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Award Number: W81XWH-11-1-0345

TITLE: Production of a Novel OX40 Ligand for Clinical Use

PRINCIPAL INVESTIGATOR: Andrew D. Weinberg, Ph.D.

CONTRACTING ORGANIZATION: Providence Portland Medical Center
Portland, OR 97213

REPORT DATE: October 2012

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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Production of a Novel OX40 Ligand for Clinical Use

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-11-1-0345

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Andrew D. Weinberg, Ph.D.

E-Mail: andrew.weinberg@providence.org

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Providence Portland Medical Center
Portland, Oregon 97213

8. PERFORMING ORGANIZATION REPORT NUMBER

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

.
Cancer cells have evolved to evade immune-mediated destruction through several documented mechanisms. Our group has developed a technique to enhance immune function in tumor-bearing hosts by targeting a protein on the surface of white blood cells, termed OX40. This type of immune modulation leads to therapeutic benefit in tumor-bearing mice. We have produced a protein that binds to the human OX40 protein and activates human white blood cells. We have a cell line that produces high quantities of this protein and our goal is to test this protein for safety and efficacy in non-human primates so that we can obtain FDA approval for clinical trials in cancer patients. The long-range goal of this proposal is translate these findings to prostate cancer patients.

15. SUBJECT TERMS- T Cells, Co-stimulation, OX40, tumor immunotherapy

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
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a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
U	U	U	UU	98	

Table of Contents

DOD Prostate Award Research Technical Reporting: 1 year Progress Report
Laboratory-Clinical Transition Award:

PC101977 – Production and Characterization of a Novel OX40 Ligand for Clinical Use.
PI -Andrew Weinberg, PhD

INTRODUCTION: Cancer cells have evolved to evade immune-mediated destruction through several documented mechanisms. Our group has developed a technique to enhance immune function in tumor-bearing hosts through the use of OX40 agonists, which can lead to regression of tumors of various histologies, including prostate cancer. In particular, we have produced a human OX40 agonist, termed OX40L:ILZ:Ig (OX40L:Ig) that has potent biologic function in vitro and is produced in large quantities by tissue culture cells. The ILZ portion of the chimeric protein was initially a trimerization domain obtained from a yeast sequence. In the past few years we have produce a fully human OX40 ligand protein and it was tested for in vivo biologic activity in non-human primates and had potent activity. The ultimate goal of the current research is to produce clinical grade human OX40L:Ig to test in clinical trials for patients suffering from prostate cancer. With that goal in mind we made a GMP compliant cell line that produces large quantities of the protein within the first year of funding. Future work will include testing this protein in monkey primates for toxicology studies, which are typically mandated by the FDA prior to approval for phase I studies to be conducted in cancer patients.

BODY: The first year of funding was spent on constructing a GMP cell line that produces a fully human version of the OX40L:Ig fusion protein. In previous work funded by the DOD we characterized the optimal sequences that gave us the most potent biologic activity for the human OX40L:Ig fusion protein. This protein was subsequently tested in non-human primate studies and the potent biologic activity observed allowed for confidence to move forward with cell line production. Since GMP cell line construction is a multi-faceted task that requires compliance on several levels, our lab is not equipped

to perform such tasks. Therefore we contracted the work to WuXi AppTec, Inc to perform all the tasks necessary to produce a cell line that the FDA would deem appropriate for manufacturing large quantities for clinical use. Prior to cell bank manufacture the cells producing the OX40L:Ig fusion protein had to be tested for sterility and mycoplasma contamination. The cells were both sterile and devoid of mycoplasma and therefore WuXi proceeded with the cell bank creation in their GMP suite. 200 vials were created and are now being stored at two separate GMP storage facilities. After the cell bank was produced a number of tests were performed to detect potential pathogens that may have contaminated the cell bank. These tests included testing for bacterial and fungal contaminants as well as several viral-specific tests all of which are mandated by the FDA. There were no pathogens found within the cell bank therefore we now have a cell line that is ready for GMP protein production. We have added documentation from WuXi showing the testing was indeed completed and the certificate of analysis is included as well (see attached reports).

During the last year of funding we licensed the OX40L:Ig fusion program to an industry sponsor for testing in cancer patients. The license deal is a collaboration between the two groups and is especially advantageous in terms of increased support for the program as far as taking this platform through phase I, II, and III clinical trials. The sponsor's protein chemistry group has taken several antibodies and Ig fusion proteins to the clinic and has vast experience communicating with the FDA to gain approval for clinical testing. Upon evaluating the OX40L:Ig sequence their group noticed that we had left the Fc gamma-binding domain of the Ig portion intact in the construct we produced. The industry sponsor mutated that sequence so that it no longer bound the Fc gamma receptor and they found that the OX40L:Ig fusion protein with the mutated sequence had a reduced capacity to enhance Fc-mediated NK killing in an in vitro assay. While they felt that mutating the OX40L:Ig sequence would lead to a "safer" profile for FDA screening purposes, it was not clear whether mutating this sequence would decrease the anti-tumor efficacy of the OX40L:Ig protein. Therefore both groups agreed that further testing of the OX40L:Ig fusion protein was warranted in tumor models. To this end we have produced the identical murine version of the OX40L:Ig fusion protein and we intend to study its anti-tumor efficacy in mice that are devoid in Fc-gamma binding in year 2 of

this grant. If these studies show that the anti-tumor efficacy is intact then we will produce the mutated mouse OX40L:Ig fusion protein and test it in our tumor models. However, if the mutation decreases the anti-tumor efficacy of the OX40L:Ig fusion protein we will proceed with the original protein construct and move forward with monkey toxicity as planned in the original aims of this grant.

KEY RESEARCH ACCOMPLISHMENTS:

- We have produced a GMP compliant cell line that can be used to manufacture the human OX40L:Ig fusion protein.
- We now know that the Fc-gamma binding portion of the human OX40L:Ig fusion protein confers increased NK cell activation and killing.
- We have produced a functional murine OX40L:Ig fusion protein and it will be used to model the human version in therapy experiments within tumor-bearing mice.

REPORTABLE OUTCOMES:

The experiments planned within this proposal are to develop an OX40L:Ig fusion protein that can be injected into cancer patients. Hence, most of the experiments somewhat confirm what is already known in the literature and thus may be difficult to publish. However the data regarding whether the OX40L:Ig fusion protein needs to bind the Fc-gamma receptor for biologic activity is an important question, which could be a publishable outcome in the future.

CONCLUSIONS:

In summary, we have produced an OX40L:Ig secreting cell line that has gone through the rigors of a GMP testing for a FDA compliant cell bank. This cell bank is now being stored in two different GMP compliant facilities and will hopefully be used to produce protein for cancer patient clinical trials. We now have the financial backing from an industry sponsor that will help take this protein into clinical trials. Upon their assessment of the protein sequence they deleted the Fc-gamma binding amino acids and found that there was less NK-cell activation and hence might be a safer profile for FDA

consideration. However, we need to assess if the NK-cell activation is important for the anti-tumor efficacy of the protein and if it is we will proceed forward with protein production from the GMP compliant cell line described above.

Amended CERTIFICATE OF ANALYSIS

Product:	**hOX40L:Ig clone 755-19 MCB**
Manufacturer:	**WuXi AppTec, Inc.**
Client :	**Providence ***
Accession Number:	**11-003668**
Lot Number:	**120170969**
Freeze Date:	**24FEB2012**
Number of Vials:	**235**
Volume / Vial:	**1.5 mL**
Cryopreservative:	**90.0% of Complete CD Opti-CHO media with 8 mM GlutaMAX, and 10.0% of DMSO.**

TEST	SPECIFICATION	RESULTS
Sterility (Test # 30744, 30744A)	Negative	Negative
Bacteriostasis / Fungistasis (Test # 30736)	No Bacteriostatic / Fungistatic activity	No Bacteriostatic / Fungistatic activity
Mycoplasma Detection with Mycoplasmastasis (Test # 32700)	Considered Negative for Mycoplasma contamination	Negative
Post Cell Bank- Cell Growth (Test # 32370)	≥ 9.0 x 10^5 cells/mL on day 2-3 at P+2	Vial # 006 = 1.1 x10^6 viable cells/mL on Day 3 at P+2 Vial # 130 = 1.3 x10^6 viable cells/mL on Day 3 at P+2 Vial # 230 = 1.6 x10^6 viable cells/mL on Day 3 at P+2

*Amendment to change the client's name.

The material referenced on this COA was manufactured in compliance with U.S. 21 CFR 210 and 211, EMEA GMP (Rules and Guidance for Pharmaceutical Manufacturers and Distributors), and applicable ICH Q7 standards for Phase I through Commercial Material.

Robyn Grunyk — Quality Assurance Area Management

04Jan13 — Date

Master Document Date Effective 18Jan12

WuXi AppTec, Inc. • 4751 League Island Blvd., Philadelphia, PA 19112 • (800) 622-8820 (215) 218-5500 FAX (215) 218-5990

E-FR-1-11-003668.30011

FINAL STUDY REPORT

STUDY TITLE: **Hamster Antibody Production (HAP) Test**

TEST PROTOCOL NUMBER: 30011.14

TEST ARTICLE IDENTIFICATION	WUXI APPTEC ACCESSION NUMBER
hOX40L:Ig clone 755-19 MCB WuXi AppTec Accession # 11-003668 Lot#120170969	11-003668

SPONSOR:

Nick Morris
Providence Portland Medical Center
Cancer Research Rm 2NC29
4805 NE Glisan Street
Portland, OR 97213

PERFORMING LABORATORY:

WuXi AppTec, Inc.
4751 League Island Blvd.
Philadelphia, PA 19112

SUBCONTRACTED TO:

Charles River Laboratories
251 Ballardvale Street
Wilmington, MA 01887

WUXI APPTEC ACCESSION NUMBER	RESULTS
11-003668	The presence of 11 hamster-specific adventitious viral contaminants was not detected in the test article.

QUALITY ASSURANCE UNIT SUMMARY

STUDY: Hamster Antibody Production (HAP) Test

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

Refer to Charles River Laboratories Final Report.

Quality Assurance

16 May 12
Date

GOOD LABORATORY PRACTICES STATEMENT

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

Study Director

17 May 12
Date

Performed at Charles River Laboratories:

Charles River Laboratories
251 Ballardvale Street
Wilmington, MA 01887

1.0 SCHEDULING

DATE SAMPLES RECEIVED:	March 6, 2012
STUDY INITIATION DATE:	March 26, 2012
STUDY COMPLETION DATE:	See page 2 for Study Director's signature and date.

2.0 TEST ARTICLE PREPARATION

On March 6, 2012, the Cell Biology Laboratory received 3 vials from QA frozen in dry ice (vial #006, 130, 230) each containing 1.0 mL of "MCB," and designated for use in protocol 32370, "Post Cell-Bank Cell Growth for Testing." Cell growth was initiated immediately. After 2 passages, the cells were transferred to protocol 30040 "Cell Growth for Assays" using Sponsor's media and instructions.

On March 22, 2012, the Cell Biology Laboratory designated 2 vials, each containing 3.0 mL of test article ($1x10^7$ cells/mL) for use in this assay. The test article was stored at ≤ -60° until shipment to the subcontractor. On March 26, 2012, 1 vial containing 3.0 mL of test article cells was shipped via overnight carrier in dry ice to the subcontractor.

3.0 RECORD RETENTION

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

charles river

FINAL REPORT

Study No.: 2012-017551

Test Article: 11-003668

Protocol No.: PR-32-7

Protocol Title: Hamster Antibody Production (HAP) Test

Report Date: 11-May-2012

Sponsor: WuXi AppTec, Inc.
4751 League Island Blvd.
Philadelphia , PA 19112

Prepared By:

CHARLES RIVER
251 Ballardvale Street
Wilmington, MA 01887 USA
Tel: 781-222-6000
Fax: 978-988-9093

charles river

Final Report

Protocol No.: PR-32-7
Protocol Title: Hamster Antibody Production (HAP) Test
Study No.: 2012-017551

Table of Contents

charles river

Final Report

Protocol No.: PR-32-7
Protocol Title: Hamster Antibody Production (HAP) Test
Study No.: 2012-017551

I. PURPOSE/JUSTIFICATION

With the growth of the biotechnology industry, biologics derived from rodent tissues and cell culture are being administered to people more frequently. To be used safely, and to comply with government regulations, these products must be tested to show that they are free of extraneous rodent viruses. Although viruses may cause disease in animals and cytopathic effects in cell culture, monitoring by these methods is not reliable, as they are often inapparent or non-specific. However, when laboratory animals are the host system, serum samples from inoculated animals can be assayed for virus-specific antibodies formed in response to infection. The purpose of this test is to determine whether the test article contains murine viruses that are known to infect hamster tissues.

In addition to the viruses recommended for inclusion on this test by the Guidance for Industry, Q5A Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin, rodent parvoviruses and Hantavirus have been added to the virus panel in this test. Hamsters are highly susceptible to infection with these viruses under experimental conditions, even though they are not indigenous to hamsters. Hantaan virus is a concern because rodent Hantaviruses are zoonotic.

II. TEST FACILITY

Charles River Laboratories
251 Ballardvale Street
Wilmington, MA 01887 USA

III. PERSONNEL

Name	Title
Rodney A. Lequillo	Study Director
Dolores Welch	Senior Technologist
Keith Provencher	Supervisor

IV. TEST SCHEDULE

The study was initiated on 30-Mar-2012.

V. QUALITY ASSURANCE

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

VI. TEST MATERIAL

A. Test Article

1.	Designation:	11-003668
2.	Lot:	12017069
3.	Species:	Hamster
4.	Cell Type:	CHO
5.	Vial Label:	30011 22Mar12 11-003668 $1x10^7$ cells/ml
6.	Quantity:	3mL
7.	Received by and Date:	Dolores Welch on 27Mar2012
8.	Condition on receipt:	Frozen on Dry Ice

B. Control Article

1.	Material Name:	Hank's Balanced Salt Solution (HBSS)
2.	Manufacturer:	Sigma
3.	Lot #:	RNBB5690
4.	Expiration Date:	10/2012

VII. TEST SYSTEM

A. Species/Strain

Hamster, *Mesocricetus auratus*/ Golden Syrian

B. Sex

Female

C. Age at Inoculation

Hamsters were 27 days old at the time of inoculation.

D. Total Number of Test Systems

Six

E. Source

All animals were obtained from a Charles River facility on which routine health monitoring is performed. Colonies from which the animals were obtained are screened for the viruses included on the panel for this test.

F. Identification

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

VIII. HOUSING AND HUSBANDRY PRACTICES

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

A. Housing

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

B. Acclimation

Hamsters were acclimated for a minimum of three days.

C. Feed and Water

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

D. Environment

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

E. Animal Welfare

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

IX. EXPERIMENTAL DESIGN

The Test Article, at two different dilutions, is inoculated into hamsters by multiple routes to increase the likelihood of infection with viruses which may be present in the test article. The hamsters 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 hamsters.

X. PROCEDURE

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

A. Test Article Receipt and Processing

1. Upon receipt, the Test Article is maintained in a dedicated storage unit at -70°C ±10° C.
2. Prior to inoculation, a sample of the Test Article was diluted 1/10 in HBSS. The undiluted Test Article was designated "Inoc I". The diluted Test Article was designated "Inoc II".

B. Animal Inoculation and Testing

Test Article Inoculation- Day 0, 03-Apr-2012

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

Animal Identification	Test Material	Dose/Route
1, 2	Inoc I (TA)	0.5mL intraperitoneal, ~0.05mL intranasal, ~0.05mL per os
3, 4	Inoc II (TA)	
5, 6	Control Article	

2. Test Article inoculated hamsters are housed together to facilitate the spread of potential low-titered virus from Inoculum I hamsters to Inoculum II hamsters.

C. Clinical Observations

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

D. Euthanasia and Blood Collection

1. 28 days post inoculation, the hamsters were euthanized using CO_2 asphyxiation.
2. A blood sample was collected from each hamster and serum samples were prepared for serologic testing.

E. Serological Assay

1. The indirect enzyme-linked immunosorbent assay (ELISA) was the primary serologic method for detection of antibodies to most viruses.
2. Results were reported as positive (+), negative (-), equivocal (+/-), or as a non-specific reaction with tissue control (TC). Samples that show +, +/-, or TC reactions are retested by an alternative serologic method.
3. Standard immune and non-immune control sera were included in all serologic tests.
4. Serum from each animal was assayed for antibodies to the viruses listed below.

