Thaw and Culture Details

Cell Line Name	WA16
WiCell Lot Number	WB0029
Provider	WiCell
Banked By	WiCell
Thaw and Culture Recommendations	WiCell recommends thawing 1 vial into 3 wells of a 6 well plate.
Culture Platform	Feeder Independent
	Medium: mTeSR™1
	Matrix: Matrigel®
Protocol	WiCell Feeder Independent mTeSR™1 Protocol
Passage Number	p18 These cells were cultured for 17 passages prior to freeze. WiCell adds +1 to the passage number at freeze so that the number on the vial best represents the overall passage number of the cells at thaw.
Date Vialed	29-June-2010
Vial Label	WB00029 WA16 p18 DF 29JUN10
Biosafety and Use Information	Appropriate biosafety precautions should be followed when working with these cells. The end user is responsible for ensuring that the cells are handled and stored in an appropriate manner. WiCell is not responsible for damages or injuries that may result from the use of these cells. Cells distributed by WiCell are intended for research purposes only and are not intended for use in humans.

Lot Specific Testing Performed by WiCell

The following tests were performed on this specific lot.

Test Description	Test Provider	Test Method	Test Specification	Result
Karyotype by G-banding	WiCell	SOP-CH-003	Expected karyotype	Pass
	Result from report: This is an abnormal karyotype, with an extra X chromosome in all cells examined. This finding is consistent with previous reports that the WA16 cell line has a 47,XXY karyotype.			
Post-Thaw Viable Cell Recovery	WiCell	SOP-CH-305	≥ 15 Undifferentiated Colonies, ≤ 30% Differentiation	Pass
Identity by STR	UW Molecular Diagnostics Laboratory	PowerPlex 1.2 System by Promega	Consistent with known profile	Pass[1]
	[1]This was the first STR performed for this cell line and therefore it establishes the STR identity for this cell line.			
Sterility - Direct transfer method	Apptec	30744	Negative	Pass
Mycoplasma	Bionique	M250	No contamination detected	Pass
Comprehensive Human Virus Panel	Charles River	ID 91/0	Negative	Pass

General Cell Line Testing Performed by WiCell

The following tests were performed on the cell line. The tests do not apply to any particular lot.

Test Description	Test Provider	Test Method
HLA	UW Molecular Diagnostics Laboratory	PowerPlex 1.2 System by Promega
ABO	American Red Cross	For ABO: Olsson ML, Chester MA. A rapid and simple ABO genotype screening method using a novel B/O2 versus A/O2 discriminating nucleotide substitution at the ABO locus. Vox Sang 1995; 69(3):242-7. For RHD: Singleton BK, Green CA, Avent ND, Martin PG, Smart E, Daka A, Narter-Olaga EG, Hawthorne LM, Daniels G. The presence of an RHD pseudogene containing a 37 base pair duplication and a nonsense mutation in Africans with the Rh D-negative blood group phenotype. Blood 2000; 95(1): 12-8.
Comprehensive Human Virus Panel	Charles River	ID 91/0

Testing Reported by Provider

Test Description	Result	Report
Karyotype	XXY	See Publication
Oct 4	Present	See Publication
SSEA4	Present	See Publication
Tra 1-60	Present	See Publication
Tra 1-81	Present	See Publication
Terataoma	3 Germ Layers Present	See Publication

Approval Date	Quality Assurance Approval
18-March-2011	8/9/2017 X AMK AMK Quality Assurance Signed by: Klade, Anjelica

WiCell Cytogenetics Report: 003466
WISC 3163

Report Date: August 05, 2010

Case Details:

