MICROBIAL PERFORMANCE EVALUATION OF UNIVERSAL TRANSPORT SYSTEM OF PURITAN MEDICAL PRODUCTS LLC (ALSO BRANDED AS TRANSPRO™ CVM)

PROJECT NO. 2560284.0001.001

FINAL TECHNICAL REPORT of OCTOBER 28, 2011

To
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OCTOBER 28, 2011

LOVELACE RESPIRATORY RESEARCH INSTITUTE
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(28 are the total number of pages in the report)

LOVELACE ORGANIZATION OVERVIEW
ALBUQUERQUE, NEW MEXICO

Lovelace Respiratory Research Institute (LRRI) is a private not-for-profit biomedical research institute headquartered in Albuquerque, New Mexico dedicated to the reduction of the nation's substantial respiratory health burden. LRRI brings a broad range of research capabilities and research alliances to bear on respiratory health issues of concern to government, industry, universities, health advocacy organizations, and the public. We are committed to the cure of respiratory diseases through research aimed at understanding their causes and biological mechanisms, eliminating exposures to causal agents, and developing improved treatments. The Institute currently employs over 100 PhDs, DVMs, or MDs.

LRRI's scientists include clinicians (physicians, veterinarians), Board-certified laboratory animal veterinarians, molecular and cellular biologists, immunologists, microbiologists, physiologists, neurobiologists, Board-certified toxicologists, Board-certified veterinary pathologists, aerosol scientists and statisticians. LRRI's scientists work in either of two locations shown below:

North and South facilities for Lovelace Respiratory Research Institute in Albuquerque, NM comprising 125,000 and >350,000 ft^2 of research space.

LRRI facilities include virology, microbiology, cellular biology, physiology, wind tunnel and molecular biology laboratories. Additional specialized facilities include biosafety level-2 (BSL-2), BSL-3, animal BSL-3, chemical agent and radiobiology laboratories. Also included are extensive AAALAC-accredited facilities for housing rodents, rabbits, ferrets, dogs and non-human primates.

MICROBIAL PERFORMANCE EVALUATION OF UNIVERSAL TRANSPORT SYSTEM OF PURITAN MEDICAL PRODUCTS LLC (ALSO BRANDED AS TRANSPRO™ CVM)

FOREWORD

This report describes a study conducted by Lovelace Respiratory Research Institute for Puritan Medical Products Company LLC (PMP). Kunapuli T. Madhusudhan, PhD served as the Technical Expert and Mehdi Karamchi served as the Sponsor Representative for the duration of the study. The study was conducted during the period March 1, 2011 (contract signature) to October 31, 2011 (final report signature).

This is to certify that the entire project work on "Microbial Performance Evaluation of Puritan Medical Products LLC Universal Transport System" (also branded as TransPRO™ CVM) was conducted at the Lovelace Respiratory Research Institute by the key personnel specified below. All results presented in this report are accurate to the best of our knowledge including scientifically valid interpretations and conclusions related to equivalency determination.

Trevor Brasel, PhD, served as the Study Director, and was responsible for the overall conduct of the study. Robert Sherwood, PhD, served as the Principal Investigator. Krystle Agans, BS, served as the Lead Virologist; Sara Jones, BS, served Lead Microbiologist; Brandi France, BS, served as Project Manager.

31 Oct 2011

Trevor Brasel, PhD — Date
Study Director

Approved:

Robert L Sherwood 31 Oct 2011

Robert Sherwood, PhD — Date
Director, Applied Life Sciences/
Sr. Scientist

MICROBIAL PERFORMANCE EVALUATION OF UNIVERSAL TRANSPORT SYSTEM OF PURITAN MEDICAL PRODUCTS LLC

SPONSOR REPRESENTATIVE APPROVAL

Signature,

Oct. 31, 2011

Mehdi Karamchi
Sponsor Representative

Date

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MICROBIAL PERFORMANCE EVALUATION OF UNIVERSAL TRANSPORT SYSTEM OF PURITAN MEDICAL PRODUCTS LLC (ALSO BRANDED AS TRANSPRO™ CVM)

EXECUTIVE SUMMARY

The objective of this study was to compare the performance of a non-propagating transport device composed of culture medium that comprises a Universal Transport Medium (UTM) System-RT with a similar commercial device. Comparison testing was accomplished through recovery, storage stability, and cytotoxicity studies. The test device was defined as the Puritan® Medical Products Company LLC (PMP) Universal Transport Medium (also branded as TransPRO™ CVM) for Viruses, Chlamydia, Mycoplasma, and Ureaplasma. The predicate device was defined as the Universal Viral Transport System of Becton, Dickinson and Company (BD) manufactured by Copan Diagnostics of Italy.