Name	Family
Sendai virus (SEND)	Paramyxoviridae
Simian virus 5 (SV-5)	Paramyxoviridae
Pneumonia virus of mice (PVM)	Paramyxoviridae
Minute virus of mice (MVM)	Parvoviridae
Kilham's Rat virus (KRV)	Parvoviridae
Toolan's H-1 virus (H-1)	Parvoviridae
Rodent parvovirus , Non-structural Protein 1 (PARV NS1)	Parvoviridae
Theiler's murine encephalomyelitis virus (GDVII)	Picornaviridae
Reovirus (REO)	Reoviridae
Lymphocytic choriomeningitis virus (LCMV)	Arenaviridae
Hantaan virus (HANT)	Bunyaviridae

XI. EVALUATION OF THE TEST RESULTS

A. Evaluation of Serological Assay Results

1. In order for the results of the assay to be evaluated:
   a) Three or more test article-inoculated hamsters must survive at least 28 days post inoculation with serum available for the assay.

b) Samples from control article-inoculated animals are seronegative for all listed viruses.

c) The standard immune and non-immune control sera used in the serology assay must yield acceptable serologic test results.

2. A serum sample is considered positive for a viral agent if a positive or equivocal result obtained in the ELISA is confirmed by an alternative method.

   A positive reaction in the primary assay will be interpreted as nonspecific, if the reaction is not substantiated by the alternative assay.

B. Evaluation of the HAP Test

1. If sera from two or more hamsters have a positive response on confirmatory testing, then the test article is considered to be contaminated by a viral agent.
2. If serum from only one hamster has a positive response on confirmatory testing, the test will be declared indeterminate for that virus and may be repeated.
3. According to the results of serologic testing for the virus-specific antibodies, rodent viruses were not detected in the test article, 11-003668.

XII. STORAGE AND ARCHIVING

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

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

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

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

XIII. APPROVALS

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

Rodney A. Legwilla — 11 May 2012

CHARLES RIVER — Date

Study Director

charles river

QUALITY ASSURANCE STATEMENT

This study (**2012-017551, PR-32-7**) has been inspected by the Quality Assurance Unit to assure conformance with the following good laboratory practice regulations: US Food and Drug Administration, Good Laboratory Practice Regulations, Final Rule, 21 CFR Part 58. Reports were submitted in accordance with SOPs as follows.

QAU INSPECTION DATES

		Dates Findings Submitted to:	
Dates of Inspection	Phase(s) Inspected	Study Director	Study Director Management
01 Sep 2010	Protocol	30 Mar 2012	30 Mar 2012
01 May 2012	HAP Bleeds	01 May 2012	01 May 2012
09 May 2012	Serology Batch Record WE 04 May 2012	09 May 2012	09 May 2012
10 May 2012	Final Report and Raw Data Audit	11 May 2012	11 May 2012

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

Rebecca Miller
Rebecca Miller
Quality Assurance Auditor

11 May 2012
Date

Charles River Research Animal Diagnostic Services
251 Ballardvale Street, Wilmington, MA 01887 USA
Tel: 781.222.6701

Serology Results Report

Sponsor: WuXi AppTec Inc. **Accession #: 2012-017551 (GLP)**

HAP Test Serology Profile

Sample #: / Code :	1 I	2 I	3 II	4 II	5 CON	6 CON
ELISA SEND	-	-	-	-	-	-
ELISA SV-5	-	-	-	-	-	-
ELISA PVM	-	-	-	-	-	-
ELISA MVM	-	-	-	-	-	-
ELISA KRV	-	-	-	-	-	-
ELISA H-1	-	-	-	-	-	-
ELISA PARV NS1	-	-	-	-	-	-
ELISA GDVII	-	-	-	-	-	-
ELISA REO	-	-	-	-	-	-
ELISA LCMV	-	-	-	-	-	-
ELISA HANT	-	-	-	-	-	-

Remarks: ELISA/IFA Results: - = Negative; +/- = Equivocal; + = Moderate to strong positive; TC = Non-specific reaction with tissue control.
These test results are valid according to the following criteria:

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

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

Attachment 2

UPS CampusShip: View/Print Label

1. **Ensure there are no other shipping or tracking labels attached to your package.** Select the Print button on the print dialog box that appears. Note: If your browser does not support this function select Print from the File menu to print the label.

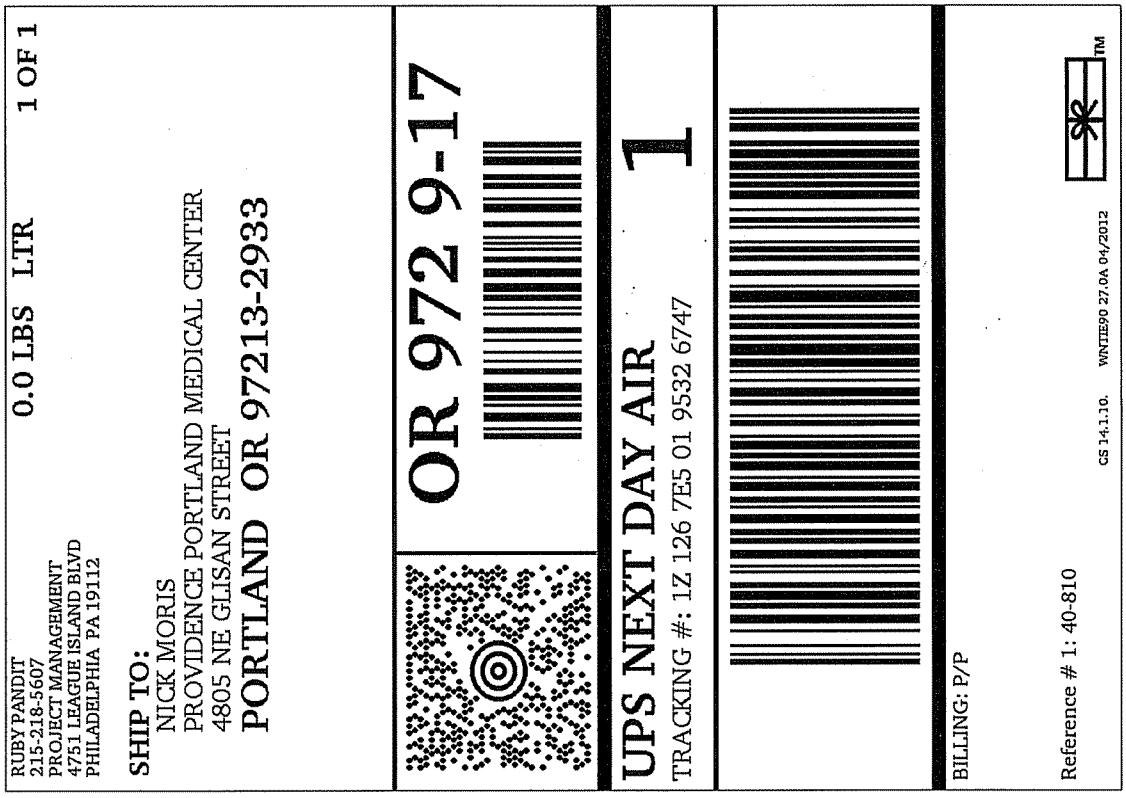
2. **Fold the printed sheet containing the label at the line so that the entire shipping label is visible. Place the label on a single side of the package and cover it completely with clear plastic shipping tape. Do not cover any seams or closures on the package with the label.** Place the label in a UPS Shipping Pouch. If you do not have a pouch, affix the folded label using clear plastic shipping tape over the entire label.

3. **GETTING YOUR SHIPMENT TO UPS**

   UPS locations include the UPS Store®, UPS drop boxes, UPS customer centers, authorized retail outlets and UPS drivers.
   Find your closest UPS location at: www.ups.com/dropoff
   Take your package to any location of The UPS Store®, UPS Drop Box, UPS Customer Center, UPS Alliances (Office Depot® or Staples®) or Authorized Shipping Outlet near you. Items sent via UPS Return Services(SM) (including via Ground) are also accepted at Drop Boxes. To find the location nearest you, please visit the Resources area of CampusShip and select UPS Locations.

   Customers with a Daily Pickup
   Your driver will pickup your shipment(s) as usual.

FOLD HERE



E-FR-1-11-003668.30236

FINAL STUDY REPORT

STUDY TITLE: **Detection of Adventitious Bovine Viruses: Extended Screening for Non-Bovine Cell Lines Grown in Bovine Serum or Products**

TEST PROTOCOL NUMBER: 30236.10

TEST ARTICLE IDENTIFICATION	WUXI APPTEC ACCESSION NUMBER
hOX40L:Ig clone 755-19 MCB WuXi AppTec Accession # 11-003668 Lot#120170969	11-003668

SPONSOR:

Nick Morris
Providence Portland Medical Center
Cancer Research Rm 2NC29
4805 NE Gilsan Street
Portland, OR 97213

PERFORMING LABORATORY:

WuXi AppTec, Inc.
4751 League Island Blvd.
Philadelphia, PA 19112

WUXI APPTEC ACCESSION NUMBER	RESULTS
11-003668	No evidence of viral contamination was detected in the test article.

QUALITY ASSURANCE STATEMENT

STUDY: Detection of Adventitious Bovine Viruses: Extended Screening for Non-Bovine Cell Lines Grown in Bovine Serum or Products

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

Phase Inspected	Date
Step 4.3.3 Handling each cell line separately, remove the medium from the Vero and BT flasks (two of each) to be inoculated with the test article. Add 1.0 mL test article to each flask.	April 12, 2012

Quality Assurance

11 Jun 12
Date

GOOD LABORATORY PRACTICES STATEMENT

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

Jason Kuchar
Study Director

11 Jun 12
Date

Professional Personnel involved in study:

Judith Franco	Elizabeth Roessner	Emily Mastrocola	Lauren Yannarella
Lauren Gasis	Kristin Daumer	Abibatou Ndoye	Babatunde Sholanke
Jason Kuchar	Laura Schina		

1.0 PURPOSE

The purpose of this study was to detect the presence of bovine viruses in a non-bovine cell lines (or harvest fluids) exposed to bovine serum, serum/plasma-derived products, or other bovine-derived products.

2.0 SPONSOR:

Providence Portland Medical Center

Cancer Research Rm 2NC29
4805 NE Gilsan Street
Portland, OR 97213

3.0 TEST FACILITY:

WuXi AppTec, Inc.
4751 League Island Blvd.
Philadelphia, PA 19112
(800) 622-8820
(215) 218-5500

4.0 SCHEDULING

DATE SAMPLES RECEIVED: March 6, 2012
STUDY INITIATION DATE: March 30, 2012
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:

hOX40L:Ig clone 755-19 MCB
WuXi AppTec Accession # 11-003668
Lot#120170969

7.0 TEST SYSTEM DESCRIPTION

Many biopharmaceutical products, including expressed proteins, viral vaccine products, and gene therapy vectors, are all derived from cell cultures, such as hybridomas, genetically altered cell lines, or other primary or continuous cell cultures. For some biopharmaceuticals and somatic cell therapies, the cells themselves are the product. In all of these different biopharmaceutical cases, the cells may have been exposed to bovine-derived serum or other products and must be demonstrated to be free of any adventitious agents.

Growth and maintenance of cell cultures typically rely on the addition of various supplements to the growth media. Bovine sera (fetal or newborn calf) and other serum-derived products, such as bovine serum albumin, are perhaps the most commonly used of these supplements. Bovine sera are usually processed from blood collected at the time of slaughter. Although methods of blood

collection and sera processing have improved significantly over the past few years, there remains the potential for contamination of sera with bacteria, fungi, mycoplasma, or viruses. Many sera have also been contaminated *in utero* and thus may contain certain cytopathic and non-cytopathic viruses. The presence of adventitious agents in media supplements may lead to the infection of test article cells. The presence of contaminants in media supplements can ultimately affect the safety, purity and potency of the final product.

As described in the 9 CFR, the introduction of test article cells or culture media to a simian kidney cell line (Vero) and a bovine cell line (bovine turbinate cells (BT)) allows the detection of a wide range of viruses.[1,2,5] The detector cells are observed for at least 21 days for the development of characteristic changes in morphology attributable to replication of viral agents. A blind passage is made on day 21, and the cells are isolated, lysates prepared and fresh Vero and BT cells are inoculated and observed for at least an additional 14 days. Upon completion of the cultivation period, the cells are stained with a cytological stain and are again observed microscopically for changes in morphology. Certain viruses may replicate in cells with the development of little or no cytopathic effects.[1,2] The presence of some of these viruses may be detected by their ability to adsorb erythrocytes (chicken and guinea pig) to the surface of infected cells.[3]

A final test is run to detect extraneous viral agents by the fluorescent antibody technique as described in the 9 CFR Section 113.47.[4] Non-bovine derived cell lines which have been exposed to bovine serum and/or serum derived products are tested for the presence of bovine viral diarrhea virus (BVDV), bovine parvovirus (BPV), bovine adenovirus (BAV), blue tongue virus (BTV), bovine respiratory syncytial virus (BRSV), reovirus (RV) and rabies virus. In this assay, the test article-inoculated Vero and BT cultures are examined with antiserum for the appropriate viruses: anti-BVDV, anti-BPV, anti-BRSV and anti-BAV on BT cultures and anti-RV and anti-BTV on Vero cultures. Anti-rabies antiserum will be used on Vero cultures. The positive controls consist of negative control monolayers inoculated with a specific virus.

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 Vero and BT cells were inoculated with Eagle's Minimum Essential Medium (EMEM) and served as the negative controls.

8.2 The test article was inoculated onto Vero and BT cells. Prior to inoculation, the sample was thawed in a 37±2°C waterbath. An additional freeze/thaw was performed using a dry ice/ethanol bath and 37±2°C waterbath, for a total of 2 freeze/thaw cycles. The lysate was clarified by low-speed centrifugation, and 1 mL per flask of the clarified supernatant was used to inoculate 2 flasks per cell line.

8.3 The positive controls for CPE were inoculated: BT cells were inoculated with bovine viral diarrhea virus (BVDV) and Vero cells were inoculated with pseudorabies virus (PrV).

8.4 Following inoculation, the cultures (negative control, test article, and positive control) were incubated for 60–90 minutes at 37±2°C in a humidified atmosphere of 5±2%CO_2. Following the adsorption period, the cultures were fed with fresh medium.

8.5 Inoculated indicator cell cultures were incubated at 37±2°C in a humidified atmosphere of 5±2%CO_2 and were observed at least twice a week for 21 days for changes in morphology. After a blind passage, the detector cells were observed for an additional 14 days, for a total of 35 days. The cells were observed for evidence of inclusion bodies, cell rounding, abnormal number of/or presence of giant cells, or any other cytopathology attributable to an extraneous agent.

8.6 The cultures were fed every 3-4 days or as needed to maintain cell health. Subculturing was performed on days 7 and 14. On day 21, a blind passage was performed: lysates were prepared from the negative control and test article-inoculated cells. The lysates were inoculated onto fresh BT or Vero cells as appropriate.

8.7 Two days prior (day 19) to hemadsorption, Vero negative cultures (at least 6 cm^2) were infected with parainfluenza type 3 (PI3) virus.

8.8 On day 21, a hemadsorption assay was performed: 0.2% suspensions of chicken and guinea pig erythrocytes were added separately to each monolayer. Cultures were observed macroscopically and microscopically for adsorption of erythrocytes to the monolayers after incubation at 2-8°C and at 20–25°C for 30 to 45 minutes.

8.9 Also on day 21, slides were made from the negative control and test article-inoculated cells. The slides were fixed and stored at -60°C or below until stained with the appropriate immunofluorescent antibodies at the test conclusion.

8.10 A final subculture was performed on day 28.

8.11 Six days prior (day 29) to the test conclusion, negative control and test article-inoculated BT cultures were infected with 100 - 300 $FAID_{50}$ units of BAV and BPV. Negative control and test article-inoculated Vero cultures were infected with 100 - 300 $FAID_{50}$ units of RV and BTV.

8.12 Three days prior (day 32) to the test conclusion, negative control and test article-inoculated BT cultures were infected with 100 - 300 $FAID_{50}$ units of BRSV.

8.13 Two days prior (day 33) to the test conclusion, negative control and test article-inoculated BT cultures were infected with 100 - 300 $FAID_{50}$ units of BVDV.

8.14 Two days prior (day 33) to hemadsorption, Vero negative cultures (at least 6 cm^2) were infected with PI3 virus.

8.15 On day 35, a hemadsorption assay was performed: 0.2% suspensions of chicken and guinea pig erythrocytes were added separately to each monolayer. Cultures were observed macroscopically and microscopically for adsorption of erythrocytes to the monolayers after incubation at 2-8°C and at 20–25°C for 30 to 45 minutes.

8.16 On day 35, representative BT and Vero monolayers were fixed. The cells were stained with a cytological stain and observed microscopically for any changes in morphology.

8.17 Replicate BT cultures (negative control, test article, interference controls, and positive controls) were prepared and fixed for subsequent examination with immunofluorescence techniques for the presence of BVDV, BPV, BAV and BRSV.