Cell Line: *WA16-WB0029 (3163)*

Passage #: *16*

Date Completed: *8/5/2010*

Cell Line Gender: *Male*

Investigator: *Wisconsin International Stem Cell Bank*

Specimen: *hESC on Matrigel*

Date of Sample: *7/26/2010*

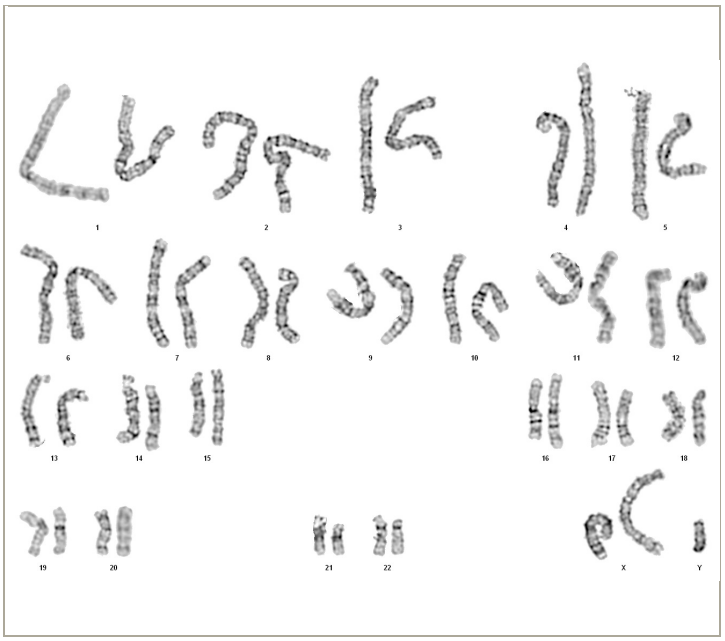
Tests,Reason for: *WB testing*

Results: *47,XXY*

Completed by [redacted] *MS, CG(ASCP), on 8/4/2010*

Reviewed and interpreted by [redacted] *PhD, FACMG, on 8/5/2010*

Interpretation: *This is an abnormal karyotype, with an extra X chromosome in all cells examined. This finding is consistent with previous reports that the WA16 cell line has a 47,XXY karyotype.*



Cell: *S01-04*

Slide: *2-11*

Slide Type: *Karyotyping*

# of Cells Counted: 20

# of Cells Karyotyped: 4

# of Cells Analyzed: 8

Band Level: 400-550

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

UW Health

University of Wisconsin
Hospital and Clinics

Histocompatibility/Molecular Diagnostics Laboratory

[redacted]

Short Tandem Repeat Analysis*

Sample Report: 3163-STR | UW HLA#: 63524 | Sample Date: 08/02/10
Lab Received 08/02/10

Requestor: WiCell Research Institute

Test Date: 08/03/10 | File Name: 100804 | Report Date: 08/06/10

Sample Name: (label on tube) 3163-STR

Description: WI Cell Research Institute provided genomic DNA
250.6 ug/mL 260/280=1.91

Locus	Repeat #	STR Genotype
D16S539	5, 8-15	9,11
D7S820	6-14	11,12
D13S317	7-15	10,12
D5S818	7-15	11,12
CSF1PO	6-15	11,12
TPOX	6-13	8,11
Amelogenin	NA	X,Y
TH01	5-11	7,9.3
vWA	11, 13-21	15,16

Comments: Based on the 3163-STR DNA submitted by WI Cell dated and received on 08/02/10, this sample (UW HLA# 63524) defines the STR profile of the human stem cell line WA16 comprising 16 allelic polymorphisms across the 8 STR loci analyzed. No STR polymorphisms other than those corresponding to the human WA16 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. This result suggest that the 3163-STR DNA samples submitted corresponds to the WA16 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%.

[redacted] 8-10 23W

Date

HLA/Molecular Diagnostics Laboratory

[redacted] 08/06/10

Date

HLA/Molecular Diagnostics Laboratory

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

File: Final STR Report

Test Facility:
[redacted]

WuXi AppTec

Report Number
842178
Page 1 of 1

WiCell Research Institute
[redacted]

August 03, 2010
P.O. #: [redacted]

STERILITY TEST REPORT

Sample Information: hES Cells, WA16-WB0029 # 5311

Date Received: July 15, 2010
Date in Test: July 19, 2010
Date Completed: August 02, 2010

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

TEST PARAMETERS	PRODUCT	
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

PRODUCT	APPROXIMATE VOLUME TESTED (each media)
1	0.37 mL
2	0.5 mL

[redacted] 08-04-10
QA Reviewer Date

[redacted] 08-04-10
Technical Reviewer Date

Testing conducted in accordance with current Good Manufacturing Practices.

