	1/3/2014 X AMC AMC Quality Assurance Signed by: [redacted]

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UW Health
University of Wisconsin
Hospital and Clinics

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

Short Tandem Repeat Analysis*

Sample Report: 1297-STR
WA09-DL-7

UW HLA#: 59442

Sample Date: 09/04/08
Received Date: 09/04/08

Requestor: WiCell Research Institute

Test Date: 09/10/08, 09/17/08

File Name: 080911, 080918

Report Date: 09/19/08

Sample Name: (label on tube) 1297-STR

Description: DNA Extracted by WiCell
229 ug/mL; 260/280 = 1.9

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

Comments: Based on the DNA 1297-STR dated and received on 09/04/08 from WI Cell, this sample (UW HLA# 59442) matches exactly the STR profile of the human stem cell line H9 comprising 12 allelic polymorphisms across the 8 STR loci analyzed. No STR polymorphisms other than those corresponding to the human H9 stem cell line were detected and the concentration of DNA required to achieve an acceptable STR genotype (signal/noise) was equivalent to that required for the standard procedure (~1 ng/amplification reaction) from human genomic DNA. These results suggest that the 1297-STR DNA sample submitted corresponds to the H9 stem cell line and it was not contaminated with any other human stem cells or a significant amount of mouse feeder layer cells. Sensitivity limits for detection of STR polymorphisms unique to either this or other human stem cell lines is ~5%. A preliminary copy of this report was issued via electronic mail to the WI Cell Research Institute on Monday, September 22, 2008.

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

Test Facility:
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.

WuXi AppTec

WiCell Research Institute
[redacted]

August 11, 2008
P.O. #: [redacted]

STERILITY TEST REPORT

Sample Information: hES Cells
1: WA09-DL-6
2: WA09-DL-7

Date Received: July 23, 2008
Date in Test: July 25, 2008
Date Completed: August 08, 2008

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

QA Reviewed: [signature] 08-12-08

Reviewed: Erika Hailes 08-11-08

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.

WuXi AppTec

WiCell Research Institute

August 11, 2008
P.O. #: RP2030

STERILITY TEST REPORT

Sample Information: hES Cells
2: WA09-DL-7

Date Received: July 23, 2008
Date in Test: July 25, 2008
Date Completed: August 08, 2008

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

TEST PARAMETERS	PRODUCT	
Approximate Volume Tested	0.5 mL	0.5 mL
Number Tested	2	2
Type of Media	SCD	FTM
Media Volume	400 mL	400 mL
Incubation Period	14 Days	14 Days
Incubation Temperature	20 °C to 25 °C	30 °C to 35 °C
RESULTS	2 NEGATIVE	2 NEGATIVE

QA Reviewed: Page 1 Signed _____

Reviewed: Page 1 Signed _____

Testing conducted in accordance with current Good Manufacturing Practices.

WuXi AppTec

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FINAL STUDY REPORT

STUDY TITLE:	**MYCOPLASMA DETECTION: "Points to Consider"**
PROTOCOL NUMBER:	30055F
TEST ARTICLE IDENTIFICATION:	WA09-DL-5
SPONSOR:	WiCell Research Institute [redacted]
PERFORMING LABORATORY:	WuXi AppTec, Inc. 2540 Executive Drive St. Paul, MN 55120
STUDY NUMBER:	108515
RESULT SUMMARY:	Considered **negative** for mycoplasma contamination

Reference PO # RP1983

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QUALITY ASSURANCE UNIT SUMMARY

STUDY: Mycoplasma Detection: "Points to Consider"

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

Critical Phase	Date	Study Director	Management
Staining of coverslips	07/11/08	07/15/08	08/14/08
Final Report	08/13/08	08/13/08	08/14/08

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

Quality Assurance Auditor: Scott W. Sherman Date: 8-14-08
Scott W. Sherman

GOOD LABORATORY PRACTICES STATEMENT

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

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

Study Director: Sheri J. Zielinski Date: 8/14/08
Sheri Zielinski

Professional Personnel Involved:

Lisa Olson, BS	Vice President of St. Paul Operations
Sheri Zielinski, BS	Study Director
Jean Mesarich, AA	Client Relations Manager

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1.0 PURPOSE

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

2.0 SPONSOR:

WiCell Research Institute
[redacted]

3.0 TEST FACILITY:

WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

4.0 SCHEDULING

DATE SAMPLE RECEIVED: 07/01/08
STUDY INITIATION DATE: 07/03/08
STUDY COMPLETION DATE: 08/14/08

5.0 TEST ARTICLE IDENTIFICATION:

WiCell Research Institute
WA09-DL-5

6.0 SAMPLE STORAGE

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

7.0 TEST ARTICLE CHARACTERIZATION

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

The Vero cells were maintained by WuXi AppTec's Cell Production Laboratory.

8.0 EXPERIMENTAL DESIGN

8.1 Overview

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

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8.2 Justification for Selection of the Test System

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

9.0 EXPERIMENTAL SUMMARY

The indirect method of detection allows visualization of mycoplasma, particularly non-cultivable strains, by growing the mycoplasma on an indicator cell line and then staining using a DNA-binding fluorochrome stain. The indicator cell line should be easy to grow, have a large cytoplasmic to nuclear area ratio and support the growth of a broad spectrum of mycoplasma species. The African green monkey kidney cell line, Vero, fits this description and was used in this assay. The assay was performed with negative and positive controls. Both a strongly cyto-adsorbing (*M. hyorhinis*) and a poorly cyto-adsorbing (*M. orale*) mycoplasma species were used as positive controls. Poor cyto-adsorbing mycoplasma species may not give reliable positive results when inoculated in low numbers. A second dilution of *M. orale* was used to ensure cyto-adsorption. Staining the cultures with DNA binding fluorochrome allows for the detection of mycoplasma based on the staining pattern observed. Only the cell nuclei demonstrate fluorescence in the negative cultures but nuclear and extra-nuclear fluorescence is observed in positive cultures.