8.18 Replicate Vero cultures (negative control, test article, interference controls, and positive controls) were prepared and fixed for subsequent examination with immunofluorescence techniques for the presence of RV and BTV. Replicate Vero cultures (negative control and test article) were prepared and fixed for subsequent examination with immunofluorescence techniques for the presence of rabies virus.

8.19 The slides were examined concurrently using appropriate virus-specific immunofluorescent antibodies. Positive control slides were compared to the test article-inoculated cultures that received positive control viruses: a measurement of potential interference was made from a comparison of these two sets of controls.

9.0 TEST ARTICLE PREPARATION

On March 6, 2012, the Cell Biology Laboratory received 3 vials (#006, 130, 230) from QA, each containing "MCB," and designated for use in protocol 32370, "Post Cell-Bank Cell Growth for Testing." Cell growth was initiated immediately. After 2 passages, the cells were transferred to protocol 30040 "Cell Growth for Assays." On March 16, 2012, the Cell Biology Laboratory generated 2 tubes, each containing 8.0 mL of $1x10^7$ cells/mL of test article cells. The tubes were stored at ≤ -60°C until the assay was initiated.

10.0 POSITIVE CONTROLS

10.1 Positive controls for CPE were BT cells infected with BVDV and Vero cells infected with PrV.

10.2 The positive control for hemadsorption was Vero cells infected with PI3 virus.

10.3 Positive controls for immunofluorescence were BT cells infected with BVDV, BAV, BRSV and BPV and Vero cells infected with BTV and RV. The positive control slides for rabies virus were obtained from the National Veterinary Services Laboratory (NVSL) as specified in 9 CFR.

11.0 NEGATIVE CONTROLS

11.1 Negative control cultures for cytopathic effects (CPE), hemadsorption and immunofluorescence were indicator cells inoculated with EMEM.

11.2 The secondary antibody control for immunofluorescence was secondary antibody inoculated onto fixed test article cells. The primary antibody was deleted.

12.0 ASSAY VALIDITY

The test is considered valid if characteristic cytopathic changes, hemadsorption and immunofluorescence are detected in the positive control cultures, the negative control cultures are negative for viral CPE, hemadsorption and immunofluorescence and the test article secondary antibody controls are negative for immunofluorescence.

13.0 TEST EVALUATION

A positive result, as judged by the development of viral cytopathic changes, and/or adsorption of erythrocytes in cultures inoculated with the test article, and/or the observation of a positive immunofluorescence pattern, would indicate the presence of adventitious agents.

If there is interference with the immunofluorescence of the controls set up for the test article inoculated cells; it may be that some level of viral infection exists in the test article. A viral infection could prevent superinfection by a second virus. As there are other potential reasons for interference, any interference noted should be further investigated.

A negative result would indicate the test article is free of detectable adventitious viruses. However, it does not indicate that the culture is free of persistent or latent viruses. Detection of the latter agents may require further immunologic, biochemical or ultrastructural studies.

14.0 RESULTS

The test was valid. The negative control cultures showed no morphological changes during this assay (Tables 1 and 2). The Vero culture infected with PrV demonstrated +4 viral CPE on day 3 and was discarded. The BT culture infected with BVDV demonstrated +3 viral CPE on day 3 and was discarded (Table 1).

Positive immunofluorescent responses were seen in cultures inoculated with BVDV, BAV, BRSV, BPV, BTV and RV and for the rabies positive control slides. The negative controls produced negative immunofluorescent responses when tested with FITC antisera to BVDV, BAV, BRSV, BPV, BTV, RV and rabies. The test article secondary antibody control produced a negative immunofluorescent response (Table 3).

Vero cultures infected with PI3 virus were positive for hemadsorption activity performed on days 21 and 35. Negative cultures did not demonstrate hemadsorption activity (Table 4).

The test article (tested on Vero and BT cells) did not induce cytopathic changes indicative of viral contamination (Tables 1 and 2) during the course of this test. The test article cultures showed negative immunofluorescent responses when tested for BVDV, BAV, BRSV, BPV, BTV, RV and rabies on days 21 and 35 (Table 3). The test article cultures did not demonstrate any adsorption of erythrocytes in the hemadsorption test run on day 21 and day 35 (Table 4).

In conclusion, no evidence of bovine viral contamination was detected in the test article.

Interference Measurement:

In a comparison of the immunofluorescence detected with positive control viruses added to the negative control slides or to the test article inoculated cultures, the results were similar for the virus/antisera combinations tested. No interference was detected. Note: Interference could not be performed for rabies.

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

Culture Inoculum	Cell Lines	
	Vero	BT
Accession #11-003668	**0**	**0**
Negative Control (EMEM)	0	0
Pseudorabies Virus (PrV)	+4	NA
Bovine Viral Diarrhea Virus (BVDV)	NA	+3

Legend:

0	No viral cytopathic changes observed during the 35-day test period.
~+1	Up to 25% of the cells in culture show viral cytopathic changes.
+1	25-50% of the cells in culture show viral cytopathic changes.
+2	50-75% of the cells in culture show viral cytopathic changes.
+3	75-90% of the cells in culture show viral cytopathic changes.
+4	90-100% of the cells in culture show viral cytopathic changes.

TABLE 2: Detection of Bovine Viruses by Cytological Staining

Culture Inoculum	Day of Test	Cell Lines	
		Vero	BT
Accession #11-003668	**35**	**-**	**-**
Negative Control (EMEM)	35	-	-

Legend:

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

TABLE 3: Detection of Bovine Viruses by Immunofluorescence

Culture Inoculum	Cell Line	Day of Test	αBVDV	αBPV	αBAV	αBRSV	αBTV	αRV	αRabies
Accession #11-003668	**BT**	**21, 35**	**-**	**-**	**-**	**-**	**NA**	**NA**	**NA**
Accession #11-003668 – Secondary Antibody Control	**BT**	**21, 35**	**-**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**
Accession #11-003668	**Vero**	**21, 35**	**NA**	**NA**	**NA**	**NA**	**-**	**-**	**-**
Negative Control (EMEM)	BT	21, 35	-	-	-	-	NA	NA	NA
Negative Control (EMEM)	Vero	21, 35	NA	NA	NA	NA	-	-	-
Negative Control Cultures + BVDV	BT	35	+	NA	NA	NA	NA	NA	NA
Negative Control Cultures + BPV	BT	35	NA	+	NA	NA	NA	NA	NA
Negative Control Cultures + BAV	BT	35	NA	NA	+	NA	NA	NA	NA
Negative Control Cultures + BRSV	BT	35	NA	NA	NA	+	NA	NA	NA
Negative Control Cultures + BTV	Vero	35	NA	NA	NA	NA	+	NA	NA
Negative Control Cultures + RV	Vero	35	NA	NA	NA	NA	NA	+	NA
Rabiesvirus Control Slide[1]	Vero	NA	NA	NA	NA	NA	NA	NA	+
Test Article 11-003668 + BVDV	BT	35	+	NA	NA	NA	NA	NA	NA
Test Article 11-003668 + BPV	BT	35	NA	+	NA	NA	NA	NA	NA
Test Article 11-003668 + BAV	BT	35	NA	NA	+	NA	NA	NA	NA
Test Article 11-003668 + BRSV	BT	35	NA	NA	NA	+	NA	NA	NA
Test Article 11-003668 + BTV	Vero	35	NA	NA	NA	NA	+	NA	NA
Test Article 11-003668 + RV	Vero	35	NA	NA	NA	NA	NA	+	NA

Legend:

NA Not applicable
\- Negative reaction indicating absence of viral agent.
\+ Positive reaction indicating presence of viral agent.
[1] Rabies positive control slide supplied by the National Veterinary Services Laboratory (NVSL) as specified in 9 CFR.

TABLE 4: Hemadsorption Activity of Indicator Cell Lines

Culture Inoculum	Cell Line	Day of Test	2 - 8°C		20–25°C	
			C	GP	C	GP
Accession #11-003668	**BT**	**21, 35**	-	-	-	-
	Vero	**21, 35**	-	-	-	-
Negative Control (EMEM)	BT	21, 35	-	-	-	-
	Vero	21, 35	-	-	-	-
Positive Control (PI3 1:10)	Vero*	21, 35	+	+	+	+
Positive Control (PI3 1:20)	Vero*	21, 35	+	+	+	+

Legend:

NA	Not applicable
-	Negative reaction indicating absence of viral agent.
+	Positive reaction indicating presence of viral agent.
*	Vero cultures inoculated 2 days prior to hemadsorption.
C	Chicken red blood cells
GP	Guinea pig red blood cells

15.0 CONCLUSION

No evidence of adventitious bovine virus 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 test protocol with all amendments, any written communication concerning the conduct of the study, test article accountability record, raw data, worksheets and an official copy of the final study report.

19.0 REFERENCES

1. Timoney JF, Gillespie JH, Scott FW, and Barlough JE, eds Hagan and Brunner's Microbiology and Infectious Diseases of Domestic Animals, 8th edition. Cornell University Press, Ithaca, NY. 1988

2. Fenner FJ, Gibbs EPJ, Murphy FA, Rott R, Studdert MJ, and White DO, eds. Veterinary Virology. Academic Press, Inc, San Diego, CA. 1993

3. Code of Federal Regulations, Title 9 part 113.46, (current version)

4. Code of Federal Regulations, Title 9 part 113.47, (current version)

5. Code of Federal Regulations, Title 9 parts 113.51, 113.52(e), 113.52(f), 113.53(c) (current version)

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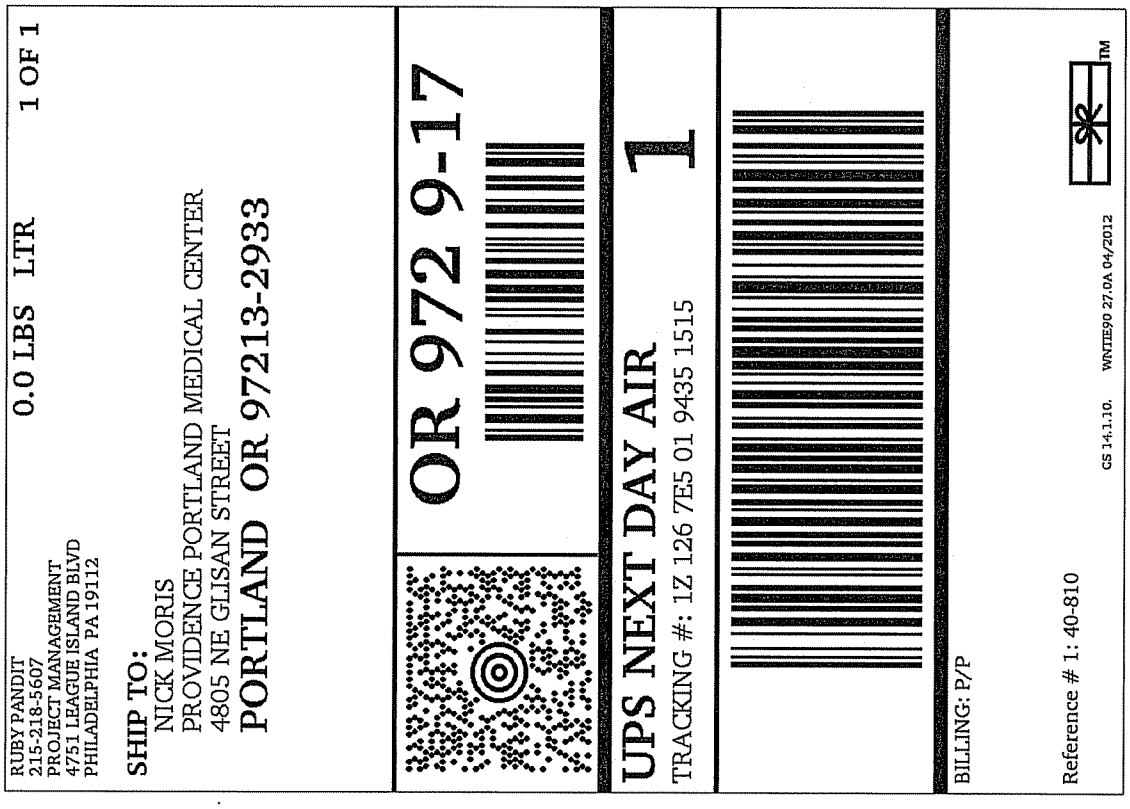


FINAL STUDY REPORT

STUDY TITLE: **Cell Line Authentication and Identification by Isoenzyme Electrophoresis (GLP)**

TEST PROTOCOL NUMBER: 30330.07

TEST ARTICLE IDENTIFICATION	WUXI APPTEC ACCESSION NUMBER
hOX40L:Ig clone 755-19 MCB WuXi AppTec Accession # 11-003668 Lot#120170969	11-003668

SPONSOR:

Nick Morris
Providence Portland Medical Center
Cancer Research Rm 2NC29
4805 NE Glisan Street
Portland OR, 97213

PERFORMING LABORATORY:

WuXi AppTec, Inc.
4751 League Island Blvd.
Philadelphia, PA 19112

WUXI APPTEC ACCESSION NUMBER	RESULTS
11-003668	The test article expressed an isoenzyme pattern representative of Hamster, Chinese cells.

QUALITY ASSURANCE UNIT SUMMARY

STUDY: Cell Line Authentication and Identification by Isoenzyme Electrophoresis (GLP)

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

Phase Inspected	Date
Step 4.2.9 Collect and measure volume of the supernatant.	April 20, 2012

Quality Assurance — Date: 01MAY12

GOOD LABORATORY PRACTICE STATEMENT

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

Study Director — Date: 01 May 2012

Personnel involved in study:

Kasia Warchol Lubov Arotsky April King Walt Ciesielka

1.0 PURPOSE

The purpose of this assay is to identify and authenticate the cell line origin (murine, human, hamster or other) of test article cells by isoenzyme electrophoresis.

2.0 SPONSOR:

Providence Portland Medical Center
Cancer Research Rm 2NC29
4805 NE Glisan Street
Portland OR, 97213

3.0 TEST FACILITY:

WuXi AppTec, Inc.
4751 League Island Blvd.
Philadelphia, PA 19112
(800) 622-8820
(215) 218-5500

4.0 SCHEDULING

DATE SAMPLES RECEIVED: March 6, 2012
STUDY INITIATION DATE: March 19, 2012
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:

hOX40L:Ig clone 755-19 MCB
WuXi AppTec Accession # 11-003668
Lot#120170969

7.0 TEST SYSTEM DESCRIPTION

Numerous reports during the past 25 years have documented instances of cross-contamination between cell cultures.[1] Due to the dramatic increase in the number of cell lines being developed and utilized, opportunities exist both for misidentification and for cross contamination. The risks are especially acute in biotechnology laboratories where workers handle many different cell lines. Identity testing for cell lines used to produce biologicals is recommended by the FDA, Center for Biologics Evaluation and Research (CBER) under Points to Consider.[2]

Isoenzyme analysis performed on homogenates of cell lines from over 25 species have clearly demonstrated the utility of this biochemical characteristic for cell line identification.[3] The procedure consists of a rapid method of cell culture species identification that utilizes cell line specific enzyme mobilities of at least four enzymes: lactate dehydrogenase (LD), purine nucleoside phosphorylase (NP), glucose-6-phosphate dehydrogenase (G6PD), and malate

dehydrogenase (MD). These four enzymes offer a sufficiently diverse range of mobilities to determine a wide range of species of cells used most often in cell culture laboratories.[4]

8.0 EXPERIMENTAL DESIGN

Cells (approximately 1 x 10^7) were harvested from a flask (using trypsin-EDTA, if anchorage dependent), washed two times in Hank's Buffered Saline Solution, and resuspended in a cell extraction buffer. The cell suspension was freeze/thawed six (6) times in an ethanol/dry ice bath followed by centrifugation. The supernatant was then extracted, measured and transferred to an eppendorf tube. An equal volume of enzyme stabilizer was added to the tube, which was either maintained on ice for immediate gel electrophoresis or stored frozen at or below –60°C until the analysis was performed.

The procedure and materials used were based on the Innovative AuthentiKit™ System.[5] The species of origin was determined following agarose gel electrophoresis for 4-7 enzymes. Species authentication was achieved by comparing electrophoretic mobilities of specific isoenzymes present in the test article extracts with those of control and standard extracts. Analysis included the use of NP, G6PD, PEP-B, and LD enzymes.

9.0 TEST ARTICLE PREPARATION

On March 6, 2012, the Cell Biology Laboratory received 3 vials of frozen MCB which were stored on dry ice until initiation of cell growth. On March 6, 2012, cell growth was initiated according to protocol 30040 (Cell Growth for Assays) using Sponsor supplied media and instructions.