[redacted]

bionique Testing Laboratories

MYCOPLASMA TESTING SERVICES

BIONIQUE® TESTING LABORATORIES, INC.

[redacted]

APPENDIX

Document ID #:	DCF9002F
Title:	QUALITY ASSURANCE REPORT - GMP
Effective Date:	03/12/10
Edition #:	01

QUALITY ASSURANCE REPORT – GMP

TEST PERFORMED	PROCEDURAL REFERENCE	TEST PERFORMED	PROCEDURAL REFERENCE
[X] M-250	SOP's 3008, 3011, 3013	[ ] M-700	SOP's 3008, 3009, 3010
[ ] M-300	SOP's 3008, 3014	[ ] M-800	SOP's 3008, 3011, 3016
[ ] M-350	SOP's 3008, 3014, 3015		

Bionique Sample ID #(s) 61955 61956 61957 61958

This testing procedure was performed in compliance with the FDA's Current Good Manufacturing Practice (cGMP) standards (to the extent that the regulations pertain to the procedures performed) as specified in the Code of Federal Regulations, Title 21 Parts 210 and 211 [21 CFR 210 & 211]. All related records derived from the test procedures have been reviewed by the Quality Assurance Department. The individual's signature below verifies that the methods and procedures referenced above have been followed and that the Final Report accurately reflects the raw data generated during the course of the procedures. All records, including raw data and final reports are archived on site for a minimum of seven years.

The specified test's procedures determine the intervals at which samples are inspected. The medium used for testing must pass quality control mycoplasmal growth promotion testing and sterility testing. Traceability of all of the components used is assured and supporting documentation can be supplied upon request.

Quality Assurance Review Date: 8/25/10

Reviewed By [redacted] QA Assistant: [redacted]

NOTE:

1. Prior to receipt at Bionique® Testing Laboratories, Inc., the stability of the test article is the responsibility of the company submitting the sample. Bionique Testing Laboratories Inc. will assume responsibility for sample stability following receipt and prior to being placed on test.
2. This test is for the detection of microbiological growth and does not require statistical validation.

REFERENCES

Regulatory:

1. Department of Health and Human Services, Food and Drug Administration (USA) [FDA]. Code of Federal Regulations [CFR], Title 21 CFR Part 210, Current Good Manufacturing Practice in Manufacturing, Processing, Packing, or Holding of Drugs; General. FDA. Office of the Federal Register, National Archives and Records Department.
2. Department of Health and Human Services, Food and Drug Administration (USA) [FDA]. Code of Federal Regulations [CFR], Title 21 CFR Part 211, Current Good Manufacturing Practice for Finished Pharmaceuticals. FDA. Office of the Federal Register, National Archives and Records Department.
3. Department of Health and Human Services, Food and Drug Administration (USA) [FDA]. Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals, Director, Center for Biologics Evaluation and Research, FDA. May, 1993. Docket No. 84N-0154.
4. Department of Health and Human Services, Food and Drug Administration (USA) [FDA]. Code of Federal Regulations [CFR], Title 21 CFR Part 610.30, General Biological Products Standards; Subpart D, Test for Mycoplasma. FDA. Office of the Federal Register, National Archives and Records Department.

General:

1. Barile MF, Kern J. Isolation of Mycoplasma arginini from commercial bovine sera and its implication in contaminated cell cultures. Proceedings of the Society for Experimental Biology and Medicine, Volume 138, Number 2, November 1971.
2. Chen, T.R. In situ detection of mycoplasma contamination in cell cultures by fluorescent Hoechst 33258 stain. Experimental Cell Research, 104: 255-262, 1977.
3. Carolyn K. Lincoln and Daniel J. Lundin. Mycoplasma Detection and Control. U. S. Fed. for Culture Collections Newsletter, Vol. 20, Number 4, 1990.
4. Fetal Bovine Serum; Proposed Guideline. National Committee For Clinical Laboratory Standards (NCCLS), Vol. 10, Number 6, 1990. (NCCLS publication M25-P).
5. McGarrity GJ, Sarama J, Vanaman V. Cell Culture Techniques. ASM News, Vol. 51, No. 4, 1985.
6. Tully JG, Razin S. Methods in Mycoplasmology, Volumes I and II. Academic Press, N.Y., 1983.
7. Barile MF, Razin S, Tully JG, Whitcomb RF. The Mycoplasmas, Volumes 1-4. Academic Press, N.Y., 1979.
8. http://www.bionique.com/ - Safe Cells Insights

bionique Testing Laboratories

MYCOPLASMA TESTING SERVICES

BIONIQUE TESTING LABORATORIES, INC.