Recovery:

The following virus strains were chosen for recovery testing: Adenovirus, Cytomegalovirus, Echovirus Type 30, Herpes Simplex Virus Types I and II, Influenza A, Parainfluenza 3, Respiratory Syncytial Virus, and Varicella Zoster Virus. The following Chlamydia strains were chosen for testing: Chlamydia pneumoniae and Chlamydia trachomatis. The following Mycoplasma strains were chosen for testing: Mycoplasma hominis and Mycoplasma pneumoniae. Ureaplasma urealyticum served as the Ureaplasma species. Prior to recovery testing, neat stocks of the test organisms were prepared and assessed for viable concentration. Viable recovery evaluations were performed following spiking of the test and predicate systems with nine different viruses, two species of Chlamydiae, two Mycoplasma strains, and one strain of Ureaplasma. The performance of the two devices was assessed by spiking swabs that accompanied each transport system with optimized dilutions of the test strains. Two dilutions of each test organism were used for recovery studies. Swabs were then transferred into the UTM, stored at different temperatures (refrigerated and room temperature) and held for 0, 24, 48, or 72 hours. Viability/stability of each test organism under the defined conditions was evaluated through cell culture and immunofluorescence staining (viruses and Chlamydia) or standard bacterial culture methods (Mycoplasma and Ureaplasma). Culture data between the test and predicate devices were statistically analyzed and compared.

For both transport systems, test viruses and bacteria could be quantified through 48 hours at the two storage temperatures. In general, refrigerated storage resulted in higher test strain recoveries for both devices. One-way analysis of variance demonstrated statistical differences ($P<0.05$) between the two devices under certain conditions that were concluded to be the result of normal microbiological variability and thus insignificant from the clinical stand point.

Storage Stability:

Storage stability studies were done to compare the performance of the test device lots beyond expiration point to newly prepared lot. The following strains were chosen for storage stability testing: Cytomegalovirus, Herpes Simplex Virus Types II, Respiratory Syncytial Virus, C. pneumoniae and M. pneumoniae. Prior to stability testing, neat stocks of the test organisms were prepared and assessed for viable concentration. Three lots of the test device beyond the expiration point and one newly prepared lot were challenged with one concentration of each neat stock. Device performance was assessed by spiking swabs that accompanied each transport system with the chosen test dilution. Swabs were then transferred into the UTM, stored at different temperatures (refrigerated and room temperature) and held for 0, 24, 48, or 72 hours.

Viability/stability of each test organism under the defined conditions was evaluated through cell culture and immunofluorescence staining (viruses and Chlamydia) or standard bacterial culture methods (Mycoplasma). Culture data between the test and predicate devices were statistically analyzed and compared.

Storage stability testing on all four test device lots at the two storage temperatures demonstrated quantification of the viruses and bacteria through 72 hours. In general, refrigerated storage resulted in higher test strain recoveries and increased stability. One-way analysis of variance demonstrated statistical differences ($P<0.05$) between the lots under certain storage conditions, primarily between refrigerated and room temperature storage; this was concluded to be insignificant from the clinical stand point. Storage stability testing indicated maintenance of the test device performance up to and including the expiration date.

Cytotoxicity:

Cytotoxicity of the test device was evaluated to ensure that the test product is not toxic to the host cell line for viruses and Chlamydia. Testing was performed using an MRC-5 cell line in conjunction with a standard Sulforhodamine B assay demonstrated no cellular toxicity associated with three lots of test device (two expired, one unexpired) when statistically compared to negative controls.

Statistical analyses conducted on results obtained following recovery, stability and cytotoxicity testing demonstrated performance equivalence between the test and predicate devices through 72 hours of storage at two temperatures. From a clinical perspective, no differences were noted between the two devices since all test organisms could be detected and quantified following all defined study conditions, including storage in expired device lots. In certain cases, the test device performed in a superior fashion to the predicate thus making it a valid system for the collection, storage, and transport of clinical specimens.