On March 16, 2012, the Cell Biology Laboratory designated 1 flask containing 30mL of test article for use in this assay. The test article was stored at 37±2°C/5±2% CO_2 until the assay was initiated. On March 19, 2012, two pellets of approximately 1x10^7 cells/pellet were harvested and stored at ≤ -60°C until the cell extraction procedure was performed

10.0 POSITIVE CONTROLS

The standard will be an extract of the cell line L929 (ATCC CCL1, mouse) and will be used to assure the reproducibility of the system regardless of the laboratory or the operator. Both standard and control will be provided as a part of the Innovative AuthentiKit system.

Another appropriate known control extract Hamster, Chinese which had been characterized and tested by isoenzyme electrophoresis and immunofluorescence was also used as an additional control.

11.0 NEGATIVE CONTROLS

The control was an extract of the HeLa cell line (ATCC CCL2, human).

12.0 ASSAY VALIDITY

The isoenzyme analysis test is considered valid when the standard and control substances and/or the known control substance (if applicable) yield the anticipated electrophoretic banding patterns with the expected mobility values. All bands should be clear and distinct enough to measure with accuracy or gel electrophoresis will be repeated with adjusted volumes as necessary.

13.0 TEST EVALUATION

Comparison of migration distances of test article bands with those of appropriate known controls and/or with the values in a reference handbook[5] permits species identification with a high degree of certainty. The presence of isoenzymes from more than one species may be detected.

14.0 RESULTS

The assay is valid according to the criteria in section 12.0.

Electrophoretic mobilities of enzymes NP, G6PD, PEP B, and LD present in an extract prepared from the cells identified the test article cells as Hamster, Chinese cells. Isoenzyme profiles of NP, G6PD, PEP B, and LD for both the controls and the test article are included in the Appendix.

15.0 CONCLUSION

Test article 11-003668 expressed an isoenzyme pattern representative of Hamster, Chinese cells. There was no evidence that cells other than Hamster, Chinese cells were present in the culture.

16.0 STATISTICAL DATA ANALYSIS

Statistical analysis of the data was not required.

17.0 DEVIATIONS / AMENDMANTS

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

No amendments to the protocol were generated.

18.0 RECORD RETENTION

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

19.0 REFERENCES

1. Peterson, Ward D. Jr., Simpson, W. & Hukku, B. (1979). Cell Culture Characterization: Monitoring for Cell Identification. *Methods in Enzymology* 58:164-178

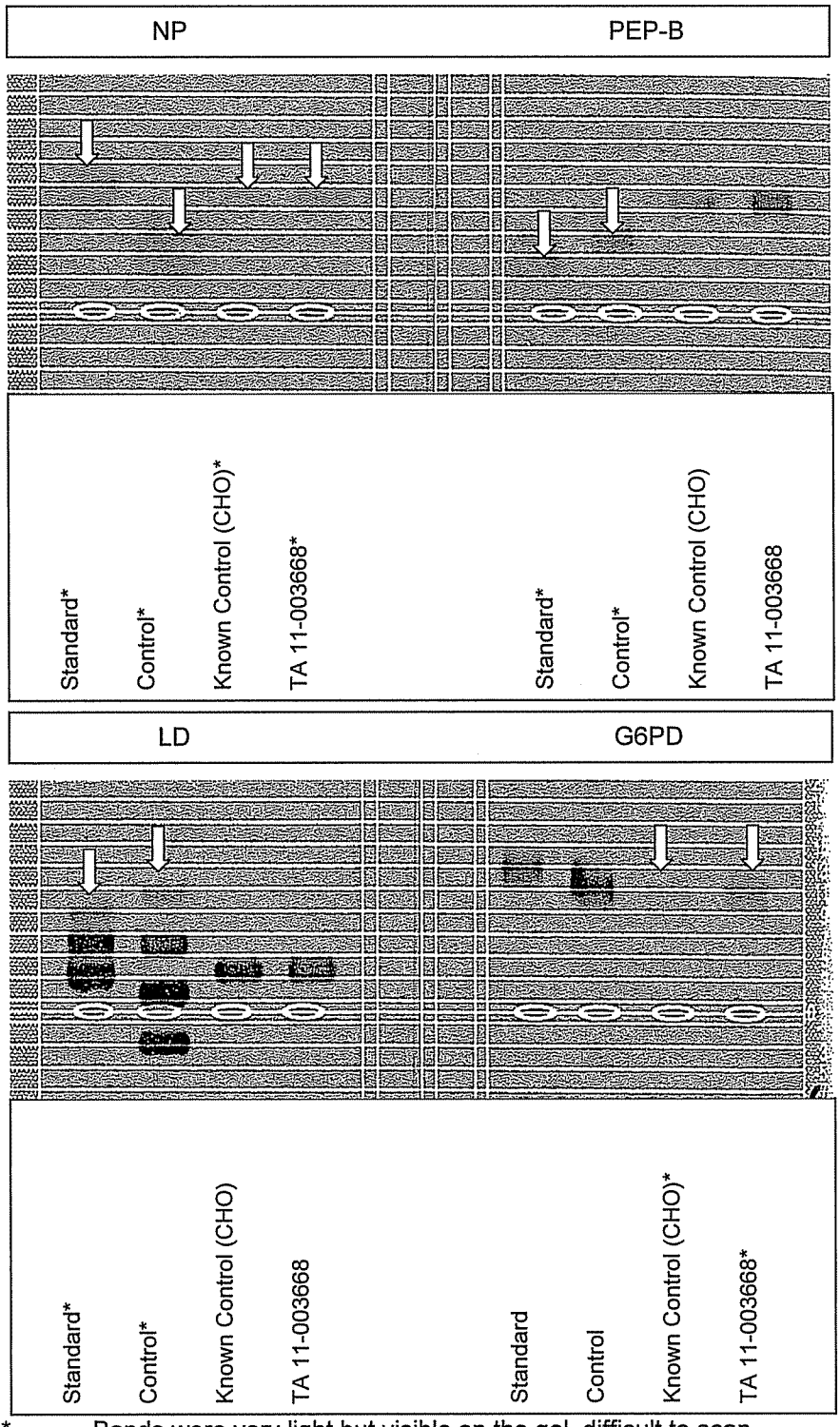
2. Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals (1993). Center for Biologics Evaluation and Research, Food and Drug Administration

3. Hay, R., The Seed Stock Concept and Quality Control for Cell Lines (1988). *Analytical Biochemistry* 171: 225-237

4. Ottenbreit, M.J., Halton, D.M., and Peterson, W.D., Jr., Rapid Isoenzyme Analysis of Cell Cultures by Agarose Electrophoresis. I. Interspecies Identification. *Journal of Tissue Culture Methods* 6:107-110

5. The AuthentiKit System, Handbook for Cell Authentication and Identification, 2nd ed., Innovative Chemistry, Marshfield, MA, 1988

Isoenzyme Profiles



* Bands were very light but visible on the gel, difficult to scan.

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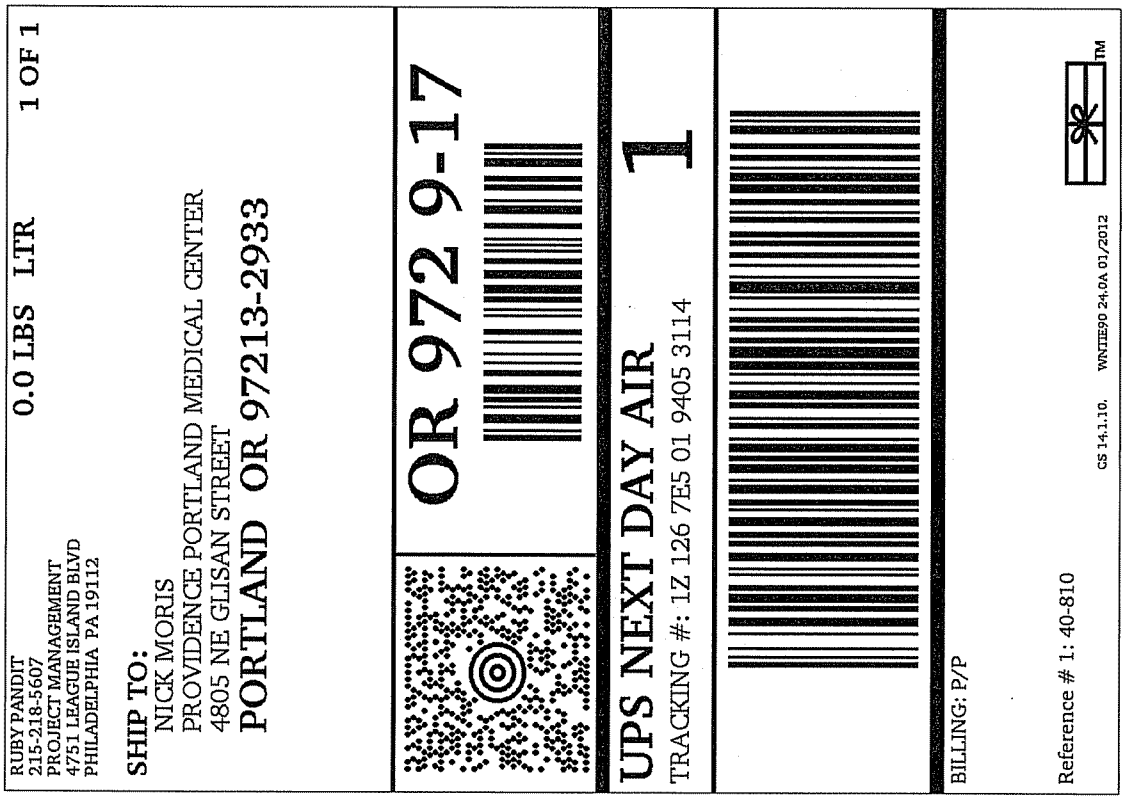

E-FR-1-11-003668.30610

FINAL STUDY REPORT

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

TEST PROTOCOL NUMBER: 30610.07

TEST ARTICLE IDENTIFICATION	WUXI APPTEC ACCESSION NUMBER
hOX40L:Ig clone 755-19 MCB WuXi AppTec Accession # 11-003668 Lot#120170969	11-003668

SPONSOR:

Nick Morris
Providence Portland Medical Center
4805 NE Gilsan Street
Portland, OR 97213

PERFORMING LABORATORY:

WuXi AppTec, Inc.
4751 League Island Blvd.
Philadelphia, PA 19112

SUBCONTRACTED TO:

Charles River Laboratories
Pathology Associates – North Carolina
4025 Stirrup Creek Drive, Suite 150
Durham, NC 27703

WUXI APPTEC ACCESSION NUMBER	RESULTS
11-003668	Transmission electron microscopic examination of 200 cells revealed no identifiable virus-like particles, nor did it reveal any other identifiable microbial agents. Retrovirus-like particles observed in the sample were intracytoplasmic A-type particles, often seen near centrioles. 8.0% of the cells expressed A-type particles. As these are CHO cells, this result is considered to be typical and expected.

QUALITY ASSURANCE UNIT SUMMARY

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

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

Phase Inspected	Date
Step 4.2.1 Aspirate the medium from the cell pellet and add 10 mL of 5% glutaraldehyde.	March 28, 2012

Quality Assurance — 05May2012 — Date

GOOD LABORATORY PRACTICES STATEMENT

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

Study Director — 07May12 — Date

Professional Personnel involved in study:

April King Kathy Laughlin Christopher Larson

1.0 PURPOSE

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

2.0 SPONSOR:

Providence Portland Medical Center
4805 NE Gilsan Street
Portland, OR 97213

3.0 TEST FACILITY:

WuXi AppTec, Inc.
4751 League Island Blvd.
Philadelphia, PA 19112
(800) 622-8820
(215) 218-5500

SUBCONTRACTOR:

Charles River Laboratories
Pathology Associates – North Carolina
4025 Stirrup Creek Drive, Suite 150
Durham, NC 27703

4.0 SCHEDULING

DATE SAMPLES RECEIVED: March 26, 2012
STUDY INITIATION DATE: March 28, 2012
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:

hOX40L:Ig clone 755-19 MCB
WuXi AppTec Accession # 11-003668
Lot#120170969

7.0 TEST SYSTEM DESCRIPTION

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

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

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

8.0 EXPERIMENTAL DESIGN

For most purposes, optimum preservation of fine structure in animal cells, viruses, and other microbial agents is the prime consideration, and procedures for ensuring this are now fairly well standardized. The cells were thawed and grown at WuXi AppTec Laboratories or were submitted as live cells in a flask. When an optimal level of $1 - 2 \times 10^7$ cells was available, the cells were harvested and a cell count determined.

8.1 The cells were fixed, while in suspension, in 5% glutaraldehyde then pelleted.

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

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

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

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

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

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

8.8 At least 200 cells were evaluated for particles with A-, B-, C-, D-, and R-type retrovirus-like morphology as described in Section 7. Electron micrographs were made to document representative examples of any virus-like particles observed. Except where noted otherwise, a bar denoting 100 nanometers was placed on each micrograph for size reference.

9.0 TEST ARTICLE PREPARATION

On March 6, 2012, the Cell Biology laboratory received 3 vials from QA frozen on dry ice (vial # 006, 130 and 230), each containing 1 ml of "MCB" and designated for use in protocol 32370, "Post Cell-Bank Cell Growth for Testing". Cell growth was initiated immediately. After 2 passages, the cells were transferred to protocol 30040 "Cell Growth for Assays". On March 26, 2012, the Cell Biology Laboratory generated a flask of cells, for use in this assay. The tubes were stored at ≤ -60°C until processed per assay protocol which took place on March 28, 2012. On April 2, 2012, 1 tube containing a fixed and pelleted cell culture was shipped on ice packs, in storage conditions of 2–8°C via overnight carrier to the subcontractor.

10.0 NEGATIVE CONTROLS

A sample consisting of agar and water was run in parallel with the test article.

11.0 ASSAY VALIDITY

The following validity criteria are evaluated:

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

12.0 TEST EVALUATION

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

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

13.0 RESULTS

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

Cellular Ultrastructure

Cells in the section were small to moderate in size and rounded to irregular in shape (J75653). Cells had microvilli (MV: J75653) unevenly distributed on the surface. Nuclei (N: J75653) tended to be rounded to irregular, with chromatin relatively evenly dispersed or clumped along the periphery. Nuclei often had one or more nucleoli (NS: J75653) that were variably located. Some cells were observed to be undergoing mitosis, with chromosomes (CH: J75659) visible.

The cytoplasm of most cells contained varying numbers of mitochondria (MI: J75658). Short profiles of rough endoplasmic reticulum (RER: J75658) were seen among the mitochondria. Ribosomes (RB: J75655) were common in the cytoplasm of most cells. Cells were observed to contain cleft-like spaces (CS: J75655), centrioles (CN: J75656) and autophagic vacuoles (AV: J75654).

General Viral Particle Evaluation

Transmission electron microscopic examination of 200 cells revealed no identifiable virus-like particles other than retrovirus-like particles, nor did it reveal any other identifiable microbial agents. Retrovirus-like particles observed in the sample were intracytoplasmic A-type particles (A in micrographs), often seen associated with centrioles.

Retrovirus-like Particle Evaluation and Tabulation

Results of retrovirus-like particle counts in the specimen were as follows:

Test Article: 11-003668
PAI EM Number: EM-12.118

Number of cells with:

Particle Type	No Particles	1-5 Particles	6-20 Particles	>20 Particles
A-Type	184	15	1	0

Percentage of cells with each type of retrovirus-like particle:

A-Type 8%

Three percent of the cells were observed to be necrotic.

14.0 CONCLUSION

Transmission electron microscopic examination of 200 cells revealed no identifiable virus-like particles, nor did it reveal any other identifiable microbial agents. Retrovirus-like particles observed in the sample were intracytoplasmic A-type particles, often seen near centrioles. 8.0% of the cells expressed A-type particles. As these are CHO cells, this result is considered to be typical and expected.

15.0 STATISTICAL DATA ANALYSIS

Statistical analysis of the data is not required.

16.0 DEVIATIONS / AMENDMENTS

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

No amendments to the protocol were generated.