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

10.0 TEST MATERIAL PREPARATION

10.1 Test Article Identification:

Test Article Name:	WA09-DL-5
Lot/Batch #:	Not Given
General Description:	hES cells
Number of Aliquots used:	1 x 15 mL
Stability (Expiration):	Not Given
Storage Conditions:	Ultracold (< -60°C)
Safety Precautions:	BSL-1
Intended Use/Application:	Not Given

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10.2 Test Sample Preparation

The test article was thawed in a water bath at 37 ± 2°C and 1:5 and 1:10 dilutions of the test article were prepared in sterile phosphate buffered saline (PBS). 1.0 mL of the undiluted sample, the 1:5 and 1:10 dilutions were then inoculated onto each of two (2) coverslips (per sample/dilution) containing Vero cells. The coverslips were incubated in incubator E770 for 1-2 hours at 37 ± 1°C / 5 ± 2% CO_2 and then 2.0 mL of EMEM, 8% Fetal Bovine Serum (FBS) was added to each coverslip. The coverslips were returned to incubator E770 at 37 ± 1°C / 5 ± 2% CO_2. After three days of incubation, the coverslips were fixed, stained, and then read using an epifluorescent microscope.

0.2 mL of the undiluted test article was then inoculated onto each of two SP-4 agar plates, and 10.0 mL was inoculated into a 75 cm^2 flask containing 50 mL of SP-4 broth. The plates were placed in an anaerobic GasPak system and incubated at 36 ± 1°C for a minimum of 14 days.

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

10.3 Controls and Reference Materials

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

10.3.2 Positive Controls

a. Indirect Assay

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

a.2 Poorly cyto-adsorbing species - *M. orale* (ATCC #23714) at 100 or fewer CFU and at approximately 100 ID_{50} per inoculum

b. Direct Assay

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

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

10.3.3 Control Preparation

a. Negative Controls

a.1 1.0 mL of sterile SP-4 broth was inoculated onto each of two (2) coverslips containing Vero cells to serve as the negative control in the indirect assay.

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a.2 0.2 mL of SP-4 broth was inoculated onto each of two (2) SP-4 agar plates to serve as the negative control in the direct assay. 10.0 mL of SP-4 broth was inoculated into a 75 cm^2 flask containing 50 mL of SP-4 broth to serve as the negative control in the direct assay.

b. Positive Controls

b.1 *M. hyorhinis, M. orale,* and *M. pneumoniae* were diluted to less than 100 CFU per inoculum in sterile SP-4 broth. 1.0 mL of *M. hyorhinis* and *M. ora*le at less than 100 CFU/mL was inoculated onto each of two (2) coverslips containing Vero cells. 1.0 mL of *M. orale* at 100 ID_{50} CFU per inoculum was also inoculated onto each of two (2) coverslips containing Vero cells. These coverslips served as the positive controls in the indirect assay.

b.2 The coverslips were incubated in incubator E770 for 1-2 hours at 37 ± 1°C / 5 ± 2% CO_2 and then 2.0 mL of EMEM, 8% Fetal Bovine Serum (FBS) was added to each coverslip. The coverslips were returned to incubator E770 at 37 ± 1°C / 5 ± 2% CO_2. After three days of incubation, the cell cultures were fixed, stained, and then read using an epifluorescent microscope.

b.3 0.2 mL of *M. orale* and *M. pneumoniae* at less than 100 CFU/plate was inoculated onto each of two (2) SP-4 agar plates. 10.0 mL of *M. orale* and *M. pneumoniae* at less than 10 CFU/mL (≤100 CFU/inoculum) were each inoculated into a 75 cm^2 flask containing 50 mL of sterile SP-4 broth.

b.4 The agar plates were placed in an anaerobic GasPak system and incubated at 36 ± 1°C for 14 days. The broth cultures were incubated aerobically at 36 ± 1°C for a minimum of 14 days and were read each working day for 14 days. On Days 3, 7, and 14, 0.2 mL from each broth culture flask was subcultured onto each of two (2) SP-4 agar plates. These subculture plates were placed in an anaerobic GasPak system and incubated at 36 ± 1°C. The subculture plates were observed microscopically for the presence of mycoplasma colonies after a minimum of 14 days incubation.

c. See Section 15.0, Results, for the results of these controls.

11.0 DATA ANALYSIS

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

12.0 STATISTICAL METHODS

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

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13.0 EVALUATION CRITERIA

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

13.1 Indirect Assay

DETECTION OF MYCOPLASMA CONTAMINATION BY INDIRECT ASSAY

CONTROLS	MYCOPLASMA FLUORESCENCE OBSERVED (AT LEAST ONE COVERSLIP REQUIRED FOR THE EVALUATION)
Negative Control	-
M. hyorhinis	+
M. orale (≤100 CFU)	+/-*
M. orale (100 ID_{50})	+

*Mycoplasma must be observed for at least one dilution of the poorly cyto-adsorbing mycoplasma species *M. orale*.