MICROBIAL PERFORMANCE EVALUATION OF PURITAN MEDICAL PRODUCTS LLC UNIVERSAL TRANSPORT SYSTEM (ALSO BRANDED AS TRANSPRO™ CVM)

1.0 INTRODUCTION

Sampling of body fluids including sputum, blood, bronchoalveolar (BAL) fluid, urine, bronchial and nasal washes, aspirates, etc. is commonly performed to test for bacterial and viral pathogens. Such procedures usually require specific equipment and well trained personnel. Swabs represent alternative tools that may be used for similar sampling that require little training and equipment. Upon sampling, swabs are often transferred into appropriate stabilizing transport media (solid, semisolid, or liquid) in order to preserve the viability and integrity of pathogens, to prevent the overgrowth of commensals, and/or to enumerate and classify specific pathogenic organisms to assess infection or efficacy of a given antibiotic treatment. Considering that primary care clinics and hospitals possess minimal microbiology laboratory facilities, a transport medium is necessary to maintain diagnostic sensitivity and specificity. For accurate identification of various bacteriological agents that cause a myriad of different pathophysiological conditions, several transport media, namely Stuart medium, Amies medium, Cary and Blair medium, and others, are commercially available (Stuart *et al*., 1954; Cary and Blair, 1964; Amies, 1967). In addition, numerous viral transport media of differing composition are described in the literature. These include Universal Transport Medium (UTM-RT; Copan and BD), Bartels Viratrans medium, M4-RT, M5 media, modified liquid Stuart's medium, Universal Collection Medium, and Multitrans medium (Starplex Scientific). Overall, however, limited media are available for the transport of viruses, *Chlamydiae* spp., *Mycoplasma* spp., and *Ureaplasma* spp. (Cornall and Urquhart, 1978; Johnson, 1990; Ogburn *et al*., 1994; Racioppi and Brinker, 1997; Taha *et al*., 2006; WHO, 2006; Adelson and Mordelchai, 2007).

Recovery of pathogens in significant proportions from clinical specimens after transport has long been recognized as a significant problem (Collee *et al*., 1974; Baron *et al*., 1993; Perry, 1997). This problem is more often encountered with intracellular pathogens such as viruses. Irrespective of the method of testing by culture or molecular methods, the sensitivity and speed of a diagnostic test is dependent on the number (i.e., concentration) of pathogens in the sample. Furthermore, the original conditions of a patient may deteriorate with time to the further detriment of treatability in the absence of accurate and timely diagnosis. Owing to the speed, cost, and sensitivity, molecular tests are increasingly used to diagnose infections caused by several viruses, *Chlamydiae* spp., *Mycoplasma* spp., and *Ureaplasma* spp. However, culture is a gold standard that is routinely performed prior to (baseline) and at intervals following the administration of antibiotic to patients to determine the success of the treatment regimen. Additionally, molecular tests may be unsuitable to certain disease conditions and culture may be required. Thus, an ideal transport medium for specimens should preserve viability and enable the analysis by culture, molecular (e.g., PCR), and immunological methods. Standard viral transport media contain a mixture of salts and nutrients (to sustain the viability of pathogens) and antibiotics to prevent growth of commensal organisms such as fungi and unrelated bacteria. Such media also maintain the capacity to detect various microbiological agents including those mentioned above. The UTM, launched by Copan Italia, was the first commercially available transport medium product that became popular and widely accepted by the clinical community. It has been extensively tested for recovery of various viruses and bacteria (Dunn *et al*, 2003; Luinstra *et al*., 2011). The Universal Viral Transport System of BD is manufactured by Copan Italia. This study was a continuation of the UTM design and evaluation effort and focused on a UTM system from Puritan Medical Products Company LLC (also branded as TransPRO™).