17.0 RECORD RETENTION

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

18.0 REFERENCES

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

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

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

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

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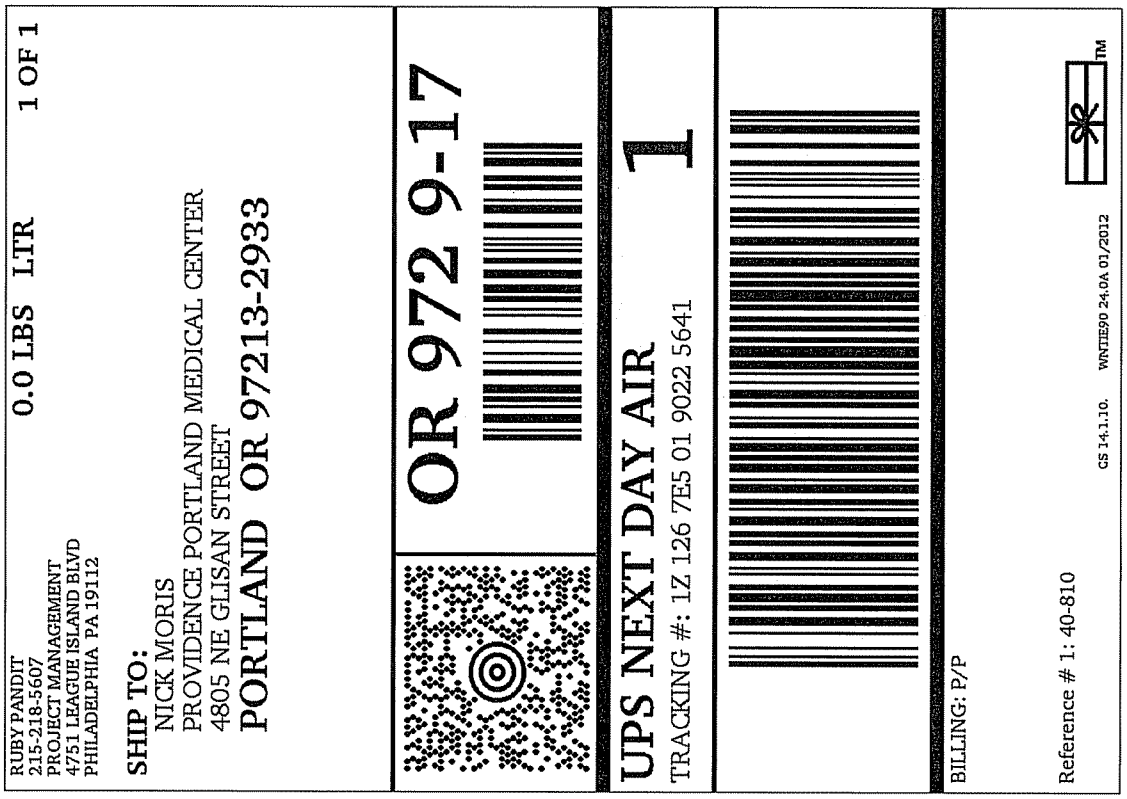

E-FR-1-11-003668.30727

FINAL STUDY REPORT

STUDY TITLE: **Detection of Bovine Polyoma Virus (BPyV) DNA by Quantitative Polymerase Chain Reaction (qPCR): (GLP)**

TEST PROTOCOL NUMBER: 30727.02

TEST ARTICLE IDENTIFICATION	WUXI APPTEC ACCESSION NUMBER
hOX40L:Ig clone 755-19 MCB WuXi AppTec Accession # 11-003668 Lot#120170969	11-003668

SPONSOR:

Nick Morris
Providence Portland Medical Center
Cancer Research Rm 2NC29
4805 NE Gilsan Street
Portland, OR 97213

PERFORMING LABORATORY:

WuXi AppTec, Inc.
4751 League Island Blvd.
Philadelphia, PA 19112

WUXI APPTEC ACCESSION NUMBER	RESULTS
11-003668	**Negative.** BPyV DNA sequences were not detected in the Sponsor's sample. The Limit of Detection of the assay was 100 copies.

QUALITY ASSURANCE UNIT SUMMARY

STUDY: Detection of Bovine Polyoma Virus (BPyV) DNA by Quantitative Polymerase Chain Reaction (qPCR): (GLP)

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

Phase Inspected	Date
DNA Isolation BR# 36198.07 Step 1.4.1 Dilute an aliquot of the test article 1:80 to 400 µl final volume in TE buffer for spectrophotometric analysis.	March 28, 2012
qPCR Protocol BR #30727.02 Step 4.4 (Step 5) A Limit of Detection (LOD) is determined by examining the data obtained from the standard line and determining the lowest standard detected above background.	March 30, 2012

Quality Assurance

Date

GOOD LABORATORY PRACTICES STATEMENT

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

Study Director

Date

Professional Personnel involved in study:

Don Schmidt
Gil Vaisberg
Michael Kim
Akash Patel
Mital Patel

1.0 PURPOSE

This is a limits test to detect human Bovine Polyoma Virus (BPyV) DNA in the Sponsor's sample using quantitative Polymerase Chain Reaction (qPCR) with Roche LightCycler technology and to provide data on assay sensitivity by including a standard line of 5 levels of BPyV DNA.

2.0 SPONSOR:

Providence Portland Medical Center
Cancer Research Rm 2NC29
4805 NE Gilsan Street
Portland, OR 97213

3.0 TEST FACILITY:

WuXi AppTec, Inc.
4751 League Island Blvd.
Philadelphia, PA 19112
(800) 622-8820
(215) 218-5500

4.0 SCHEDULING

DATE SAMPLES RECEIVED: March 6, 2012
STUDY INITIATION DATE: March 26, 2012
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:

hOX40L:Ig clone 755-19 MCB
WuXi AppTec Accession # 11-003668
Lot#120170969

7.0 TEST SYSTEM DESCRIPTION

Bovine Polyoma Virus (BPyV) was first isolated as a contamination in cell culture of monkey kidney cells and was initially thought to be of simian origin. Since then other isolates of simian cell lines suggested a bovine origin of the virus and bovine sera as the likely source through which the virus had been introduced into cell cultures. More recently, it has been shown in PCR that BPyV is a frequent contaminant in commercial batches of fetal bovine serum.[1-2]

PCR* is the method of choice for detection of viral DNA in cell substrates because of its great sensitivity and specificity for detecting DNA sequences. The technique may be used to detect as few as three copies of a target DNA in a background of half a million cells. By defining the borders of a selected region of the BPyV sequence with a pair of oligonucleotide primers, it is possible to

* The PCR process is covered by U.S. patents owned by Hoffmann La-Roche Inc.

amplify the target sequence with a thermostable DNA polymerase by a factor of 10 billion in a few hours. [3-4]

Quantitative PCR offers a unique combination of sensitivity and quantification that had been previously difficult to achieve with classic endpoint PCR. The Roche LightCycler™ is a particularly well-suited tool with which to perform these types of studies. It offers extremely rapid cycling times, with PCR runs being completed in 15-20 minutes, and a unique single chamber design, ensuring complete well-to-well uniformity. Sequence-specific hybridization probes are designed to allow detection and analysis of PCR products on the LightCycler without the need for any post-PCR sample manipulation.[5]

The PCR primers and probes used in this study are derived from BPyV large T antigen motif.[6] The assay may be used to detect as few as 10 - 100 copies of BPyV DNA. The assay can be completed within a single day.

8.0 EXPERIMENTAL DESIGN

8.1 The test article was maintained according to the Sponsor's instructions. DNA was isolated from the test article by use of a commercial kit. The purified DNA was resuspended in a volume of 1X TE buffer that resulted in the concentration of 0.1 μg/μL.

8.2 A master mix of reagents containing the appropriate primers, probes, PCR-grade water, $MgCl_2$, and the LightCycler reaction buffer was prepared and dispensed to every reaction in the assay.

8.3 Five aliquots of test article DNA were dispensed into capillaries. Three aliquots of test article DNA were dispensed into capillaries with no additional DNA supplements. The other aliquots were spiked with 100 and 1000 copies of BPyV DNA, respectively.

8.4 Three aliquots of reagent control were dispensed into capillaries. One aliquot of reagent control was dispensed into a capillary with no additional DNA supplements and was used as a negative control. The other two aliquots of reagent control were spiked with 100 and 1000 copies of BPyV DNA, respectively.

8.5 Four aliquots of H9 DNA were dispensed into capillaries. Two aliquots of H9 DNA were dispensed into capillaries with no additional DNA supplements and were used as negative controls. The other two aliquots of H9 DNA were spiked with 100 and 1000 copies of BPyV DNA, respectively.

8.6 Five levels of BPyV DNA from 10 copies to 10^5 copies were prepared to provide a standard line from which linear regression measurements can be performed.

9.0 TEST ARTICLE PREPARATION

On February 28, 2012, WuXi AppTec Inc. received 3 vials of "MCB" frozen in liquid nitrogen vapor phase and designated for use in protocol 32370 "Post Cell-Bank Cell Growth for Testing." On March 6, 2012, the Cell Biology Laboratory received 3 vials from QA frozen in dry ice (vial #006, 130, 230), each containing 1.0 mL of "MCB," and cell growth was initiated. On March 22, 2012, the Cell Biology Laboratory generated 4 pellets of test article ($1x10^7$ cells/pellet) for use in the qPCR assays. The pellets were stored at ≤ -60°C until the assay was initiated. DNA was isolated from $2x10^7$ cells of test article and a portion of the DNA was used in this assay.

10.0 POSITIVE CONTROLS

A plasmid DNA containing the target BPyV sequence was used as a positive control.

11.0 NEGATIVE CONTROLS

11.1 Purified DNA from the human H9 cell line was used as a negative control.

11.2 A reaction tube containing all of the components except a DNA template served as a reagent control.

12.0 ASSAY VALIDITY

The following validity criteria were evaluated:

12.1 The 1000 copies of BPyV DNA spiked H9 DNA must show a fluorescence signal above background.

12.2 The 100 copies of BPyV DNA standard must show a fluorescence signal above background.

12.3 Fluorescence signals ≥ the Limit of Detection must not be detected in the reagent control.

12.4 Fluorescence signals ≥ the Limit of Detection must not be detected in the unspiked H9 DNA samples.

12.5 The R^2 value of the standard curve must be ≥ 0.80.

13.0 TEST EVALUATION

All results will be judged by analysis of the quantitative fluorescence figures (F2/F1 vs. Cycles).

A Limit of Detection (LOD) is determined by examining the data obtained from the standard line and determining the lowest standard detected above background.

A negative result, as judged by the fluorescence signal below the LOD in all of the replicates of the PCR-amplified unspiked test article DNA, would indicate that BPyV sequences specific for the primers and probes used are not present in detectable quantities in the Sponsor's sample. The result will be reported as, "Negative. BPyV DNA sequences were not detected in the Sponsor's sample. The Limit of Detection of the assay was *X* copies."

A positive result, as judged by the presence of a specific fluorescence signal equal to or above the LOD in any two of the replicates of the PCR-amplified unspiked test article DNA, in contrast to the absence of such fluorescence signals in the negative controls, would indicate that BPyV sequences are present in the Sponsor's sample. The result will be given as a mean score and will be reported as, "Positive. *X* copies of BPyV DNA per mass or volume of sample tested/extracted were detected in the Sponsor's sample. The Limit of Detection of the assay was *X* copies."

If a specific fluorescence signal equal to or above the LOD is detected only in one of the replicates of the PCR-amplified unspiked test article DNA, the result will be reported as "Putative Positive: requires verification."

If fluorescence signals are not detected above background in both the test article spiked with 100 and 1000 copies of BPyV DNA, the results will be reported as, "Test article interferes with the qPCR reaction." The Sponsor will be contacted, and an additional test of a 1:10 and/or 1:100 diluted test article DNA will be initiated upon the Sponsor's approval.

14.0 RESULTS

The test was valid. The 1000 copies BPyV DNA spiked H9 DNA and the 100 copies BPyV DNA standard both showed fluorescence signals above background. Fluorescence signals equal to or above the LOD were not detected in the reagent control and the unspiked H9 DNA samples. The R^2 value of the standard curve was 1.00.

The test article produced a negative result for BPyV. BPyV specific probes did not hybridize to the PCR-amplified test article DNA giving fluorescence signals equal to or above the LOD, indicating that BPyV DNA sequences specific for the primers and probes used were not present in detectable quantities in the Sponsor's sample. The LOD was 100 copies.

BPyV specific fluorescence signals were detected in 1000 copies of BPyV DNA spiked into DNA isolated from the test article. BPyV specific fluorescence signals were detected in 1000 copies of BPyV DNA spiked into DNA isolated from H9 control cells. Therefore, the test article and H9 control did not contain material that inhibited the PCR reaction.

DNA was isolated from $2x10^7$ cells of test article. DNA extracted from the test article was re-suspended in 100 µL of 1x TE buffer. 5 µL of DNA was used for the OD scan. The concentration by OD was determined to be 0.56 µg/µL. Therefore the total yield was 100 µL x 0.56 µg/µL = 56 µg of DNA.

$2x10^7$ cells of test article generated 56 µg of DNA. Subsequently, the DNA was diluted to a final concentration of 0.1 µg/µL with 1x TE buffer. 0.5 µg of DNA was therefore obtained from 0.5 µg of DNA x $2x10^7$ cells of test article ÷ 56 µg of DNA = $1.8x10^5$ cells. Since 0.5 µg of DNA was used per reaction and the reaction was run in triplicate, an equivalent of $1.8x10^5$ cells x 3 = $5.4x10^5$ cells of original test article was tested.

A printout of the qPCR results is included as Appendix A to this Final Report.

15.0 CONCLUSION

BPyV DNA sequences were not detected in the Sponsor's sample.

16.0 STATISTICAL DATA ANALYSIS

Linear regression analysis was used to calculate an R^2 value for the standard line. The copy number of the test article was determined by comparison to the standard line.

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 test protocol with all amendments, any written communication concerning the conduct of the study, test article accountability record, raw data, worksheets, and an official copy of the final study report.

19.0 REFERENCES

1. Schurman R, van Steenis B, van Strien A, van der Noordaa J, and Sol C (1991). "Frequent Detection of Bovine Polyomavirus in commercial batches of calf serum by using the Polymerase chain reaction." *J. Gen. Virology* 2739-2745

2. "Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use" (June 1, 1987). The Director, Office of Biologics Research and Review, Center for Drugs and Biologics, FDA, Bethesda, MD 20892

3. Mullis KB and Faloona FA (1987). "Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction." *Methods in Enzymology* 155: 335-350

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5. Wittwer C, Herrmann M, and Moss A (1997). "Continuous fluorescence monitoring of rapid cycle DNA amplification." *BioTechniques* 22 (1): 130-138

6. Kappeler A, Lutz-Wallace C, Sapp T, and Sidhu M (1996). "Detection of Bovine Polyoma virus contamination in fetal bovine sera and modified live viral vaccines using polymerase chain reaction." *Biologicals* 24: 131-135

LightCycler Data Analysis (Lightcycler) - [Quantification: 11-003668 BPyV DJS 30MAR12; F2/F1; CYCLE seg 2]

File Quantification Report Window Help

Analysis
- (•) Fit Points
- ( ) Second Derivative Maximum

Baseline Adjustment
- ( ) None
- ( ) Arithmetic
- (•) Proportional
- ( ) Normalized

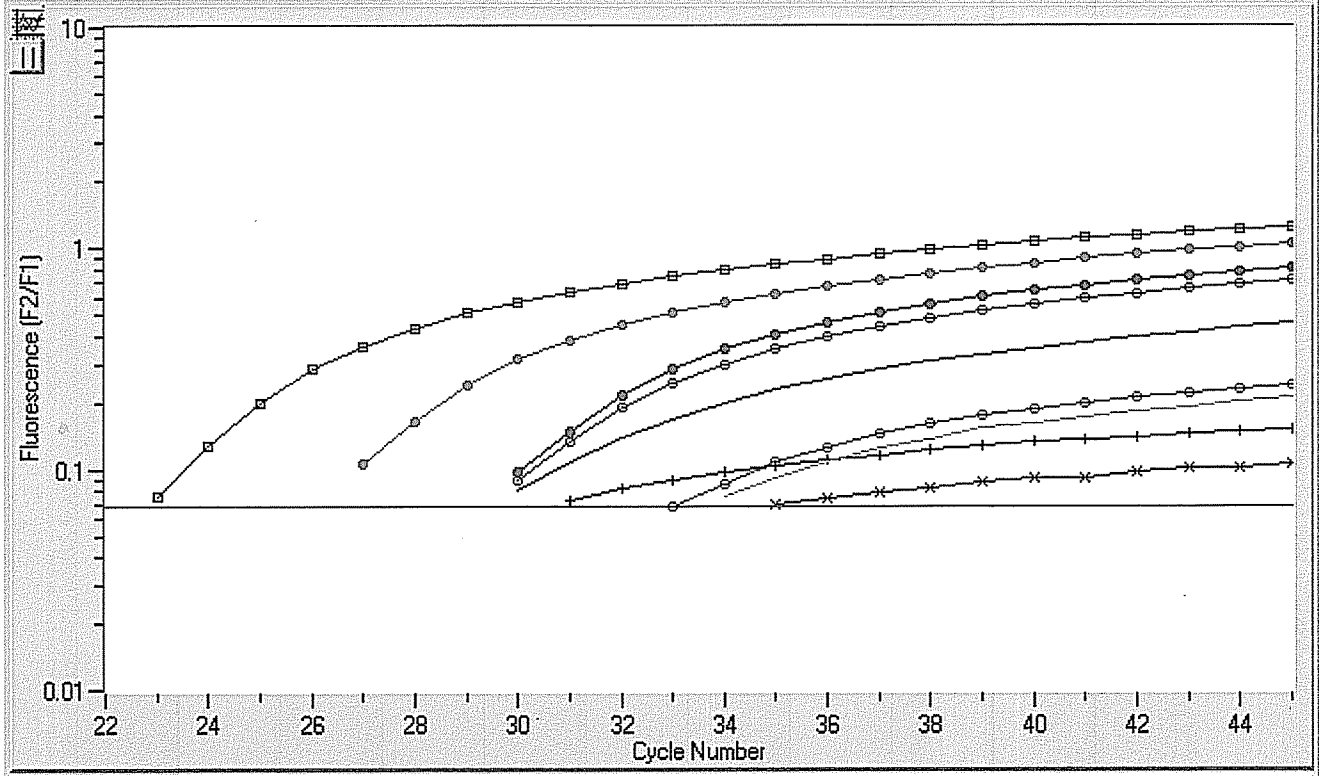
Number of Points
2

[ ] Show Fit Points

Analysis Notes
APPENDIX A. BK 30727 DOS 30MAR12

P...	Name	Standard	Calculat...	Cro...
1	RC			
2	RC 100	1.000E+02	1.138E+02	32.92
3	RC 1000	1.000E+03	1.369E+03	29.14
4	H9 NS			
5	H9 NS			
6	H9 100	1.000E+02		
7	H9 1000	1.000E+03	5.827E+02	30.44
8	STD 10	1.000E+01		
9	STD 100	1.000E+02	8.466E+01	33.37
10	STD 1000	1.000E+03	1.246E+03	29.28
11	STD 10000	1.000E+04	1.062E+04	26.02
12	STD 100000	1.000E+05	8.928E+04	22.78
13	11-003668 NS			
14	11-003668 NS			
15	11-003668 NS			
16	11-003668 100	1.000E+02	3.452E+01	34.74
17	11-003668 1000	1.000E+03	1.124E+03	29.44