13.2 Direct Assay

DETECTION OF MYCOPLASMA CONTAMINATION BY DIRECT ASSAY

	NEGATIVE CONTROL	*M. PNEUMONIAE*	*M. ORALE*
Broth (Color change or turbidity change)	-	+ / -	+ / -
Agar Day 0 (at least one plate)	-	+	+
Agar Day 3, 7, 14 (at least one plate on one day)	-	+	+
Results	-	+	+

14.0 TEST EVALUATION

14.1 Indirect Assay

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

14.2 Direct Assay

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

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

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14.3 Indirect Assay and Direct Assay Results Interpretation

IF:	TEST ARTICLE				
Mycoplasmal fluorescence	-	+	+/-	+/-	-
Broth (Color change or turbidity change)	-	+/-	+/-	+/-	+*
Agar - Day 0 (at least one plate)	-	+/-	+/-	+	-
Agar - Day 3, 7, 14 (at least one plate on one day)	-	+/-	+	+/-	-
THEN: OVERALL FINAL RESULT	**-**	**+**	**+**	**+**	**-**

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

14.4 Positive Results

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

14.5 Negative Results

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

15.0 RESULTS

Indirect Assay and Direct Assay Results

	INDIRECT	DIRECT		OVERALL
		BROTH FLASKS	AGAR PLATES	
Test Article: WA09-DL-5	Negative	Negative	Negative	Negative
Negative Control	Negative	Negative	Negative	Negative
M. hyorhinis	Positive			Positive
M. orale	Positive	Positive	Positive	Positive
M. pneumoniae		Positive	Positive	Positive

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

16.0 ANALYSIS AND CONCLUSION

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

16.2 These findings indicated that the test article, WA09-DL-5, is considered negative for the presence of mycoplasma contamination.

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17.0 DEVIATIONS: None.

18.0 AMENDMENTS: The study directorship was reassigned to Sheri Zielinski.

19.0 RECORD RETENTION

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

20.0 TECHNICAL REFERENCES

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

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

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

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

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

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

(For Laboratory Use Only)

WuXi AppTec Study # 10515

Accession # N/A

PROTOCOL TITLE: MYCOPLASMA DETECTION: "POINTS TO CONSIDER"

TEST CODE: 30055

PERFORMING LABORATORY: WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

EFFECTIVE DATE: 3 June 2008

GLP PROTOCOL: 30055F

Technical Management

Quality Assurance has reviewed this protocol for compliance with applicable regulatory requirements and internal procedures.

PROPRIETARY INFORMATION

This document is provided with the understanding that the recipient shall recognize it contains WuXi AppTec proprietary information, that it shall be kept confidential by the person and/or company to whom it is addressed, and that it shall be used for no other purpose than assessing and approving the described services to be performed by WuXi AppTec or for the purpose of regulatory submission.

2540 Executive Drive • St. Paul, MN 55120 • 888.794.0077 • 651.675.2000 • Fax: 651.675.2005

Client Protocol

MYCOPLASMA DETECTION: "POINTS TO CONSIDER"

1.0 PURPOSE

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

2.0 TEST FACILITY: WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

3.0 SCHEDULING / DISCLAIMER

3.1 Test protocol initiation is generally within 10 working days after receipt of the test article, a signed Client Protocol Approval form, and a signed sample submission form. The Client Protocol Approval form and the sample submission form serve as addenda to this protocol. Written notification of the proposed initiation and completion dates will be provided at the time the test article and signed protocol are received by the laboratory. The estimated testing time is 28 days. Verbal results will be available from the Study Director upon completion of the study with the written quality assurance audited report to follow approximately 10 working days after completion of the study.

3.2 If a test, or a portion of it, must be repeated due to failure by WuXi AppTec to adhere to specified procedures, it will be repeated free of charge. If a test must be repeated, or a portion of it, due to failure of internal controls or failure to meet assay validity requirements, it will be repeated free of charge. "Methods Development" fees shall be assessed, however, if the test article and test system require modifications due to complexity and difficulty of testing.

3.3 If the Sponsor requests a repeat test, they will be charged for an additional test.

3.4 Neither the name of WuXi AppTec nor any of its employees are to be used in advertising or other promotion without written consent from WuXi AppTec.

3.5 The Sponsor is responsible for any rejection of the final report by the regulatory agency concerning report format, pagination, etc. To prevent rejection, the Sponsor should carefully review the WuXi AppTec final report and notify WuXi AppTec of any perceived deficiencies in these areas before submission of the report to the regulatory agency. WuXi AppTec will make reasonable changes deemed necessary by the Sponsor, without altering the technical data.

4.0 TEST ARTICLE IDENTIFICATION

Test article information to be included in the final report will be provided solely by the Sponsor on the WuXi AppTec sample submission form attached to this protocol.

5.0 TEST ARTICLE CHARACTERIZATION

The Sponsor is responsible for all test and control article characterization data as specified in the GLP regulations. The identity, strength, stability, purity, and chemical composition 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. Furthermore, it is the responsibility of the Sponsor to ensure that the test article submitted for testing is representative of the final product that will be subjected to materials characterization. Any special requirements for handling or storage must be arranged in advance of receipt and the test article must be received in good condition.

The test article will be maintained according to the Sponsor's instructions. The Vero cells are maintained by WuXi AppTec's Cell Production Laboratory.

6.0 EXPERIMENTAL DESIGN

6.1 Overview

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

The indirect method (indicator cell culture) of detection allows visualization of mycoplasma, particularly non-cultivable strains, by growing the mycoplasma on an indicator cell line and then staining using a DNA-binding fluorochrome stain (Hoechst stain). The indicator cell line should be easy to grow, have a large cytoplasmic to nuclear area ratio and support the growth of a broad spectrum of mycoplasma species. The African green monkey kidney cell line, Vero, fits this description and is frequently used.