MICROBIAL PERFORMANCE EVALUATION OF PURITAN MEDICAL PRODUCTS LLC UNIVERSAL TRANSPORT SYSTEM

1.0 INTRODUCTION

Sampling of body fluids including sputum, blood, bronchoalveolar (BAL) fluid, urine, bronchial and nasal washes, aspirates, etc. is commonly performed to test for bacterial and viral pathogens. Such procedures usually require specific equipment and well trained personnel. Swabs represent alternative tools that may be used for similar sampling that require little training and equipment. Upon sampling, swabs are often transferred into appropriate stabilizing transport media (solid, semisolid, or liquid) in order to preserve the viability and integrity of pathogens, to prevent the overgrowth of commensals, and/or to enumerate and classify specific pathogenic organisms to assess infection or efficacy of a given antibiotic treatment. Considering that primary care clinics and hospitals possess minimal microbiology laboratory facilities, a transport medium is necessary to maintain diagnostic sensitivity and specificity. For accurate identification of various bacteriological agents that cause a myriad of different pathophysiological conditions, several transport media, namely Stuart medium, Amies medium, Cary and Blair medium, and others, are commercially available (Stuart *et al*., 1954; Cary and Blair, 1964; Amies, 1967). In addition, numerous viral transport media of differing composition are described in the literature. These include Universal Transport Medium (UTM-RT; Copan and BD), Bartels Viratrans medium, M4-RT, M5 media, modified liquid Stuart's medium, Universal Collection Medium, and Multitrans medium (Starplex Scientific). Overall, however, limited media are available for the transport of viruses, *Chlamydiae* spp., *Mycoplasma* spp., and *Ureaplasma* spp. (Cornall and Urquhart, 1978; Johnson, 1990; Ogburn *et al*., 1994; Racioppi and Brinker, 1997; Taha *et al*., 2006; WHO, 2006; Adelson and Mordelchai, 2007).

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2.0 MATERIALS AND METHODS

An overall flow chart of the virus and bacteria recovery study design is presented below in Figure 1. Where appropriate, controls are discussed in the sections that follow.

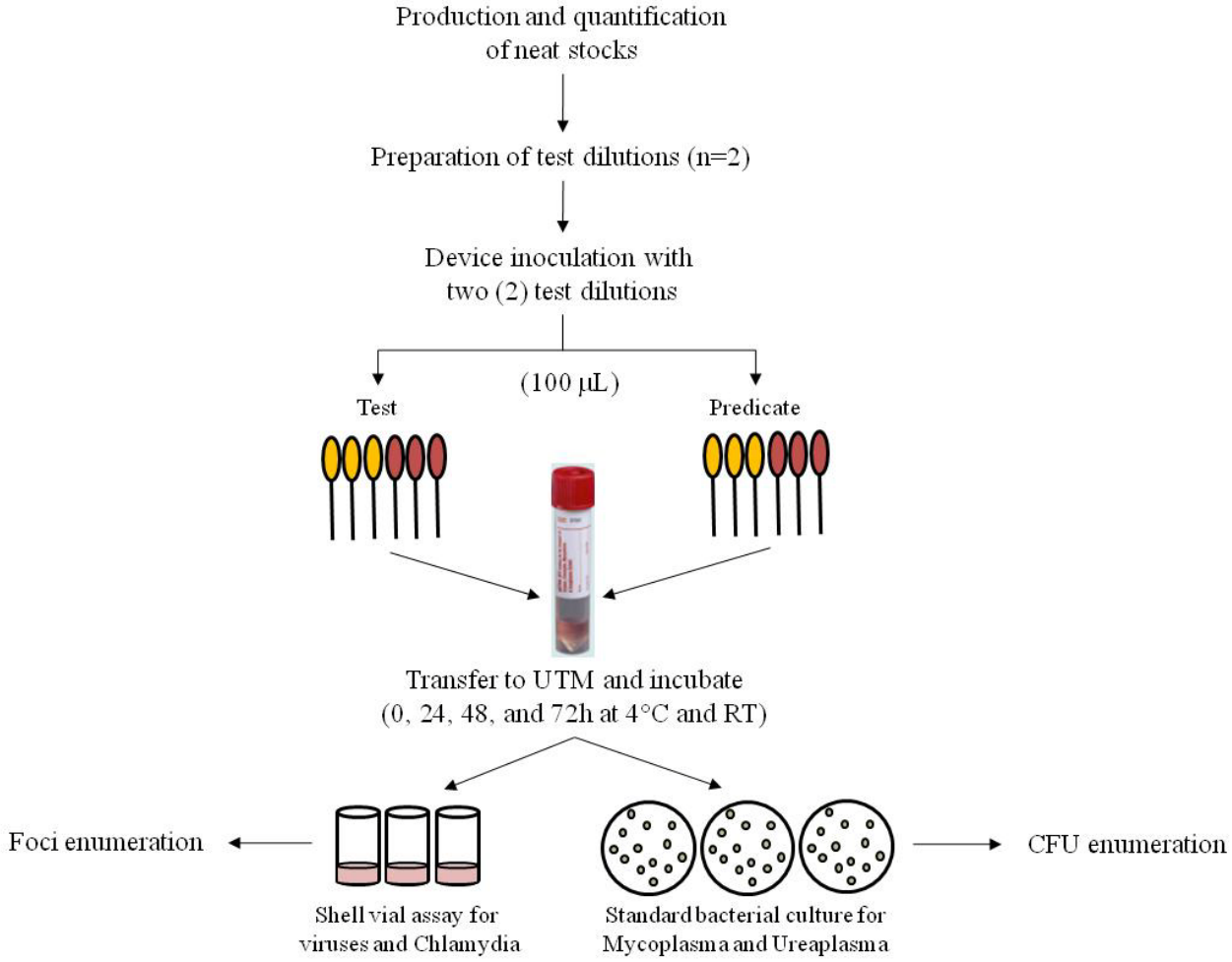

Figure 1. Flow chart for device comparison: virus and bacteria recovery testing.