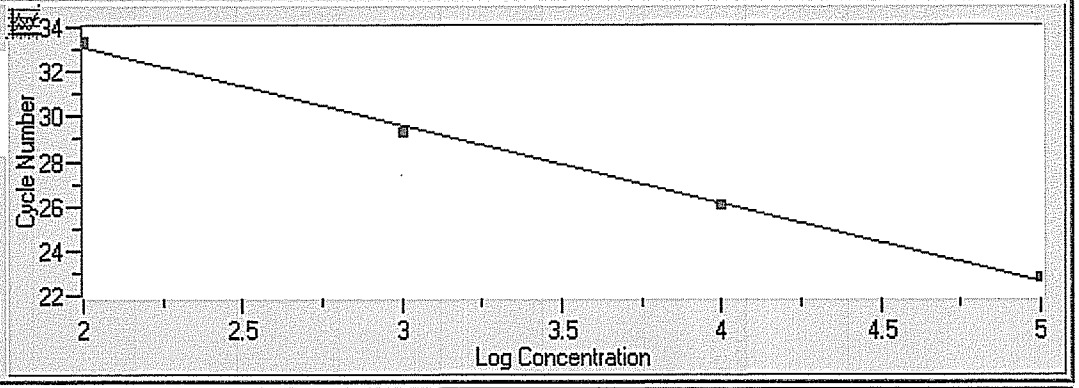
Step 1: Baseline | Step 2: Noise Band | Step 3: Analysis



Linear Regression
Crossing Points

Slope = -3.504
Intercept = 40.13
Error = 0.116
r = -1.00

Minimize Error



ERIN KILLEEN
215-218-5539
PROJECT MANAGEMENT
4751 LEAGUE ISLAND BLVD
PHILADELPHIA PA 19112

0.0 LBS LTR 1 OF 1

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NICK MORIS
PROVIDENCE PORTLAND MEDICAL CENTER
4805 NE GLISAN STREET
PORTLAND OR 97213-2933

OR 972 9-17

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TRACKING #: 1Z 126 7E5 01 9287 2368

BILLING: P/P

Reference # 1: Erin Killeen

CS 14.1.10. WNTE90 24.0A 01/2012

E-FR-1-11-003668.31912

FINAL STUDY REPORT

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

TEST PROTOCOL NUMBER: 31912.02

TEST ARTICLE IDENTIFICATION	WUXI APPTEC ACCESSION NUMBER
hOX40L:Ig clone 755-19 MCB WuXi AppTec Accession # 11-003668 Lot#120170969	11-003668

SPONSOR:

Nick Morris
Providence Portland Medical Center
Cancer Research Rm 2NC29
4805 NE Glisan Street
Portland, OR 97213

PERFORMING LABORATORY:

WuXi AppTec, Inc.
4751 League Island Blvd.
Philadelphia, PA 19112

WUXI APPTEC ACCESSION NUMBER	RESULTS
11-003668	The presence of adventitious virus was not detected in the test article.

QUALITY ASSURANCE UNIT SUMMARY

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

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

Phase Inspected	Date
Step 4.4.5 Inoculate Test Article plates.	March 31, 2012

Quality Assurance

03May12
Date

GOOD LABORATORY PRACTICES STATEMENT

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

Study Director

03May12
Date

Professional Personnel involved in Study:

Joseph V. Hughes	Chief Scientist of Virology and VP ofService Development
Stephanie Smith	Director of Virology Operations - Virology & Viral Clearance
Amanda Ferzetti	Associate Director, Virology Operations
Amy Werts	Study Director, Virology Operations
Dana Cipriano	Senior Director of Operations

1.0 PURPOSE

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

2.0 SPONSOR:

Providence Portland Medical Center
Cancer Research Rm 2NC29
4805 NE Glisan Street
Portland, OR 97213

3.0 TEST FACILITY:

WuXi AppTec, Inc.
4751 League Island Blvd.
Philadelphia, PA 19112
(800) 622-8820
(215) 218-5500

4.0 SCHEDULING

DATE SAMPLES RECEIVED: March 6, 2012
STUDY INITIATION DATE: March 27, 2012
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:

hOX40L:Ig clone 755-19 MCB
WuXi AppTec Accession # 11-003668
Lot#120170969

7.0 TEST SYSTEM DESCRIPTION

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

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

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

8.0 EXPERIMENTAL DESIGN

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

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

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

8.3 The positive cultures were established: MRC-5 cells were inoculated with Encephalomyocarditis Virus (EMC), VERO cells were inoculated with Adenovirus Type 5 (Ad 5) Virus and CHO cells were inoculated with Vesicular Stomatitis Virus (VSV).

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

8.5 Cultures were fed on days 3, 10, 17 and 24. Subcultivation was performed on days 7, 14, and 21.[2,9]

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

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

9.0 TEST ARTICLE PREPARATION

On March 6, 2012, the Cell Biology Laboratory received 3 vials of "MCB" from QA, frozen in dry ice and designated for use in protocol 32370, "Post Cell-Bank Cell Growth for Testing." Cell growth was initiated immediately. After growth, the cells were transferred to protocol 30040, "Cell Growth for Assays." On March 16, 2012, 2 tubes, each containing 6.0 mL of $1x10^7$ test article cells/mL in conditioned media were generated and designated for use in this assay. The tubes were stored at ≤ -60°C until the assay was initiated.

On the day of inoculation, March 31, 2012, the test article was thawed using a 37±2°C waterbath and was subjected to one additional freeze/thaw cycle using a dry ice/ethanol bath and a 37±2°C waterbath. The test article was clarified by low-speed centrifugation and the supernatant was then inoculated as per step 8.2 in the experimental design section.

10.0 POSITIVE CONTROLS

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

Positive controls for CPE were:

1) MRC-5 cultures infected with EMC
2) VERO cultures infected with Ad 5
3) CHO cultures infected with VSV

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

10.3 The positive control for hemagglutination was influenza type A virus.

11.0 NEGATIVE CONTROLS

MRC-5, VERO and CHO cultures inoculated with EMEM served as the negative control cultures for CPE, hemadsorption, and hemagglutination.

12.0 ASSAY VALIDITY

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

13.0 TEST EVALUATION

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

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

14.0 RESULTS

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

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

Influenza type A virus was positive for hemagglutination when used as the positive control and the negative control cultures demonstrated no hemagglutination activity (Table 3).

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

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

Culture Inoculum	Cell Line		
	MRC-5	VERO	CHO
Accession #11-003668	-	-	-
EMEM (Negative Control)	-	-	-
Encephalomyocarditis (Positive Control)	+	NA	NA
Adenovirus type 5 (Positive Control)	NA	+	NA
Vesicular Stomatitis Virus (Positive Control)	NA	NA	+

Legend:

- \- Negative reaction indicating absence of viral agent
- \+ Positive reaction indicating presence of viral agent
- NA Not Applicable

TABLE 2: Hemadsorption Activity of Indicator Cell Lines

Culture Inoculum	Cell Line	Day of Test	2 - 8°C			20 – 25°C		
			C	GP	H	C	GP	H
Accession #11-003668	**MRC-5**	**28**	-	-	-	-	-	-
	VERO	**28**	-	-	-	-	-	-
	CHO	**28**	-	-	-	-	-	-
EMEM (Negative Control)	MRC-5	28	-	-	-	-	-	-
	VERO	28	-	-	-	-	-	-
	CHO	28	-	-	-	-	-	-
Parainfluenza Type 3 Virus (Positive Control) (1:10)	VERO	28	+	+	+	+	+	+
Parainfluenza Type 3 Virus (Positive Control) (1:20)	VERO	28	+	+	+	+	+	+

Legend:

H Human red blood cells
C Chicken red blood cells
GP Guinea pig red blood cells
- Negative reaction indicating absence of viral agent
+ Positive reaction indicating presence of viral agent

TABLE 3: Hemagglutination Activity of Indicator Cell Lines

Sample	Supernatant From	2 - 8°C			37 ± 2°C		
		C	GP	H	C	GP	H
Accession #11-003668	**MRC-5**	-	-	-	-	-	-
	VERO	-	-	-	-	-	-
	CHO	-	-	-	-	-	-
Negative Control Supernatant	MRC-5	-	-	-	-	-	-
	VERO	-	-	-	-	-	-
	CHO	-	-	-	-	-	-
Positive Assay Control (Influenza Type A)	NA	+	+	+	+	+	+
Negative Assay Control (EMEM)	NA	-	-	-	-	-	-

Legend:

H Human red blood cells
C Chicken red blood cells
GP Guinea pig red blood cells
- Negative reaction indicating absence of viral agent
+ Positive reaction indicating presence of viral agent

15.0 CONCLUSION

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

16.0 STATISTICAL DATA ANALYSIS

Statistical analysis of the data was not required.

17.0 DEVIATIONS / AMENDMENTS

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

No amendments to the protocol were generated.

18.0 RECORD RETENTION

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

19.0 REFERENCES

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

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

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

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

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

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

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

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

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

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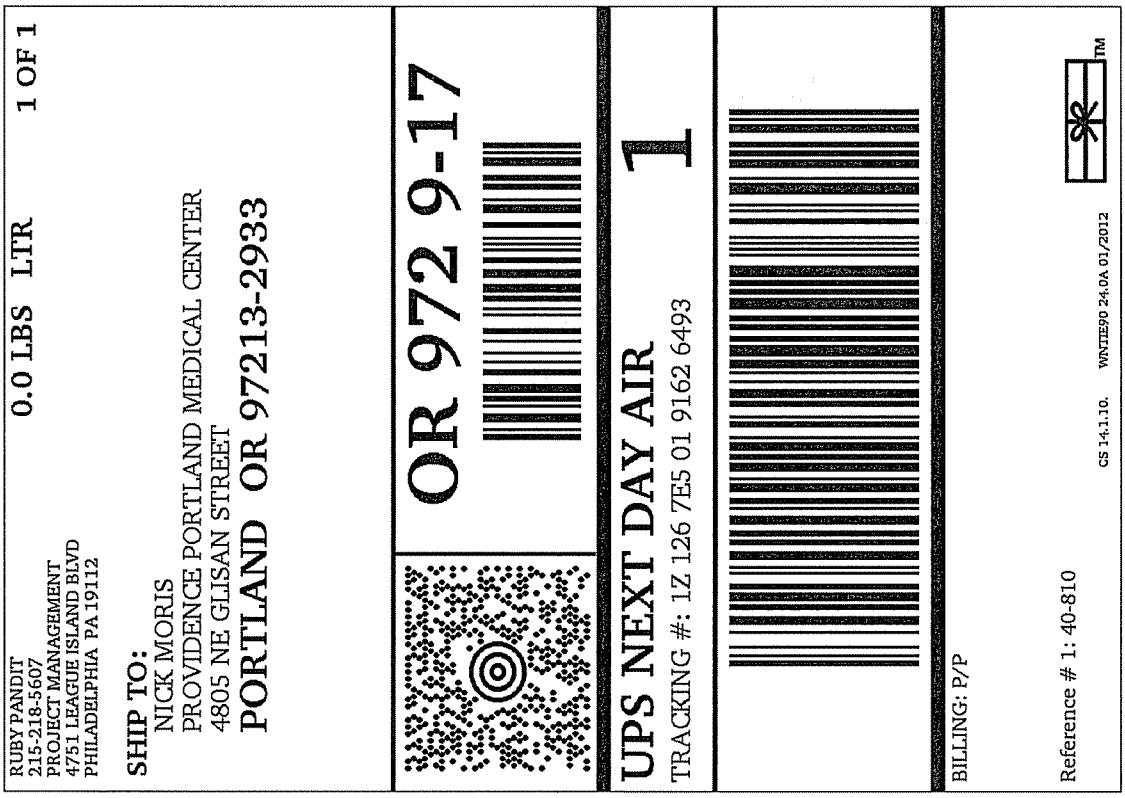

FINAL STUDY REPORT

STUDY TITLE:	**MYCOPLASMA DETECTION WITH MYCOPLASMASTASIS: EUROPEAN PHARMACOPOEIA AND UNITED STATES PHARMACOPEIA GUIDELINES**
PROTOCOL NUMBER:	32700-1
TEST ARTICLE IDENTIFICATION:	hOX40L:Ig clone 755-19 MCB WuXi AppTec Accession# 11-003668 Lot # 120170969
SPONSOR:	Providence Portland Medical Center Cancer Research Rm 2NC29 4805 NE Glisan Street Portland, OR 97213
PERFORMING LABORATORY:	WuXi AppTec, Inc. 2540 Executive Drive St. Paul, MN 55120
ACCESSION NUMBER: **STUDY NUMBER:**	11-003668 167429
RESULT SUMMARY:	Considered **negative** for mycoplasma contamination and is **non-inhibitory** for the detection of mycoplasma

QUALITY ASSURANCE UNIT SUMMARY

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

Critical Phase	Date	Study Director	Management
Dilution of Controls	03/23/12	03/26/12	05/01/12
Final Report	04/27/12	04/27/12	05/01/12

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

Quality Assurance Auditor: Sheri Handy Date: 5/1/12

Sheri Handy

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: Date: 5/1/12

Todd Quinn

Professional Personnel Involved:

Lisa Olson, BS	Vice President Testing and Service Development
Teri Tanquist, BS	Vice President of Process Improvement and Operations
Todd Quinn, BS	Manager, Mycoplasma Testing Laboratory, Study Director
Jean Mesarich, AA	Client Relations Manager

1.0 PURPOSE

To demonstrate that a test article consisting of a master or working cell bank, virus seed lot, control cells, virus harvest, bulk vaccine or final lot and media is free of mycoplasmal contamination, according to European Pharmacopoeia (EP) and United States Pharmacopoeia (USP) criteria.

This study included a mycoplasmastasis (test article inhibition) assay to evaluate for the presence of test article (product) specific inhibition per EP and USP guidelines. The mycoplasmastasis assay should be repeated each time there is a change in production method that may affect the detection of mycoplasmas.

2.0 TEST FACILITY:

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

3.0 SCHEDULING

TEST ARTICLE RECEIVED:	03/20/12
INITIATION DATE:	03/22/12
COMPLETION DATE:	05/01/12

4.0 TEST ARTICLE IDENTIFICATION

Test Article Name:	hOX40L:Ig clone 755-19 MCB WuXi AppTec Accession# 11-003668
Lot/Batch #:	120170969
General Description:	MCB
Number of Aliquots used:	2 x 23 mL
Storage Conditions:	Ultracold (< -60 °C)

5.0 TEST ARTICLE CHARACTERIZATION

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

6.0 SAMPLE STORAGE

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

7.0 SAFETY

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

8.0 EXPERIMENTAL DESIGN

8.1 Experimental Summary

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

The indirect (indicator cell culture) method of detection allows visualization of mycoplasma, particularly non-cultivable strains, by inoculation into an indicator cell line and then staining using a DNA-binding fluorochrome (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 was used. The indicator cells are maintained by WuXi AppTec's Cell Production Laboratory.