[redacted]

Document#: DCF3013D
Edition#: 10
Effective Date: 07/15/2003
Title: **M-250 FINAL REPORT SHEET**

M-250 FINAL REPORT

Direct Specimen Culture
Procedure 3008, 3011, 3013

TO: WiCell QA
WiCell Research Institute

[redacted]

BTL SAMPLE ID#: **61955** P.O.#: [redacted] DATE REC'D: **07/27/2010**

TEST/CONTROL ARTICLE:

WA16-WB0029 #3163

LOT#: **NA**

DIRECT CULTURE SET-UP (DAY 0) DATE: **07/28/2010**

INDICATOR CELL LINE (VERO) SEE DNA FLUOROCHROME RECORD SHEET

			DATE
THIOGLYCOLLATE BROTH	DAY 7	+ ⊖	08/04/2010
	DAY 28	+ ⊖	08/25/2010
BROTH-FORTIFIED COMMERCIAL			
0.5 mL SAMPLE	DAY 7	+ ⊖	08/04/2010
6.0 mL BROTH	DAY 28	+ ⊖	08/25/2010
BROTH-MODIFIED HAYFLICK			
0.5 mL SAMPLE	DAY 7	+ ⊖	08/04/2010
6.0 mL BROTH	DAY 28	+ ⊖	08/25/2010
BROTH-HEART INFUSION			
0.5 mL SAMPLE	DAY 7	+ ⊖	08/04/2010
6.0 mL BROTH	DAY 28	+ ⊖	08/25/2010

(See Reverse)

Document#: DCF3013D
Edition#: 10
Effective Date: 07/15/2003
Title: M-250 FINAL REPORT SHEET

SAMPLE ID#: **61955**		AEROBIC	MICROAEROPHILIC	DATE
AGAR PLATES-FORTIFIED COMMERCIAL	DAY 7	+ ⊖	+ ⊖	08/04/2010
	DAY 14	+ ⊖	+ ⊖	08/11/2010
	DAY 21	+ ⊖	+ ⊖	08/18/2010
AGAR PLATES-MODIFIED HAYFLICK	DAY 7	+ ⊖	+ ⊖	08/04/2010
	DAY 14	+ ⊖	+ ⊖	08/11/2010
	DAY 21	+ ⊖	+ ⊖	08/18/2010
AGAR PLATES-HEART INFUSION	DAY 7	+ ⊖	+ ⊖	08/04/2010
	DAY 14	+ ⊖	+ ⊖	08/11/2010
	DAY 21	+ ⊖	+ ⊖	08/18/2010
BROTH SUBCULTURES (DAY 7)		DATE: 08/04/2010		
AGAR PLATES-FORTIFIED COMMERCIAL	DAY 7	+ ⊖	+ ⊖	08/11/2010
	DAY 14	+ ⊖	+ ⊖	08/18/2010
	DAY 21	+ ⊖	+ ⊖	08/25/2010
AGAR PLATES-MODIFIED HAYFLICK	DAY 7	+ ⊖	+ ⊖	08/11/2010
	DAY 14	+ ⊖	+ ⊖	08/18/2010
	DAY 21	+ ⊖	+ ⊖	08/25/2010
AGAR PLATES-HEART INFUSION	DAY 7	+ ⊖	+ ⊖	08/11/2010
	DAY 14	+ ⊖	+ ⊖	08/18/2010
	DAY 21	+ ⊖	+ ⊖	08/25/2010

RESULTS: No detectable mycoplasmal contamination

8/25/10
Date

[redacted]
Laboratory Supervisor
[redacted]