The indirect assay is performed with negative and positive controls. Both a strongly cyto-adsorbing (*M. hyorhinis*) and a poorly cyto-adsorbing (*M. orale*) mycoplasma species are used as positive controls. Poorly cyto-adsorbing mycoplasma species may not give reliable positive results when inoculated in low numbers. A second dilution of *M. orale* may be used to ensure cyto-adsorption. Staining the cultures with DNA-binding fluorochrome allows for the detection of mycoplasma based on the staining pattern observed. Only the cell nuclei demonstrate fluorescence in the negative cultures but nuclear and extra-nuclear fluorescence is observed in positive cultures.

Direct cultivation is a sensitive and specific method for the detection of mycoplasma. The agar and broth media employed supply nutrients necessary for the growth of cultivable mycoplasmas. These media also supply a source of carbon and energy, and favorable growth conditions. The direct assay is performed using negative and positive controls. A fermentative (*M. pneumoniae*) as well as a non-fermentative (*M. orale*) mycoplasma species are used as positive controls.

The procedures employed in this study are based on the methods described in the 1993 Attachment # 2 to the "Points To Consider" document.

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6.2 Justification For Selection Of The Test System

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

7.0 PROCEDURE FOR IDENTIFICATION OF THE TEST SYSTEM

7.1 Controls And Reference Materials

7.1.1 Sterile SP-4 glucose broth will serve as the negative control inoculum for the direct and indirect assays.

7.1.2 Optional: Cell culture medium may be added as an additional negative control for the indirect assay.

7.1.3 Positive Controls

a. Indirect Assay

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

a.2 Poorly cyto-adsorbing species - *M. orale* (ATCC #23714) at 100 or fewer CFU per inoculum and at approximately 100 ID_{50}.

b. Direct Assay

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

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

7.2 Testing is performed in strict adherence to WuXi AppTec Standard Operating Procedures (SOPs) which have been constructed to cover all aspects of the work including, but not limited to, receipt, identification, log-in, and tracking of test article(s). Additionally, each test is assigned a unique Project Number. This number is used for identification during the course of the test.

8.0 TEST METHOD

8.1 Indirect (DNA-staining) Assay

8.1.1 Inoculate no less than 1 mL of the negative control and test article directly onto coverslips containing previously incubated Vero cells. Two (2) or more coverslips will be used for each control and test article. Additional dilutions of the test article may be made to eliminate interference of cellular debris with mycoplasma detection.

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Not Official

8.1.2 For media samples (EMEM, DMEM, FBS, etc.) that are free of cellular material, inoculate 1 mL of undiluted test article onto four (4) coverslips containing previously incubated Vero cells. Three (3) coverslips will be observed and the fourth will serve as a backup.

8.1.3 For trypsin samples, add an equal volume of previously tested and released fetal bovine serum (FBS) to inactivate the trypsin. 1 mL of the inactivated test article will be inoculated onto four (4) coverslips containing previously incubated Vero cells. Three (3) coverslips will be observed and the fourth will serve as a backup.

8.1.4 The positive controls, *M. hyorhinis* and *M. orale,* are inoculated in the same manner, using 100 or fewer CFU per inoculum for *M. hyorhinis* and 100 or fewer CFU and at approximately 100 ID_{50} for *M. orale*.

8.1.5 Following reincubation for 3 to 5 days, cells are fixed and then stained with a DNA-binding fluorochrome (Hoechst 33258 stain), and are evaluated for the presence of mycoplasma using epifluorescent microscopy.

8.2 Direct (Microbiological) Assay

8.2.1 At least two (2) SP-4 glucose agar plates are inoculated with no less than 0.2 mL per plate of the negative control (sterile SP-4 glucose broth).

8.2.2 50 mL of sterile SP-4 glucose broth is inoculated with no less than 10 mL of the negative control.

8.2.3 All test articles are tested neat for the Direct Assay unless dilution is requested by the Sponsor. Sterile phosphate buffered saline (PBS) should be used to prepare dilutions unless an alternate diluent is requested by the Sponsor. The diluent that is used should be free from antibiotics or other materials that are known to interfere with the detection of mycoplasma.

8.2.4 The test article is inoculated onto two (2) or more SP-4 glucose agar plates (0.2 mL per plate) and into one (1) broth flask (no less than 10 mL into 50 mL of sterile SP-4 glucose broth).

8.2.5 The positive controls, *M. pneumoniae* and *M. orale,* are inoculated in the same manner, using 100 or fewer CFU per inoculum onto each of two (2) or more plates and 10 mL (100 or less CFU per inoculum) into 50 mL of sterile SP-4 glucose broth.

8.2.6 Agar plates are incubated anaerobically in a GasPak system at 36 ± 1°C for at least 14 days

8.2.7 Broth culture flasks are incubated aerobically at 36 ± 1°C. Broth cultures are observed daily (normal working days only) for changes in color or turbidity.

8.2.8 The broth culture flasks are subcultured on Days 3, 7, and 14 onto SP-4 agar plates. Two (2) or more plates (0.2 mL broth / plate) are inoculated and incubated anaerobically in a GasPak system for at least 14 days.

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8.2.9 The agar plates are examined microscopically for the presence of mycoplasma colonies after 14 or more days of incubation.