The indirect assay was performed with both negative and positive controls. Both a strongly cyto-adsorbing (*M. hyorhinis*) and a poorly cyto-adsorbing (*M. orale*) species were used as positive controls. At least one sub-culture of the test article and controls on indicator cells was performed. Staining the cells with DNA-binding fluorochrome allowed for the detection of mycoplasma based on the staining pattern observed. Only the cell nuclei fluoresce in the negative cultures, but nuclear and extra-nuclear fluorescence is observed in positive cultures.

Direct (agar plates and broth culture) 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, energy, and favorable growth conditions. The direct assay was performed using negative and positive controls. A fermentative mycoplasma species (*M. pneumoniae*) as well as a non-fermentative species (*M. orale*) was used as positive controls.

A mycoplasmastasis assay (test article inhibition) was performed to evaluate for the presence of product-specific inhibitory substances. In this assay, the test article was spiked with known concentrations of the positive control organisms and tested in both the direct and indirect assays. A comparison of the spiked test article result to the positive control result determined the presence or absence of inhibitory substances.

The mycoplasmastasis assay should ideally be performed prior to routine testing to allow for the inclusion of methods (dilution or neutralization) that may be necessary to counter potential product-specific inhibition. However, mycoplasmastasis may be performed concurrently.

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 EP, USP, and other regulatory agencies. This assay is a compendial method following guidelines set down in the EP, Section 2.6.7 Mycoplasmas and USP <63> Mycoplasma Tests.

8.3 Amendments / Deviations

None.

9.0 TEST AND CONTROL MATERIAL PREPARATION

9.1 Test Sample Preparation

Cells were grown at WuXi AppTec – Philadelphia using BR-30040 – Cell Growth for Assays. Four tubes of 23 mL each were prepared and sent to WuXi AppTec – St. Paul for mycoplasma testing. Two tubes of 23 mL each were thawed in a water bath at 37 ± 2 °C, pooled, and 1:5 and 1:10 dilutions were prepared in sterile phosphate buffered saline (PBS).

1 mL of the undiluted sample, the 1:5 and 1:10 dilutions were then inoculated into each of two 25 cm^2 flasks (per sample/dilution) containing previously incubated Vero cells. The flasks were incubated in incubator E770 for 1-2 hours at 36 ± 1 °C / 5 - 10% CO_2 and then 2 mL of EMEM, 8% fetal bovine serum (FBS) was added to each flask. The flasks were returned to incubator E770 at 36 ± 1 °C / 5 - 10% CO_2. After three days of incubation, the cell cultures were trypsinized and the resulting cell suspensions were added to two blank coverslips per dilution (one coverslip per flask). After three days incubation, these coverslips were fixed, stained, and then read using an epifluorescent microscope.

0.2 mL of the undiluted test article was inoculated onto each of three SP-4 agar plates which were incubated microaerophilically at 36 ± 1 °C for a minimum of 14 days.

10 mL of the undiluted test article was inoculated into one 75 cm^2 flask containing 100 mL of sterile SP-4 broth. The tightly stoppered flask was incubated at 36 ± 1 °C for a minimum of 21 days. The broth culture flasks were observed for turbidity and color changes each working day for 21 days. On Days 3, 7, and 14, 0.2 mL from each flask was subcultured onto two SP-4 agar plates and incubated microaerophilically at 36 ± 1 °C for a minimum of 14 days. On day 21, 0.2 mL from each broth culture flask was subcultured onto each of two SP-4 agar plates and incubated microaerophilically at 36 ± 1 °C for a minimum of 7 days. At the completion of the incubation period, the agar plates were observed microscopically for the presence of mycoplasma colonies.

9.2 Spiking of Test Article

9.2.1 2.7 mL of the undiluted test article was spiked with 0.3 mL of ≤ 1000 CFU/mL of *M. hyorhinis* for a final concentration of ≤ 100 CFU/mL.

9.2.2 1.8 mL of undiluted test article was spiked with 0.2 mL of ≤ $5x10^3$ CFU/mL of *M. orale* for a final concentration of ≤ 500 CFU/mL.

9.2.3 2.7 mL of undiluted test article was spiked with 0.3 mL of ≤ 1000 CFU/mL of *M. orale* for a final concentration of ≤ 100 CFU/mL.

9.2.4 9.9 mL of undiluted test article was spiked with 1.1 mL of ≤ 100 CFU/mL of *M. orale* for a final concentration of ≤ 10 CFU/mL.

9.2.5 2.7 mL of undiluted test article was spiked with 0.3 mL of ≤ 5 x 10^3 CFU/mL of *M. pneumoniae* for a final concentration of ≤ 500 CFU/mL.

9.2.6 9.9 mL of the undiluted test article was spiked with 1.1 mL of ≤ 100 CFU/mL of *M. pneumoniae* for a final concentration of ≤ 10 CFU/mL.

9.2.7 Spiked test articles were inoculated in the same manner and in the same concentrations as the positive controls.

9.3 Controls and Reference Materials

9.3.1 SP-4 broth served as the negative control for the direct and indirect assays.

9.3.2 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 per inoculum.

b. Direct Assay

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

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

9.3.3 Control Preparation

a. Negative Controls

a.1 One mL of SP-4 broth was inoculated into each of two 25 cm^2 flasks containing previously incubated Vero cells to serve as the negative control in the indirect assay.

a.2 0.2 mL of SP-4 broth was inoculated onto each of three SP-4 agar plates to serve as the negative control in the direct assay. 10 mL of SP-4 broth was inoculated into one 75 cm^2 flask containing 100 mL of SP-4 broth to serve as the negative control in the direct assay.

a.3 The cell culture flasks were incubated in incubator E770 for 1-2 hours at 36 ± 1 °C / 5 - 10% CO_2 and then 2 mL of EMEM, 8% fetal bovine serum (FBS) was added to each flask. The flasks were returned to incubator E770 at 36 ± 1 °C / 5 - 10% CO_2. After three days of incubation, the cell cultures were trypsinized and the resulting cell suspensions were added to blank coverslips (one coverslip per culture flask). After three days of incubation, the coverslips were fixed, stained, and then read using an epifluorescent microscope.

b. Positive Controls

b.1 *M. hyorhinis, M. orale,* and *M. pneumoniae* were diluted to ≤ 100 CFU per inoculum in sterile SP-4 broth. 1 mL of *M. hyorhinis* and *M. orale* at ≤ 100 CFU/mL was inoculated into each of two 25 cm^2 flasks containing previously incubated Vero cells.

b.2 The inoculated cell culture flasks were incubated in incubator E1071 for 1-2 hours at 36 ± 1 °C / 5 - 10% CO_2 and then 2 mL of EMEM, 8% fetal bovine serum (FBS) was added to each flask. The flasks were returned to incubator E1071 at 36 ± 1 °C / 5 - 10% CO_2. After three days of incubation, the cell cultures were trypsinized and the resulting cell suspensions were added to blank coverslips (one coverslip per culture flask). After three days of incubation, the coverslips were fixed, stained, and then read using an epifluorescent microscope.

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

b.4 The inoculated agar plates were incubated microaerophilically at 36 ± 1 °C for 14 days. The tightly stoppered broth culture flasks were incubated at 36 ± 1 °C for 21 days and observed each working day for color change or turbidity. On Days 3, 7, and 14, 0.2 mL from each broth culture flask was subcultured onto two SP-4 agar plates and incubated microaerophilically at 36 ± 1 °C for a minimum of 14 days.

On Day 21, 0.2 mL from each broth culture flask was subcultured onto each of two SP-4 agar plates and incubated microaerophilically at 36 ± 1 °C for a minimum of 7 days. At the completion of the incubation period, the agar plates were observed microscopically for the presence of mycoplasma colonies.

c. See Section 18.0, Analysis and Conclusion, for the final results of this assay.

10.0 TEST EVALUATION

10.1 Indirect Assay

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

10.2 Direct Assay

Change in color or turbidity of broth culture can be an indicator of the presence of mycoplasma growth. Fermentative mycoplasma produce acid from the carbohydrates in the medium causing the pH of the medium to drop and the broth to turn yellow in color. Nonfermentative mycoplasma produce ammonia by arginine hydrolysis causing the pH to rise and the broth culture 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.

10.3 Interpretation of Assay Results

10.3.1 Possible results scenarios for the indirect and direct assay and reported results

IF:	TEST ARTICLE RESULTS				
Indirect Assay - Mycoplasmal fluorescence	-	+	+/-	+/-	-
Direct Assay - Broth (color change or turbidity change)	-	+/-	+/-	+/-	+*
- Agar – Day 0 (at least one plate)	-	+/-	+	+/-	-
- Sub-culture Agar Plates (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).

10.3.2 The test article is considered as negative if both the direct assay and the indirect assay show no evidence of mycoplasma contamination and resemble the negative control for each procedure.

10.3.3 If the test article shows a positive result in either the indirect or direct assay, then the sample is considered to have failed the test and is positive.

11.0 MYCOPLASMASTASIS (TEST ARTICLE INHIBITION)

11.1 Direct Assay

11.1.1 A test article is considered inhibitory if growth of the control organism on the positive control subculture plates is observed more than one (1) subculture sooner than in the corresponding spiked test article. A test article is also considered inhibitory if plates directly inoculated (Day 0 agar plates) with the spiked test article are not within a 0.5-log unit range of the number of colonies of the corresponding Day 0 positive controls (*M. orale* or *M. pneumoniae*).

11.1.2 Equivocal Results (Growth Enhancement)
If the number of colonies on the plates directly inoculated (Day 0 agar plates) with the spiked test article is greater than 0.5-log unit higher than the corresponding Day 0 positive controls (*M. orale* or *M. pneumoniae*) the result is considered equivocal. In this case the directly inoculated agar plate portion of the direct assay should be repeated to confirm the result.

11.2 Indirect Assay
A test article is considered inhibitory if growth of the control organism is observed in the positive control, but not in the corresponding spiked test article.

11.3 Repeat Testing for Products Containing Inhibitory Substances

If a test article is found to cause inhibition, the inhibitory substance(s) must be neutralized or counteracted by some other method such as passage in a substrate not containing inhibitors, or dilution. If dilution is used, larger media volumes may be used or the inoculum volume may be divided among several flasks. The effectiveness of the neutralization or other process is confirmed by repeating the mycoplasmastasis portion of the assay.

12.0 EVALUATION CRITERIA

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

12.1 Indirect Assay

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 coverslip.

12.1.3 The positive control mycoplasma *M. orale* at ≤ 100 CFU per inoculum is detected in at least one coverslip.

DETECTION OF MYCOPLASMA CONTAMINATION BY INDICATOR CELL CULTURE ASSAY

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

12.2 Direct Assay

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

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

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

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)	-	+	+
Sub-culture agar plates (at least one plate on one day)	-	+	+
Results	-	+	+

13.0 METHOD FOR CONTROL OF BIAS: Not applicable.

14.0 DATA ANALYSIS

For the Day 0 (directly inoculated) agar plates, the average CFU per plate was calculated. A comparison of the log values for the positive control growth (average CFU per plate) in the presence and absence of the test article was made.

15.0 STATISTICAL METHODS

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

16.0 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.

17.0 TEST ARTICLE DISPOSITION:

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

18.0 ANALYSIS AND CONCLUSION

Table 1 - Indirect Assay and Direct Assay Results

Sample	Indirect	Direct		Overall
		Broth Flasks	Agar Plates	
Test Article	Negative	Negative	Negative	Negative
Test Article spiked with *M. orale*	Non-inhibitory Positive	Non-inhibitory Positive	Non-inhibitory Positive	Non-inhibitory Positive
Test Article spiked with *M. hyorhinis*	Non-inhibitory Positive			Non-inhibitory Positive
Test Article spiked with *M. pneumoniae*		Non-inhibitory Positive	Non-inhibitory Positive	Non-inhibitory Positive
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 1:5 dilution of the test article were read and determined negative. The coverslips for the undiluted test article could not be read due to insufficient cell attachment.

18.1 Mycoplasmastasis (Product-Specific Growth Inhibition)

18.1.1 Indirect assay

For the indirect assay, the test article spiked with *M. hyorhinis* at ≤ 100 CFU per inoculum as well as those spiked with *M. orale* at ≤ 100 CFU per inoculum were positive and resembled the corresponding positive controls. No growth inhibition was observed. See Table 1.

18.1.2 Direct assay – Day 0 Agar Plates

$$\text{Average CFU / Plate} = \frac{(\text{Plate 1 CFU} + \text{Plate 2 CFU} + \text{Plate 3 CFU})}{3}$$

Control $\log_{10}$ - TA $\log_{10} > 0.5$ then product-specific growth inhibition has occurred

$|\text{Control } \log_{10} - \text{TA } \log_{10}| \leq 0.5$ then product-specific growth inhibition has not occurred

Control $\log_{10}$ - TA $\log_{10} < -0.5$ then the results are considered equivocal

TABLE 2 – DAY 0 AGAR PLATES

TEST ARTICLE SPIKED WITH	POSITIVE CONTROL		SPIKED TEST ARTICLE		CONTROL LOG_{10} - TA LOG_{10}	INHIBITORY / NON-INHIBITORY
	AVE. CFU / PLATE	LOG_{10}	AVE. CFU / PLATE	LOG_{10}		
M. orale	108	2.0	137	2.1	- 0.1	Non-inhibitory
M. pneumoniae	31	1.5	24	1.4	0.1	Non-inhibitory

18.1.3 Direct assay – Subculture Plates

The subculture plates for the test article spiked with *M. orale* at ≤ 100 CFU per inoculum and those spiked with *M. pneumoniae* at ≤ 100 CFU per inoculum yielded a positive result on the same subculture day as the corresponding positive control plates. No growth inhibition was observed.

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

18.3 These findings indicate that the test article is considered **negative** for the presence of mycoplasma contamination and is **non-inhibitory** to the detection of mycoplasma.

19.0 TECHNICAL REFERENCES

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

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

19.3 European Pharmacopoeia, 6th Edition, Section 2.6.7, Mycoplasmas.

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

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

19.6 United States Pharmacopoeia, <63> Mycoplasma Tests, current revision.

19.7 WuXi AppTec SOP: MTL–32700, Mycoplasma Detection with Mycoplasmastasis: According to European Pharmacopoeia and United States Pharmacopoeia Guidelines (current revision).

Copy of Original

Protocol and Sample Submission Form

(For Laboratory Use Only)
WuXi AppTec Study # 167429
Accession # 11-003668

PROTOCOL TITLE: MYCOPLASMA DETECTION WITH MYCOPLASMASTASIS: EUROPEAN PHARMACOPOEIA AND UNITED STATES PHARMACOPOEIA GUIDELINES

TEST CODE: 32700

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

EFFECTIVE DATE: 21 October 2010

GLP PROTOCOL: 32700-1

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

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1.0 PURPOSE

To demonstrate that a test article consisting of a master or working cell bank, virus seed lot, control cells, virus harvest, bulk vaccine or final lot and media is free of mycoplasmal contamination, according to European Pharmacopoeia and United States Pharmacopoeia (USP) criteria.

This protocol includes a mycoplasmastasis (test article inhibition) assay to evaluate for the presence of test article (product) specific inhibition per EP and USP guidelines. The mycoplasmastasis assay should be repeated each time there is a change in production method that may affect the detection of mycoplasmas.

2.0 TEST FACILITY:

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

3.0 SCHEDULING AND DISCLAIMER

3.1 Test protocol initiation is generally within 10 working days after receipt of the test article, a signed protocol/Client Approval form, and a signed sample submission form. The Client 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 report to follow approximately 10 working days after completion of the study.

3.2 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.

3.3 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, or a portion of it, must be repeated 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.

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

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.

3.6 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.

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.

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5.0 TEST ARTICLE CHARACTERIZATION

The Sponsor is responsible for all test article characterization data as specified in the Good Laboratory Practices (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.

6.0 SAFETY

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

7.0 EXPERIMENTAL DESIGN

7.1 Experimental Overview

Whereas no single test is capable of detecting all mycoplasma strains, freedom from mycoplasma contamination may be demonstrated by the use of both an indirect and a direct procedure. The procedures employed in this protocol are based on methods found in the EP and USP guidelines.

The indirect (indicator cell culture) method of detection allows visualization of mycoplasma, particularly non-cultivable strains, by inoculation into an indicator cell line and then staining using a DNA-binding fluorochrome (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 Chinese hamster ovary cell line, CHO-K1, or Swiss mouse embryo cell line, NIH/3T3, may also be used. The indicator cells are maintained by WuXi AppTec's Cell Production Laboratory.