M-250 Procedural Summary: The objective of this test is to ascertain whether or not detectable mycoplasmas are present in an in vitro cell culture sample, be it a primary culture, hybridoma, master seed stock or cell line. This procedure combines an indirect DNA staining approach to detect non-cultivable mycoplasmas with a direct culture methodology utilizing three different mycoplasmal media formulations. The indirect approach involves the inoculation of the sample into a mycoplasma-free VERO (ATCC) indicator cell line and performing a DNA fluorochrome assay after 72-120 hours of incubation. The direct culture aspect of the test utilizes three different mycoplasmal media including both broth and agar formulations. The sample is inoculated into each of the 3 broth formulations and also onto duplicate plates (0.1 mL/plate) for each of the 3 agar formulations. Subculture from broth to fresh agar plates is carried out after 7 days incubation. Agar plates are incubated aerobically and microaerophillically in order to detect any colony forming units morphologically indicative of mycoplasmal contamination. Issuance of the final report with signature of the Laboratory Director signifies that the required controls were performed concurrently with the test sample(s) as detailed in the referenced SOPs and that all test conditions have been found to meet the required acceptance criteria for a valid test, including the appropriate results for the positive and negative controls.

UW Health
University of Wisconsin
Hospital and Clinics

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

Date: 03/18/2011 08:53:45

To: WiCell Research Institute
Cytogenetics Lab
[redacted]

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, 3163-HLA	**DQB SSP**		01:01	08:01	07:01g	03:01				
64813 /	**A,B,C SSP**	03/17/2011								
03/17/2011	**DRB Seq**	03/17/2011	Class I comment: C*07:01g includes *07:01/06/18							
			Class II comment: HLA Allele database: IMGT/A 3.3.0 2011-01-14							

[redacted]
nager
HLA/Molecular Diagnostics Laboratory
3-18-11 09 W
Date

[redacted]
ctor
HLA/Molecular Diagnostics Laboratory
3/22/11
Date

New York Blood Center
Laboratory of Immunohematology and Genomics
45-01 Vernon Blvd., Long Island City, N.Y. 11101
718-752-4771 • Fax 718-752-4747

March 28, 2011

WiCell Research Institute
Attn: Quality Assurance
505 South Rosa Road, Suite 120
Madison, WI 53719

SAMPLE: DNA 3163-ABO (MA#109-11)

Date Received: 03/15/11
Sample Date: not provided

HISTORY: DNA from cell line.

TEST REQUESTED: Genotype for *ABO* and common *RH*

TESTING PERFORMED: *ABO:* Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) testing for nucleotide (nt) positions 261 (O^1), 467 (A^2), 703 (B), and 1096 (B and O^2). *RH:* Multiplex PCR-RFLP for *RHD* and *RHCE*C/c*. PCR-RFLP for *RHCE* Exon 5 (676C>G for E/e).

DNA RESULTS: PCR-RFLP indicated homozygous for nt 261G characteristic of O^1 alleles.

Result	Test Method
ABO* B/O^1	PCR-RFLP
RHD positive for exons 4, 7 and no inactivating pseudogene	Multiplex PCR
RHCE*C/c	Multiplex PCR
RHCE*e/e	PCR-RFLP

Predicted phenotype: Group B, RhD+C+E–c+e+

[redacted]
Manager, Genomics

[redacted]SBB, CQA(ASQ)
Director of Operations, Immunohematology

These *in vitro* diagnostic tests were developed and their performance characteristics established in the Molecular Analysis Laboratory. The tests have not been submitted to the Food and Drug Administration (FDA) for clearance or approval and; therefore, are not FDA-licensed tests. The Molecular Analysis Laboratory is certified under the Clinical Laboratory Improvement Amendment (CLIA) of 1988 as qualified to perform high complexity clinical testing. The New York Blood Center has been approved, by the New York State Department of Health to perform these tests under its current Clinical Laboratory Permit. These results are intended to predict a blood group antigen profile in a patient or donor, and are not intended for clinical diagnosis or as the sole means for patient management decisions. There are situations where testing DNA of a person may not reflect the red cell phenotype and not all performance characteristics have been determined. Nucleotide changes that inactivate gene expression or rare new variant alleles may not be identified in these assays.