2.1 Culture Stocks and Cell Lines

All culture stocks and cell lines were obtained from the American Type Culture Collection (ATCC) to establish traceability. Chosen test strains and cell lines (where applicable) are detailed below in Table 1.

Table 1. ATCC Strains Chosen for Testing

Test Strain	Abbreviation[a]	Test Strain		Host Strain		
		ATCC Cat. #	ATCC Lot #	Cell Type[a]	ATCC Cat #[a]	ATCC Lot #[a]
Adenovirus	n/a	VR-1	58134353	A549	CCL-185	58483235
Cytomegalovirus	CMV	VR-977	58087775	HEP-2	CCL-23	57922397
Echovirus Type 30	n/a	VR-692	217120	A549	CCL-185	58483235
Herpes Simplex Type 1	HSV-1	VR-733	5006411	Vero-E6	CRL-1586	58027482
Herpes Simplex Type 2	HSV-2	VR-734	5006412	Vero-E6	CRL-1586	58027482
Influenza A	n/a	VR-1469	58101202	MDCK	CCL-34	7643577
Parainfluenza 3	n/a	VR-93	58102987	MDCK	CCL-34	7643577
Respiratory Syncytial Virus	RSV	VR-1540	58224956	HEP-2	CCL-23	57922397
Varicella Zoster Virus	VZV	VR-1367	58205693	MRC-5	CCL-171	59151521
Chlamydia pneumoniae	*C. pneumoniae*	VR-1360	5040953	McCoy	CRL-1696	58494149
Chlamydia trachomatis	*C. trachomatis*	VR-880	5841720	McCoy	CRL-1696	58494149
Mycoplasma hominis	*M. hominis*	14027	3526149	n/a	n/a	n/a
Mycoplasma pneumoniae	*M. pneumoniae*	15293	3965781	n/a	n/a	n/a
Ureaplasma urealyticum	*U. urealyticum*	27619	58304691	n/a	n/a	n/a

[a]n/a, not applicable

2.2 Pilot Study and Definitive Study Preparation

Prior to conducting the full length testing, a Pilot Study was conducted with select viruses, two hardy (VZV and Echovirus Type 30) and two sensitive (Adenovirus and Parainfluenza Type 3), one Chlamydia strain (*C. trachomatis*), and one Mycoplasma strain (*M. hominis*), at one holding temperature (room temperature [22°C ± 1°C]) and storage time (72 hours) in order to gauge the concentration of neat stock required to conduct the full length testing.

Testing was based on the presumption of a gradual reduction of most test viruses or bacterial counts with time, particularly after 72 hours of storage. The objective of this task was to determine the initial inoculum level required to obtain reasonable number of surviving organisms after storage for ensuing study tasks. Procedures for neat stock preparation, determination of $TCID_{50}$ values (for viruses and Chlamydia), UTM inoculation and incubation, shell vial cultures (for viruses and Chlamydia), and culturing of bacteria are described in the sections below. When possible, procedures as specified by the ATCC and/or the available literature (Forbes *et al*., 2007; Garcia, 2010) were followed.

2.2.1 Preparation of Neat Stocks

For each strain, neat stocks were prepared for use on the pilot and definitive studies. For viruses and *Chlamydiae*, cells were propagated in-house according to standard operating procedures following ATCC recommendations. Cells were seeded in culture flasks and allowed to incubate under optimal conditions until appropriate confluency was reached (generally, 80-90%). Upon monolayer formation, cells were inoculated with virus or *Chlamydiae* and placed in an incubator set for optimal propagation. Culture flasks were inspected daily until 80% cytopathic effect (CPE) was observed or until control cell flasks began to show signs of cellular degradation. Flasks were then subjected to three (3) rapid freeze-thaw cycles to lyse the cells and release virus/*Chlamydiae*. Following the last freeze-thaw, culture suspensions were harvested and centrifuged briefly to pellet cellular debris. The resulting supernatants containing the virus or *Chlamydiae* were diluted in cryomedium and stored frozen (-80°C) until needed.