The indirect assay will be performed with both negative and positive controls. Both a strongly cyto-adsorbing (*M. hyorhinis*) and a poorly cyto-adsorbing (*M. orale*) species will be used as positive controls. At least one sub-culture of the test article and controls on indicator cells will be performed. Staining the cells with DNA-binding fluorochrome allows for the detection of mycoplasma based on the staining pattern observed. Only the cell nuclei fluoresce in the negative cultures, but nuclear and extra-nuclear fluorescence is observed in positive cultures.

Direct (agar plates and broth culture) 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, energy, and favorable growth conditions. The direct assay will be performed using negative and positive controls. A fermentative mycoplasma species (*M. pneumoniae*) as well as a non-fermentative species (*M. orale*) will be used as positive controls.

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A mycoplasmastasis assay (test article inhibition) will be performed to evaluate for the presence of product-specific inhibitory substances. In this assay the test article will be spiked with known concentrations of the positive control organisms and tested in both the direct and indirect assays. A comparison of the spiked test article result to the positive control result will determine the presence or absence of inhibitory substances. The mycoplasmastasis assay should ideally be performed prior to routine testing to allow for the inclusion of methods (dilution or neutralization) that may be necessary to counter potential product-specific inhibition. However, mycoplasmastasis may be performed concurrently.

7.2 ***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 EP, USP, and other regulatory agencies. This assay is a compendial method following guidelines set down in the EP, Section 2.6.7 Mycoplasmas and USP <63> Mycoplasma Tests.

7.3 **Amendments / Deviations**

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.

8.0 IDENTIFICATION OF THE TEST SYSTEM

8.1 Controls And Reference Materials

8.1.1 Sterile SP-4 broth will serve as the negative control for both the direct and indirect assays.

8.1.2 **Positive Controls**

a. **Indirect Assay**

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

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

b. **Direct Assay**

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

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

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9.0 TEST METHOD

9.1 Indirect (DNA-Staining) Assay

9.1.1 1.0 mL of the negative control will be inoculated into two or more culture flasks containing previously incubated indicator cells.

9.1.2 1.0 mL of the test article will be inoculated into two or more culture flasks containing previously incubated indicator cells. Additional dilutions of the test article may be prepared to eliminate cellular debris that could interfere with mycoplasma detection.

9.1.3 For trypsin samples, an equal volume of previously tested and released fetal bovine serum (FBS) will be added to the test article. This 1:2 dilution is made to inactivate the trypsin sample and will be used for the Indirect Assay only.

9.1.4 To test for inhibitory substances, the test article will be spiked with *M. hyorhinis* and *M. orale* at ≤ 100 CFU per inoculum. Two or more culture flasks containing previously incubated indicator cells will be inoculated with each spiked test article.

9.1.5 The positive controls, *M. hyorhinis* and *M. orale* at ≤ 100 CFU per inoculum will be inoculated in the same manner. Two or more culture flasks containing previously incubated indicator cells will be inoculated with 1.0 mL of each positive control.

9.1.6 The cell cultures will be grown in the presence of test article, spiked test article, and controls for at least 3 days and then sub-cultured into culture dishes or other appropriate vessels containing blank coverslips (at least one coverslip / culture flask).

9.1.7 Following sub-culturing (incubate aerobically for 3 to 5 days at 36 ± 1 °C / 5 - 10% CO_2) cells will be fixed and stained with a DNA-binding fluorochrome (Hoechst stain), and will be evaluated for the presence of mycoplasma using epifluorescent microscopy.

9.2 Direct (Microbiological) Assay

9.2.1 At least two agar plates will be inoculated with 0.2 mL per plate of the negative control (sterile SP-4 broth).

9.2.2 10 mL of negative control will be inoculated into one flask containing 100 mL of sterile SP-4 broth.

9.2.3 The test article will be inoculated onto two or more agar plates (0.2 mL per plate) and into one broth flask (10 mL into 100 mL sterile SP-4 broth).

9.2.4 To test for inhibitory substances, the test article will be spiked with *M. pneumoniae* and *M. orale* at ≤ 100 CFU per inoculum. Each spiked test article will be inoculated onto two (2) or more agar plates (0.2 mL per plate) and into one broth flask (10 mL into 100 mL sterile SP-4 broth)

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9.2.5 The positive controls, *M. pneumoniae* and *M. orale*, will be inoculated in the same manner, using 100 or fewer CFU per inoculum onto each of two or more plates and 10 mL into one flask containing 100 mL of sterile SP-4 broth.

9.2.6 Day 0 agar plates will be incubated microaerophilically at 36 ± 1 °C for at least 14 days.

9.2.7 The tightly stoppered broth culture flasks will be incubated at 36 ± 1 °C for 20-21 days and will be observed every working day for changes in color or turbidity.

9.2.8 The broth culture flasks will be subcultured on Days 2, 3, or 4; 6, 7, or 8; and 13, 14, or 15 onto SP-4 agar plates. Two or more plates using 0.2 mL broth/plate will be inoculated and incubated microaerophilically at 36 ± 1 °C for at least 14 days.

9.2.9 The broth culture flasks will be subcultured on Day 19, 20, or 21. 0.2 mL from each broth culture will be inoculated onto two or more agar plates and incubated microaerophilically at 36 ± 1 °C for at least 7 days.

10.0 TEST EVALUATION

10.1 Indirect Assay

Hoechst stain will bind to most DNA containing organisms and organelles present in the culture; this includes indicator cell nuclei, prokaryotes including mycoplasma and cell debris. The source of DNA is determined by the staining pattern. The indicator cell nuclei fluoresce brightly and are generally 10 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.

10.2 Direct Assay

Change in color or turbidity of broth culture can be an indicator of the presence of mycoplasma growth. Fermentative mycoplasma produce acid from carbohydrates in the medium causing the pH to drop and the broth to turn yellow. 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.

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10.3 Interpretation of Test Results

10.3.1 Possible results scenarios for the indirect and direct assays and the reported results

IF:	TEST ARTICLE RESULTS				
Indirect Assay - Mycoplasmal fluorescence	-	+	+/-	+/-	-
Direct Assay - Broth (color change or turbidity change)	-	+/-	+/-	+/-	+*
- Agar – Day 0 (at least one plate)	-	+/-	+	+/-	-
- Sub-culture Agar Plates (at least one plate on one day)	-	+/-	+/-	+	-
THEN: OVERALL FINAL RESULT	-	+	+	+	-

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

10.3.2 The test article will be considered as negative if both the direct assay and the indirect assay show no evidence of mycoplasma contamination and resemble the negative control for each procedure.

10.3.3 If the test article shows a positive result in either the indirect or direct assay, then the sample will be considered to have failed the test and will be positive.

10.3.4 Further confirmation by PCR or DNA sequencing may be performed to verify the result, if requested [additional fees will apply].

11.0 MYCOPLASMASTASIS (TEST ARTICLE INHIBITION)

11.1 Direct Assay

11.1.1 A test article is considered inhibitory if growth of the control organism on the positive control subculture plates is observed more than one (1) subculture sooner than in the corresponding spiked test article. A test article is also considered inhibitory if plates directly inoculated (Day 0 agar plates) with the spiked test article are not within a 0.5-log unit range of the number of colonies of the corresponding Day 0 positive controls (*M. orale* or *M. pneumoniae*).

11.1.2 Equivocal Results (Growth Enhancement)
If the number of colonies on the plates directly inoculated (Day 0 agar plates) with the spiked test article is greater than 0.5-log unit higher than the corresponding Day 0 positive controls (*M. orale* or *M. pneumoniae*) the result is considered equivocal. In this case the directly inoculated agar plate portion of the direct assay should be repeated to confirm the result.

11.2 Indirect Assay
A test article is considered inhibitory if growth of the control organism is observed in the positive control, but not in the corresponding spiked test article.

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11.3 Repeat Testing for Products Containing Inhibitory Substances
If a test article is found to cause inhibition, the inhibitory substance(s) must be neutralized or counteracted by some other method such as passage in a substrate not containing inhibitors, or dilution. If dilution is used, larger media volumes may be used or the inoculum volume may be divided among several flasks. The effectiveness of the neutralization or other process is confirmed by repeating the mycoplasmastasis portion of the assay.

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

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 coverslip.

12.1.3 The positive control mycoplasma *M. orale* at ≤100 CFU per inoculum is detected in at least one coverslip.

DETECTION OF MYCOPLASMA CONTAMINATION BY INDICATOR CELL CULTURE ASSAY

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

12.2 Direct Assay

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

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

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

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)	-	+	+
Sub-culture agar plates (at least one plate on one day)	-	+	+
Results	-	+	+

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13.0 METHOD FOR CONTROL OF BIAS: Not applicable.

14.0 DATA ANALYSIS

For the Day 0 (directly inoculated) agar plates, the average CFU per plate will be calculated. A comparison of the log values for the positive control growth (average CFU per plate) in the presence and absence of the test article will be made.

15.0 STATISTICAL METHODS

The results of this study will be qualitative, therefore, no statistical analysis will be required.

16.0 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.

17.0 *RECORD RETENTION*

17.1 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:

17.1.1 All handwritten and equipment generated raw data for control(s) and test article(s).
17.1.1 Any protocol amendments/deviation notifications.
17.1.2 Memoranda, specifications, and other study specific correspondence relating to interpretation and evaluation of data, other than those documents contained in the final study report.
17.1.3 Original signed protocol.
17.1.4 Certified copy of final study report.
17.1.5 Study-specific SOP deviations made during the study.
17.1.6 QA reports for each QA inspection with comments.

17.2 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:

17.2.1 SOPs which pertain to the study conducted.
17.2.2 Non study-specific SOP deviations made during the course of this study which may affect the results obtained during this study.
17.2.3 Methods which were used or referenced in the study conducted.
17.2.4 Facility Records: Temperature Logs (ambient, incubator, etc.), Instrument Logs, Calibration and Maintenance Records.
17.2.5 Current job descriptions and summary of experience and training for all personnel involved in the study.

18.0 COMPLIANCE

GLP STATUS: The study will be conducted under GLP compliance (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.

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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.

20.0 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 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).

20.3 European Pharmacopoeia, 6th Edition, Section 2.6.7, Mycoplasmas.

20.4 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).

20.5 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).

20.6 United States Pharmacopoeia, <63> Mycoplasma Tests, current revision.

20.7 WuXi AppTec SOP: MTL–32700, Mycoplasma Detection with Mycoplasmastasis: According to European Pharmacopoeia and United States Pharmacopoeia Guidelines (current revision).

21.0 RESPONSIBILITIES

This protocol was written by a WuXi AppTec – St. Paul Study Director and will be approved by a WuXi AppTec Study Director, Management, and Quality Assurance. Qualified laboratory personnel will perform the testing and a mycoplasma Study Director will review and assemble the results and final report. The final report will be reviewed by a Study Director and Quality Assurance and approved by a Study Director, Quality Assurance, and Management.

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CLIENT PROTOCOL APPROVAL FORM

PROTOCOL TITLE: Mycoplasma Detection With Mycoplasmastasis: European Pharmacopoeia and United States Pharmacopoeia Guidelines

PROTOCOL NUMBER: 32700-1

SPONSOR:

Nick Morris
Agonox (at Providence Cancer Center)
4805 N.E. Glisan Street
Cancer Research Rm. 2NC29
Portland, OR 97213 U.S.A.

AGX03
Phone #: 503-215-3927
E-mail: nicholas.morris@providence.org

PRIMARY APPROVAL STATEMENT

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 number is issued.
2. The Primary Approver's position with the stated company is terminated or changes, whichever may occur first.

NICHOLAS P. MORRIS
Primary Approver (Print Name)

PROJECT MANAGER
Title

Nicholas P. Morris
Signature

MARCH 16, 2012
Date

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 number 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.

ANDREW WEINBERG P.I.
Name of Associate (Print Name)

Name of Associate (Print Name)

Name of Associate (Print Name)

Name of Associate (Print Name)

WUXI APPTEC, INC.:

Todd Quinn
Study Director (Print Name)

Study Director (Signature)

3/22/12
Date

167427

WuXi 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 type of sample.

WUXI APPTEC USE ONLY Accession #: 11-003068

For tips when completing electronically, hover your mouse over the fields/sections.

CLIENT CONTACT INFORMATION

Contact for Final Report:	PHONE	FAX	EMAIL
Nick Morris	503-215-3927	503-215-6841	nick.morris@providence.org
Secondary Contact (if applicable/needed):			

Company Name and Address:
Providence Portland Medical Center
Cancer Research Rm 2NC29
4805 NE Glisan Street
Portland OR, 97213

Client Account Number:

WuXi AppTec Quote Number:

Purchase Order Number:

SAMPLE IDENTIFICATION & INFORMATION

If resubmitting sample material, provide original WuXi AppTec sample Accession #:

KD 24Feb12

Sample Designation for Final Report:
hOX40L:Ig clone 755-19 MCB
WuXi AppTec Accession# 11-003068
Lot# 180 170969

TYPE of Sample (General Description of Material):
MCB

Commercial/Marketed Product? ☐ Yes ☐ No

If requesting GMP testing, provide product name referenced in WuXi AppTec qualification or validation:
hOX40L:Ig clone 755-19 MCB

Species of Origin: hamster

Biosafety Level:

Stability (Expiration Date):

Sample Matrix or Buffer Components (if applicable):

SAMPLE SHIPMENT & STORAGE

See Philly CCC, 3/22/12

Controlled Storage Temperature: ☐ Room Temperature ☐ Refrigerated (2° to 8°C) ☐ Frozen (-10° to -60°C) ☒ 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, WuXi 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 & 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.)	
Chinese hamster Ovary cell line (CHO)	☒ Suspension Cells ☐ Adherent Cells	☐ Requires Trypsin ☐ Requires Trypsin/EDTA	☒ No Trypsin / Cells are sensitive ☐ Other:

Medium Requirements, including Supplements/Concentrations:
NOTE: WuXi 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.

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16 7429

FOR WUXI APPTEC USE

CLIENT	DATE	ACCESSION #

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	
Post Cell-Bank Growth for testing	32370				
Cell Growth for Assays	30040C				
Determination of Cel. Morph. & Growth Char.	30117				
Cell Line Spec. Identity by Isoenz. Electrophor	30330				
Sterility Test	30744				
Sterility Test	30744A				
Biologics B/F	30736				
Mycoplasma	32700				
Adventitious Viral Contaminants	31912				
In Vivo Assay for Viral Contaminants	30194				

IF ORDERING PROTOCOL # 37000, PROVIDE THE FOLLOWING INFORMATION AS APPLICABLE

If ordering Protocol # 37000, cell lines must be listed below. *(Selections for vessel, duration and optional additional endpoint are also required.)*	VESSEL *Check one:* ☐ Plates ☐ Flasks	ASSAY DURATION *Check one:* ☐ 14-day ☐ 28-day	ADDITIONAL ASSAY ENDPOINT Perform hemagglutination? *(Check one)* ☐ Yes ☐ No *Hemabsorption is the standard endpoint. If "yes" is selected, hemagglutination will also be performed.*

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 WuXi 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 WuXi AppTec).* Failure to provide this information could result in testing delays or other issues. WuXi 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.

Signature	Print Name	Date

WuXi AppTec – Philadelphia • 4751 League Island Blvd. • Philadelphia, PA 19112 • 800.622.8820 • 215.218.5500 • FAX 215.218.5990
WuXi AppTec – Atlanta • 1265 Kennestone Circle • Marietta, GA 30066 • 888.847.6633 • 770.514.0262 • FAX 770.514.0294
WuXi AppTec – St. Paul • 2540 Executive Drive • St. Paul, MN 55120 • 888.794.0077 • 651.675.2000 • FAX 651.675.2005

16 7429

FOR WUXI APPTEC USE

CLIENT	DATE	ACCESSION #

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	
Hamster antibody production test	30011				
MVM DNNA	30910				
Thin section EM	30610	XD 28 Feb 12			
Co-Cultivation	30210 ~~30201~~				
F-PBRT	30357				
Detection of BPyV	30727				
Detection of Adventitious Bovine Viruses	30236				

PROVIDE THE FOLLOWING INFORMATION AS APPLICABLE

If ordering Protocol # 3700D, cell lines must be listed below. *[Selections for vessel, duration and optional additional endpoint are also required.]*	VESSEL *Check one:* ☐ Plates ☐ Flasks	ASSAY DURATION *Check one:* ☐ 14-day ☐ 28-day	ADDITIONAL ASSAY ENDPOINT Perform hemagglutination? *(Check one)* ☐ Yes ☐ No *Hemabsorption is the standard endpoint. If "yes" is selected, hemagglutination will also be performed.*

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 WuXi 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 WuXi AppTec)*. Failure to provide this information could result in testing delays or other issues. WuXi 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.

Nicholas P. Morris	NICHOLAS P. MORRIS	FEB 21, 2012
Signature	Print Name	Date

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