9.0 METHOD FOR CONTROL OF BIAS: Not applicable.

10.0 DATA ANALYSIS
The results of this study are based on visual observations; therefore no data analysis is required.

11.0 STATISTICAL METHODS
The results of this study are qualitative; therefore no statistical analysis is required.

12.0 ASSAY VALIDITY
Final evaluation of the validity of the assay and test article results will be based upon the criteria listed below and scientific judgment.

12.1 Indirect Assay

DETECTION OF MYCOPLASMA CONTAMINATION BY INDIRECT ASSAY

CONTROLS	MYCOPLASMA FLUORESCENCE OBSERVED (AT LEAST ONE COVERSLIP REQUIRED FOR THE EVALUATION)
Negative Control	-
M. hyorhinis	+
M. orale (≤100 CFU)	+/-*
M. orale (100 ID_{50})	+

*Mycoplasma must be observed for at least one dilution of the poorly cytoadsorbing mycoplasma species *M. orale*.

12.1.1 The negative controls do not exhibit fluorescence typical of mycoplasma contamination.

12.1.2 The positive control mycoplasma, *M. hyorhinis,* at ≤100 CFU per inoculum is detected in at least one (1) coverslip.

12.1.3 The positive control mycoplasma, *M. orale,* is detected in at least one (1) coverslip at one (1) concentration (either ≤100 CFU per inoculum or 100 ID_{50}).

12.2 Direct Assay

DETECTION OF MYCOPLASMA CONTAMINATION BY DIRECT ASSAY

	NEGATIVE CONTROL	*M. PNEUMONIAE*	*M. ORALE*
Broth (Color change or turbidity change)	-	+ / -	+ / -
Agar Day 0 (at least one plate)	-	+	+
Agar Day 3, 7, 14 (at least one plate on one day)	-	+	+
Results	-	+	+

12.2.1 The negative controls are negative for mycoplasma throughout the observation period.

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12.2.2 The positive control mycoplasmas, *M. pneumoniae* and *M orale*, are positive on at least one (1) Day 0 agar plate.

12.2.3 The positive control mycoplasmas, *M. pneumoniae* and *M orale*, are positive on at least one (1) sub-culture plate.

13.0 TEST EVALUATION

13.1 Indirect Assay

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

13.2 Direct Assay

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

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

13.3 Indirect Assay and Direct Assay Results Interpretation

IF:	TEST ARTICLE				
Mycoplasmal fluorescence	-	+	+/-	+/-	-
Broth (Color change or turbidity change)	-	+/-	+/-	+/-	+*
Agar - Day 0 (at least one plate)	-	+/-	+/-	+	-
Agar - Day 3, 7, 14 (at least one plate on one day)	-	+/-	+	+/-	-
THEN: OVERALL FINAL RESULT	**-**	**+**	**+**	**+**	**-**

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

13.3.1 Negative Results

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

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

13.4 Repeat Assays
A test will be repeated in part or in total if a control failure occurs.

14.0 PROTOCOL CHANGES
If it becomes necessary to make changes in the approved protocol, the revisions and reasons for changes will be documented, signed by the Study Director, dated, maintained with the protocol and reported to the Sponsor. If an event occurs which may have an effect on the validity of the study, the Sponsor will be notified as soon as is practical. If the Study Director is unable to complete the study, an alternate Study Director with full responsibility and authority regarding the study will be assigned.

15.0 FINAL REPORT
The final report will include but will not be limited to: the date of the study initiation and completion, the purpose as stated in the approved protocol, changes in the approved protocol, identification of the test system, a description of the methods used and conclusion as it relates to the test.

16.0 RECORD RETENTION

16.1 Study Specific Documents
All of the original raw data developed exclusively for this study shall be retained according to WuXi AppTec, Inc.'s standard operating procedures for archival. These original data include, but are not limited to the following:

16.1.1 All handwritten and equipment generated raw data for control(s) and test article(s).
16.1.2 Any protocol amendments/deviation notifications.
16.1.3 Memoranda, specifications, and other study specific correspondence relating to interpretation and evaluation of data, other than those documents contained in the final study report.
16.1.4 Original signed protocol.
16.1.5 Certified copy of final study report.
16.1.6 Study-specific SOP deviations made during the study.

16.2 Facility Specific Documents
The following records shall also be retained according to WuXi AppTec, Inc.'s standard operating procedures for archival. These documents include, but are not limited to, the following:

16.2.1 SOPs which pertain to the study conducted.
16.2.2 Non study-specific SOP deviations made during the course of this study which may affect the results obtained during this study.
16.2.3 Methods which were used or referenced in the study conducted.

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16.2.4 QA reports for each QA inspection with comments.
16.2.5 Facility Records: Temperature Logs (ambient, incubator, etc.), Instrument Logs, Calibration and Maintenance Records.
16.2.6 Current job descriptions and summary of experience and training for all personnel involved in the study.

17.0 REFERENCES

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

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

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

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

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

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

18.0 COMPLIANCE

GLP STATUS: This study will be performed in accordance with FDA, 21 CFR, Part 58 - Good Laboratory Practice for Nonclinical Laboratory Studies. The study will be inspected during at least one phase and the final report will be audited by the WuXi AppTec Quality Assurance Unit.

19.0 TEST ARTICLE DISPOSITION

It is the responsibility of the Sponsor to retain a sample of the test material. All unused test material will be discarded following study completion unless otherwise requested by Sponsor.