For the Mycoplasma and Ureaplasma strains, lyophilized material received from the ATCC was resuspended as instructed and subcultured onto A8 agar (*M. hominis* and *U. urealyticum*) or into SP-4 broth (*M. pneumoniae*). Bacteria were harvested following optimal incubation, resuspended in cryomedium, and stored frozen (-80°C) until needed.

2.2.2 Determination of $TCID_{50}$

The 50% tissue culture infective dose ($TCID_{50}$) for neat virus and *Chlamydiae* stocks was determined as described by Garcia (2010) and as specified in LRRI SOP BSF-1907, Determination of Median Tissue Culture Infectious Dose ($TCID_{50}$) for a Viral Sample. In brief, neat stocks were serially diluted (1:2 or 1:10) and added to confluent cell monolayers (as appropriate for the strain) in a 96-well format. Negative control wells lacked the addition of the test strain. Five replicates wells were tested for each dilution. Inoculated plates were allowed to incubate under optimal conditions for 7 to 14 days during which they were inspected daily for CPE. Upon initial CPE observation, incubation was ceased and positive and negative wells were enumerated. These data were used to calculate the $TCID_{50}$ value via the Reed-Muench method (Reed and Muench, 1938).

2.2.3 UTM Inoculation and Incubation

For each device replicate, 100 µL of test suspension were inoculated onto the device swab tip and immediately placed into the test or predicate transport medium followed by incubation at specified holding temperatures. This was performed in triplicate for each dilution at each holding time (0 and 72 hours for the pilot study; 0, 24, 48, and 72 hours for the definitive study) and temperature (room temperature for the pilot study; room temperature and refrigerated [4°C ± 1°C] for the definitive study). Viability was assessed via modified shell vial cultures (for viruses and *Chlamydiae*) or agar plate culture (for Mycoplasmas and Ureaplasma) as described below in Sections 2.2.4 and 2.2.5, respectively. Uninoculated devices served as negative controls.

2.2.4 Shell Vial Cultures and Immunofluorescence Staining

Shell vial cultures were prepared with modifications as described by Garcia, 2010. Cells were affixed to 12 mm glass cover slips in 24-well cell culture plates until proper confluency was achieved. At 0, 24, 48, or 72 hours post-inoculation, 200 µL of spiked UTM from each device were removed (following thorough mixing) and inoculated into the cultures. Following inoculation, each culture plate was centrifuged at room temperature for 30 minutes at 500 to 700 *x g* after which the inoculum was aspirated and replaced with maintenance medium at a volume sufficient to cover the cell monolayer. Cultures were incubated under optimal conditions for each strain. Monolayers were examined daily until the expected CPE (as specified in Tables 3-5) was observed or, in the case of poor CPE formation, the negative control presented with cellular degradation. At this point, the maintenance medium was aspirated from each well and the monolayer was gently washed with 1 mL of phosphate buffered saline (PBS). Following the PBS rinse, the cover slip was placed onto a glass slide. To each cover slip, 1 mL of cold acetone was added and the monolayer was fixed for 10 minutes. Upon completion of cell fixation, any remaining acetone was aspirated after which the cover slip was allowed to dry to completion. The samples were then considered ready for immunofluorescence staining.

Immunofluorescence staining was performed using the commercially available kits specified in Table 2:

Table 2. Immunofluorescence Diagnostic Kits

Test Strain	Abbreviation[a]	Immunofluorescence Diagnostic Kit[b]	Cat. #	Lot Number(s)	Expiration Date(s)
Adenovirus	n/a	LIGHT DIAGNOSTICS™ Adenovirus DFA Kit	3130	JH1889185 and JBC1850179	8/2012 and 4/2012, respectively
Cytomegalovirus	CMV	LIGHT DIAGNOSTICS™ CMV DFA Kit	3245	JBC1880886	8/2012
Echovirus Type 30	n/a	LIGHT DIAGNOSTICS™ Echovirus 30 IFA Kit	3340	JH1883140 and JH1B33313	9/2012 and 2/2012, respectively
	n/a	Echovirus 30 Monoclonal Antibody	3315	JBC1880893	11/2012
	n/a	LIGHT DIAGNOSTICS™ Goat Anti-Mouse IgG Antibody FITC Reagent	5008	JH1889190, JH1843654, and JBC1864663	12/2012, 8/2012, and 10/2012, respectively
Herpes Simplex Type 1	HSV-1	LIGHT DIAGNOSTICS™ SimulFluor® HSV/VZV Kit	3295	JBC1850180	8/2012
Herpes Simplex Type 2	HSV-2	LIGHT DIAGNOSTICS™ SimulFluor® HSV/VZV Kit	3295	JBC1850180	8/2012
Influenza A	n/a	LIGHT DIAGNOSTICS™ Influenza A & B DFA Kit	3123	JH1832757 and JH1880888	4/2012 and 9/2012, respectively
Parainfluenza 3	n/a	LIGHT DIAGNOSTICS™ Parainfluenza 1, 2, and 3 DFA Kit	3120	JH1887260	8/2012
Respiratory Syncytial Virus	RSV	LIGHT DIAGNOSTICS™ Respiratory Syncytial Virus DFA Kit	3125	JBH1883141	6/2012
Varicella Zoster Virus	VZV	LIGHT DIAGNOSTICS™ Varicella-Zoster Virus DFA	3430	JH1844781	4/2012
Chlamydia pneumoniae	*C. pneumoniae*	D3 DFA Chamydiae Culture Confirmation Kit	3120	CH071511	16DEC2012
Chlamydia trachomatis	*C. trachomatis*	D3 DFA Chamydiae Culture Confirmation Kit	3120	CH071511	16DEC2012

[a]n/a, not applicable

[b]DFA, Direct Fluorescent Antibody; IFA, Indirect Fluorescent Antibody

Prior to staining, all reagents were allowed to equilibrate to room temperature. Staining was performed as per manufacturer recommendations. In brief, primary antibody was added to each cover slip (4-6 drops or enough to cover the monolayer) and allowed to incubate until optimal conditions for a target of 30 minutes. Following incubation, reagent was aspirated and each cover slip was gently washed three times with 1 mL of PBS/Tween 20. After the final wash, remaining PBS/Tween 20 was aspirated and the cover slip was gently transferred to a new glass slide where it was mounted cell side down with provided mounting fluid. Once mounted, each sample was examined microscopically using an EVOS® Digital Fluorescent Microscope equipped with LED cube technology illumination, digital imaging software and computer display (Advanced Microscopy Group, Bothell, WA). The number of infectious particles was established by counting and recording the number of fluorescent foci present on the stained cover slip under 4-10X magnification.

For all test virus and Chlamydia strains, positive and negative control shell vials were processed in parallel. Positive controls consisted of the prepared slides that accompanied each of the

immunofluorescence diagnostic kits. Negative controls consisted of cells lacking the addition of the test organism.

2.2.5 Mycoplasma and Ureaplasma Cultures

At 0, 24, 48, 72 hours post-inoculation, 100 µL of spiked UTM from each device were removed (following thorough mixing) and inoculated onto the appropriate agar (specified in Tables 3 and 6) via a standard spread plate method. This was performed in duplicate for each test dilution. Following optimal incubation, agar plates were examined macroscopically (*M. hominis*) or microscopically (*M. pneumoniae* and *U. urealyticum*) for the presence of pure colonies. Upon purity confirmation, colonies were enumerated and recorded.

Finalized culture conditions and noted measurable responses for the pilot study strains are summarized below in Table 3. Remaining strains used in the definitive studies are presented in Tables 4-6:

Table 3. Pilot Study Strain Culture Conditions and Measurable Responses

Test Strain	Abbreviation[a]	Culture Conditions used on Study	Measurable Response[a]
Adenovirus	n/a	37°C, 5.0% CO_2 for 2-7 days	Shortening and swelling of A549 cells with possible vacuolation and syncytia formation
Echovirus Type 30	n/a	37°C, 5.0% CO_2 for 24-48 hours	A549 cell rounding and clumping/sloughing; refractile cells observed
Parainfluenza 3	n/a	37°C, 5.0% CO_2 for 3-5 days	MDCK cell rounding and plaque formation
Varicella Zoster Virus	VZV	36°C, 5.0% CO_2 for 4-7 days	MRC-5 cell rounding and plaque formation
Chlamydia trachomatis	*C. trachomatis*	35°C-37°C, 5.0% CO_2 for 2 days	Presence of intracellular inclusion bodies in McCoy cells best visualized by fluorescent staining
Mycoplasma hominis	*M. hominis*	Broth: 10B broth, aerobic, 37°C with shaking (180 rpm) for 24-48h Agar: A8 agar, 37°C, 5.0% CO_2 for 1 to 4 days	Broth: Alkaline shift noted via color change from yellow to pink Agar: Presence of colonies 3-4 mm in diameter following optimal incubation

[a]n/a, not applicable

2.3 Virus Quantification

The objective of this task was to conduct the definitive comparative testing using the nine (9) virus strains defined in Table 1. UTM (test and predicate) inoculation was performed as described in Section 2.2.3. Immunofluorescence was conducted as described under Section 2.2.4.