New York BloodCenter

Immunohematology
Telephone: 718-752-4771
Genomics
Telephone: 718-752-4637

Sample: MA109-11; 3163-ABO

Test: RH/ABO - GF

CPT CODE	Description/Molecular Testing	RH/ABO
83890	Isolation /extraction	X1
83892	Enzymatic digestion	X3
83894	Separation by electrophoresis	X1
83912	Interpretation and report	X1

Charles River Research Animal Diagnostic Services
251 Ballardvale Street, Wilmington, MA 01887 USA
Tel: 800-338-9680 Fax: 978-658-7698

Sponsor: WiCell Research Institute **Accession #: 2010-034920**

Diagnostic Summary Report

[redacted]

Attn: Jessica Martin

Tel: 608-577-6625

Received: 27 Jul 2010
Approved: 28 Jul 2010, 16:40

Bill Method: [redacted]
Test Specimen: Human Cells Human

Sample Set	Service (# Tested)	Profile	Assay	Tested	+	+/-	?
#1	Infectious Disease PCR (2)	All Results Negative					

+ = Positive, +/- = Equivocal, ? = Indeterminate

Service Approvals

Service	Approved By*	Date
Infectious Disease PCR	[redacted]	28 Jul 2010, 16:40

To assure the SPF status of your research animal colonies, it is essential that you understand the sources, pathobiology, diagnosis and control of pathogens and other adventitious infectious agents that may cause research interference. We have summarized this important information in infectious agent **Technical Sheets**, which you can view by visiting http://www.criver.com/info/disease_sheets.

**This report has been electronically signed by laboratory personnel. The name of the individual who approved these results appears in the header of this service report. All services are performed in accordance with and subject to General Terms and Conditions of Sale found in the Charles River Laboratories-Research Models and Services catalogue and on the back of invoices.*

Charles River Research Animal Diagnostic Services
251 Ballardvale Street, Wilmington, MA 01887 USA
Tel: 800-338-9680 Fax: 978-658-7698

Sponsor: WiCell Research Institute **Accession #: 2010-034920**

Product: Not Indicated **Test Specimen:** Human Cells Human **Received:** 27 Jul 2010

Molecular Diagnostics Infectious Disease PCR Results Report

Department Review: Approved by [redacted], 28 Jul 2010, 16:40*

Human Comprehensive Viral PCR Panel

Sample #: / Code :	1 WA16-WB0029 0988	2 WA21-WB0034 5961
John Cunningham virus	-	-
BK virus	-	-
Herpesvirus type 6	-	-
Herpesvirus type 7	-	-
Herpesvirus type 8	-	-
Parvovirus B19	-	-
Epstein-Barr Virus	-	-
Hepatitis A virus	-	-
Hepatitis B virus	-	-
Hepatitis C virus	-	-
HPV-16	-	-
HPV-18	-	-
Human T-lymphotropic virus	-	-
Human cytomegalovirus	-	-
HIV-1	-	-
HIV-2	-	-
Adeno-associated virus	-	-
Human Foamy Virus	-	-
LCMV PCR	-	-
Hantavirus Hantaan PCR	-	-
Hantavirus Seoul PCR	-	-
Mycoplasma Genus PCR	-	-
DNA Spike	**PASS**	**PASS**
RNA Spike	**PASS**	**PASS**
NRC	**PASS**	**PASS**

Remarks: - = Negative; I = Inhibition, +/- = Equivocal; + = Positive.

Sample Suitability/Detection of PCR Inhibition:
Sample DNA or RNA is spiked with a low-copy number of a exogenous DNA or RNA template respectively. A spike template-specific PCR assay is used to test for the spike template for the purpose of determining the presence of PCR inhibitors. The RNA spike control is also used to evaluate the reverse-transcription of RNA. Amplification of spike template indicates that there is no detectable inhibition and the assay is valid.

NRC:
The nucleic acid recovery control (NRC) is used to evaluate the recovery of DNA/RNA from the nucleic acid isolation process. The test article is spiked with a low-copy number of DNA/RNA template prior to nucleic acid isolation. A template-specific PCR assay is used to detect the DNA/RNA spike.

**This report has been electronically signed by laboratory personnel. The name of the individual who approved these results appears in the header of this service report.*