CLIENT PROTOCOL APPROVAL FORM

Copy of Original

PLEASE NOTE THAT TESTING CANNOT BE INITIATED UNTIL THIS FORM IS COMPLETED WITH AN AUTHORIZED SIGNATURE AND THE ORIGINAL IS RETURNED TO WUXI APPTEC.

SPONSOR:

[redacted]

[redacted]

Primary Approval Statement

I have read WuXi AppTec, Inc.'s client protocol, 30055F - Mycoplasma Detection: "Points to Consider". I accept the test method described. I understand that my approval will be valid until one or both of the following occur:

1. The protocol is revised and a new version letter is issued.
2. The Primary Approver's position with the Sponsor company is terminated or changes, whichever may occur first.

[redacted]

TITLE: QA Manager

DATE: 10/17/08

Associate(s) Approval Statement

The Primary Approver (above) has authorized the following Associate(s) to accept the responsibility for submitting samples for testing under this protocol. Each associate understands that their authorization for submission will be valid until one or more of the following has occurred:

1. The protocol has been revised and new version letter has been issued.
2. The primary Approver's position with the Sponsor company is terminated or changes, whichever may occur first.
3. Any of the Associate's positions with the Sponsor company are terminated or change, whichever may occur first.
4. The Primary Approver has removed any Associate's authorization by sending a signed and dated letter to WuXi AppTec, ATTN: Client Services.

☐ I do not wish to have an Associate(s) authorized to initiate testing of samples under this protocol.

☐ I do wish to have the following Associate(s) authorized to initiate testing of samples under this protocol.

Name of Associate (please print)	Name of Associate (please print)
Name of Associate (please print)	Name of Associate (please print)

WUXI APPTEC, INC.:

NAME: Todd Quinn
Study Director

SIGNATURE: _____
Study Director

DATE: 7/30/08

AppTec

Biopharmaceutical / Biological Products
SAMPLE SUBMISSION FORM

Complete all applicable areas on both pages of this form and enclose in sample shipment. A separate completed form is required for each lot and/or type of sample.

APPTEC USE ONLY
Accession #: 108515

of Original

CLIENT CONTACT INFORMATION PHONE FAX EMAIL

[redacted]

Company Name and Address:

[redacted]

Client Account Number: 4000252

[redacted]

J & INFORMATION *If resubmitting sample material, provide original AppTec sample Accession #:*

Sample Designation for Final Report:	TYPE of Sample *(General Description of Material)*:
WA09-TDL-5	hES cells

Commercial/Marketed Product?	Species of Origin:	Stability (Expiration Date):	Biosafety Level:	Sample Matrix or Buffer Components *(if applicable)*:
☐ Yes ☒ ~~No~~	human		1	

SAMPLE SHIPMENT & STORAGE

Controlled Storage Temperature: ☐ Room Temperature ☐ Refrigerated (2° to 8°C) ☑ Frozen (-10° to -60°C) (either) ☑ Ultracold (< -60°C) *

☐ Liquid Nitrogen *(Available only in Philadelphia)* ***NOTE:*** *For virology testing, sample storage conditions will be determined by the Study Director.*

NOTE: *For small-quantity liquid samples, AppTec has found the use of cryogenic vials with internal thread closures an effective way to help prevent leaking that can result in loss of sample volume and possible sample contamination during shipping.*

☑ Do Not Freeze/Thaw Sample for Reuse

☐ May Freeze/Thaw # of Times:

Upon test completion, samples to be: ☑ Discarded ☐ Returned (Additional fee applies)

Provide courier company and acct. # for shipping:

SAMPLE CHARACTERIZATION INFORMATION

Sample characterization information, such as strength, homogeneity, purity and stability, and others that may directly impact the testing performed:

☐ **IS ATTACHED** to this sample submission form. ☑ **IS UNAVAILABLE.** ☐ **IS CONSIDERED PROPRIETARY INFORMATION.**

IF SUBMITTING A CELL LINE, COMPLETE THIS SECTION

NOTE: Cells submitted for expansion will be stored in liquid nitrogen.

Species and Cell Type:	Check One:	Subculturing: *(Check appropriate box.)*	
	☐ Suspension Cells ☐ Adherent Cells	☐ Requires Trypsin ☐ Requires Trypsin/EDTA	☐ No Trypsin / Cells are sensitive ☐ Other:

Medium Requirements, Including Supplements/Concentrations: *NOTE: AppTec in-house medium and supplements will be utilized unless supplied by client with sample.*	Temperature and % CO_2 for Growth: °C = % CO_2 =

FOR CRYOPRESERVED CELLS:	Seeding Density:	Cells Per Vial:

PASSAGE INFORMATION	Cell Concentration / Confluence Prior to Passage:	Seeding Concentration OR Split Ratio:	Number of Days Between Passages:	Passage Level / Limit:

See Page 2 of this form to provide additional information and sample submission authorization.

* stored at <-60°C at WuXi AppTec St. Paul, C 7/3/08

FOR APPTEC USE

CLIENT	DATE	ACCESSION #

Copy of Original

REQUESTED TESTING *If more space is needed to list requested assays, attach a second copy of this sheet.*

BRIEF NAME / DESCRIPTION OF ASSAY	PROTOCOL NUMBER	For each protocol, indicate if R & D	AMOUNT OF MATERIAL ALLOCATED PER PROTOCOL		Check if requesting STAT [Additional fees apply]
			# of Tubes	Vol. In Tube	
Mycoplasma	30055		1	15ml	

PROVIDE THE FOLLOWING INFORMATION, AS APPLICABLE

If ordering Protocol # 37000, list cell lines:

Check one: ☐ Plates ☐ Flasks

Check one: ☐ 14-day ☐ 28-day

Concentration per _____ (*write in unit of measurement*) **For Patient or Test Dose** Units: _____

Check One: ☐ Particles ☐ PFU **For DNA Detection Assays** Amount of material to test: _____

FOR STERILITY TESTING: Has this TYPE of sample been submitted before? ☐ Yes ☐ No

Has B/F been conducted on this sample? ☐ Yes ☐ No Does sample contain antibiotics? ☐ Yes ☐ No

COMMENTS / SAMPLE PREPARATION & SPECIAL INSTRUCTIONS (*Including pre-testing information such as dilutions, reconstitution, etc.*)

IMPORTANT INSTRUCTIONS REGARDING THIS FORM. READ CAREFULLY BEFORE SIGNING AUTHORIZATION BELOW.

This sample submission form–which must accompany each submitted sample –acts as the official record for what is being requested/required of AppTec regarding this particular sample. It is essential that clients provide complete information on this form for ALL areas (as applicable to the sample). If the required information cannot be provided within the spaces on this form, client should attach any additional information that may be critical regarding sample description, handling, preparation, etc. ***(even if this information may have been provided previously to AppTec).*** Failure to provide this information could result in testing delays or other issues. AppTec will not be held responsible for information not provided by client. In addition, if re-testing is required because of missing or incomplete information, charges for both the initial testing and retesting will be the client's responsibility.

SAMPLE SUBMISSION AUTHORIZATION

For testing to be initiated, this section must be signed by the same sponsor representative who approved the protocol(s) listed above.

[redacted] 12-7-08

Signature Date

AppTec – Philadelphia • 4751 League Island Blvd. • Philadelphia, PA 19112 • 800.622.8820 • 215.218.5500 • FAX 215.218.5990
AppTec – Atlanta • 1265 Kennestone Circle • Marietta, GA 30066 • 888.847.6633 • 770.514.0262 • FAX 770.514.0294
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GLP COMPLIANT TEST PROTOCOL AMENDMENT

1.0 Amendment Number: 1

2.0 Effective Date: August 11, 2008

3.0 Amendment Date: August 11, 2008

4.0 Sponsor: WiCell Research Institute

[redacted]

5.0 Test Facility: WuXi AppTec Inc.
2540 Executive Drive
St. Paul, MN 55120

6.0 WuXi AppTec Protocol Number: 30055F

7.0 WuXi AppTec Project Number: 108515

8.0 Modification to Protocol:
The Study Directorship of this assay was reassigned to Sheri Zielinski.

9.0 Reason for Change:
Study director change

10.0 Impact to Protocol Interpretation:
none

11.0 Review
Signatures below represent authorization of the amendment (e.g. authorize change to the SOP and protocol).

Sheri J. Zielinski
Study Director

8/13/08
Date

NA
Sponsor (if applicable)

NA
Date

Check applicable box as task is completed:
NA
☐ ☑ Amendment attached to protocol
☑ ☐ Annotation made in protocol
☑ ☐ Special Instructions Worksheet/Study Worksheets Updated
☑ ☐ IACUC Protocol Amended/Updated

The following should be completed for amendments that alter the study schedule:
☑ ☐ Project Schedules updated
☑ ☐ Worksheets updated
☑ ☐ Project Folder Annotations/Stamps updated
☑ ☐ Critical Phase Audited Re-scheduled
☑ ☐ Cage Card Termination Date updated
☑ ☐ Special Instruction Worksheet updated

Updates Complete:
Mary Ellen Anderson
Study Director or Manager

8/14/08
Date

WiCell Cytogenetics Report: 000637-070208
NSCB 3942

Report Date: June 26, 2013

Case Details:

Cell Line: *WA09-DL-5*

Passage #: *p29*

Date Completed: *7/8/2008*

Cell Line Gender: *female*

Investigator: *National Stem Cell Bank*

Specimen: *hESC on MEF feeder*

Date of Sample: *7/2/2008*

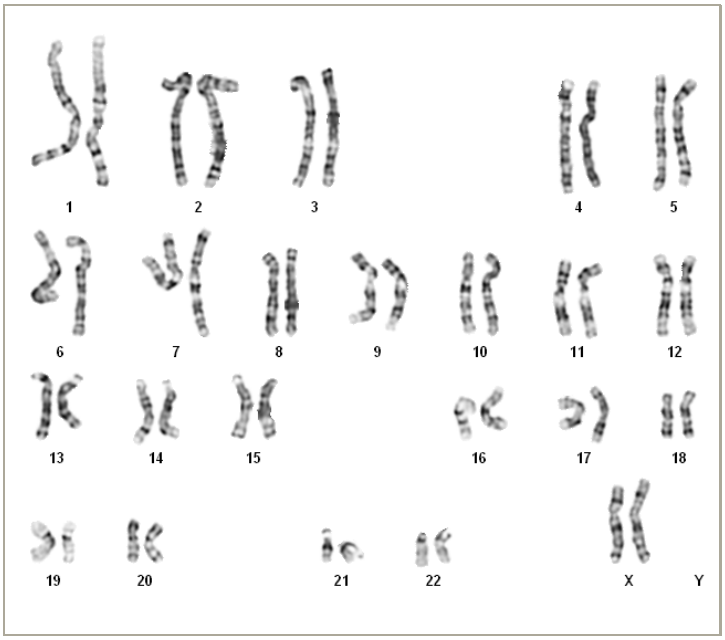
Tests,Reason for: *Confirm normal karyotype.*

Results: *46,XX*

Completed by ST, CLSp(CG), on 7/7/2008

Reviewed and interpreted by KDM, PhD, FACMG, on 7/8/2008

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



Cell: *S01-02*

Slide: *A*

Slide Type: *Karyotyping*

Cell Results: *Karyotype: 46,XX*

# of Cells Counted: *20*

# of Cells Karyotyped: *3*

# of Cells Analyzed: *6*

Band Level: *450-550*

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

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

WiCell Cytogenetics Report: 000137 NSCB3942

Report Date: *December 4, 2009*

Case Details:

Cell Line: *WA09 DL-5 (Female)*

Reference: *WA01 p37 (Male)*

Investigator: *National Stem Cell Bank*

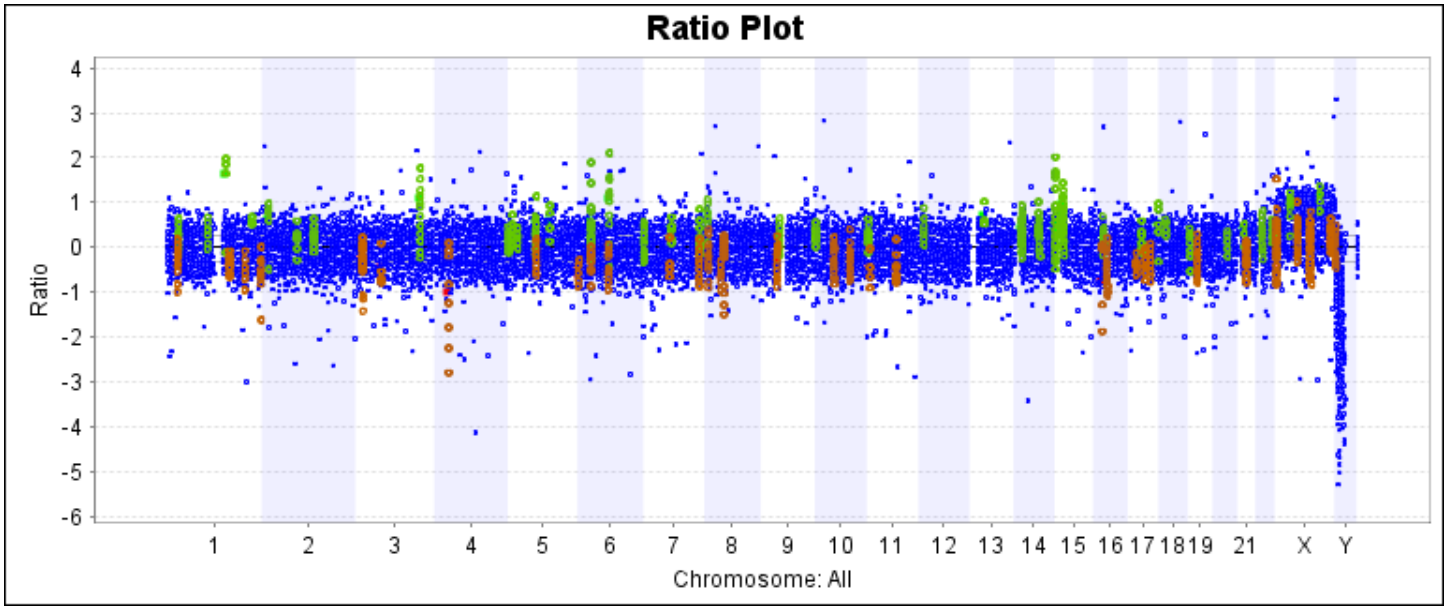
Specimen: *hES cells on MEFs*

Date of Sample: *7/3/2008*

Reason for Testing: *NSCB Testing*

GEO Accession #: *GSM325499*

aCGH Results:
Results are given in the attached Excel spreadsheet labeled "report." There were 95 copy number gains and losses identified by modified circular binary segmentation[1]. The analysis summary is depicted in the ratio plot below with copy number gains shown in green and losses in red. This data was generated using OneClickCGH™ software.



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

X-chromosome Gains or Losses at Pseudoautosomal Loci[3]	*2 of 2*
Published Copy Number Changes[5,6]	*2 of 8*
Reference DNA Copy Number Changes[2]	*13 of 17*
Select Differentially Expressed Genes	*0 of 88*

These results are consistent with karyotype results [46,XX] as reported in 000637-070208,3942-KAR.

Test sample gain or loss is consistent with the opposite gender reference standard. Additional analysis of this data was performed using different ratio settings and different window averaging.

Results Completed By: [redacted] *MS, CLSp(CG)*
Reviewed and Interpreted By: [redacted] *, 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.*
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***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:**_____

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

Cell: WA09-DL-5
Passage p33
SampleID: 3942-FAC

Date of: *(mm/dd/yy)*
acquisition: 07/24/08
file creation: 08/01/08
file submission: 08/01/08

antigen2:	SSEA4 - antigen2 +	SSEA4 + antigen2 +	SSEA4 + antigen2 -	SSEA4 - antigen2 -	ALL SSEA4 +	ALL antigen2 +
SSEA3	0.047	94.6	0.93	1.41	9.19	94.647
TRA1-60	0.013	95.6	2.85	1.54	98.45	95.613
TRA1-81	0.028	91.7	6.62	1.61	98.32	91.728
Oct-4	0.66	89.9	6.6	2.84	96.5	90.56
SSEA1	1.88	23.7	72.6	1.87	96.3	25.58