To further compare the test and predicate devices, percent infectivity for viruses was calculated for each test dilution. Percent infectivity was defined as the ratio of total immunofluorescent foci (obtained through microscopic analysis) to the total number of viruses inoculated (based on $TCID_{50}$ values; see Section 2.2.2). This value was expressed as a percentage following

adjustment of host cell confluence. Similar methods have been described in the literature (Azenabor *et al*., 2007).

Finalized culture conditions and noted measurable responses for the definitive study strains (excluding those specified for the pilot study) are summarized in Table 4:

Table 4. Definitive Study Culture Conditions and Measurable Responses: Viruses

Test Strain	Abbreviation[a]	Culture Conditions used on Study	Measurable Response
Cytomegalovirus	CMV	37°C, 5.0% CO_2 for 2-7 days	HEP-2 cell rounding and clumping/sloughing; refractile cells observed
Herpes Simplex Type 1	HSV-1	34°C, 5.0% CO_2 for 1-2 days	Vero-E6 cell rounding and clumping with eventual slumping
Herpes Simplex Type 2	HSV-2	34°C-35°C, 5.0% CO_2 for 24-72 hours	Vero-E6 cell rounding and clumping with eventual slumping
Influenza A	n/a	35°C, 5.0% CO_2 for 2-4 days	MDCK cell rounding, degeneration, and sloughing
Respiratory Syncytial Virus	RSV	35°C-37°C, 5.0% CO_2 for 3-10 days	HEP-2 cell syncytium formation

[a]n/a, not applicable

2.4 Chlamydia Quantification

The objective of this task was to conduct the definitive comparative testing using the two (2) Chlamydia strains defined in Table 1. UTM (test and predicate) inoculation was performed as described in Section 2.2.3. Immunofluorescence was conducted as described under section 2.2.4.

Percent infectivity for the two Chlamydia strains was conducted as described under Section 2.3.

Finalized culture conditions and noted measurable responses for the *C. pneumoniae* are summarized below in Table 5. Similar parameters for *C. trachomatis* were presented previously in Table 3.

Table 5. *C. pneumoniae* Culture Conditions and Measurable Responses

Test Strain	Abbreviation	Culture Conditions used on Study	Measurable Response
Chlamydia pneumoniae	*C. pneumoniae*	35°C-37°C, 5.0% CO_2 for 3 days	Presence of intracellular inclusion bodies in McCoy cells best visualized by fluorescent staining

2.5 Mycoplasma and Ureaplasma Quantification

The objective of this task was to conduct the definitive comparative testing using the Ureaplasma and two (2) Mycoplasma strains defined in Table 1. UTM inoculation was performed as described in Section 2.2.3. Bacterial culture was conducted as described in Section 2.2.5 and according to according to the available literature (e.g., Bailey and Scott's Diagnostic Microbiology, 12th edition and Clinical Microbiology Procedures Handbook, Vol. 1).

Finalized culture conditions and noted measurable responses for *Mycoplasma pneumoniae* and *Ureaplasma urealyticum* are summarized below in Table 6. Similar parameters for *M. hominis* were presented previously in Table 3.

Table 6. *M. pneumoniae* and *U. urealyticum* Culture Conditions and Measurable Responses

Test Strain	Abbreviation	Culture Conditions used on Study	Measurable Response
Mycoplasma pneumoniae	*M. pneumoniae*	Broth: SP-4 broth, aerobic, 37°C with shaking (180 rpm) for 9-13 days Agar: SP-4 or PPLO agar, 37°C, aerobic (SP-4) or 5% CO_2 (PPLO) for 5-6 days	Broth: pH shift noted via color change from pink/red to orange/light yellow Agar: Presence of microscopic colonies 10-100 µm in diameter following optimal incubation
Ureaplasma urealyticum	*U. urealyticum*	Broth: 10B broth, aerobic, 37°C with shaking (180 rpm) for 48h Agar: A8 agar, anaerobic, 37°C, for 3-5 days	Broth: Alkaline shift noted via color change from yellow to pink Agar: Presence of microscopic colonies 15-60 µm in diameter following optimal incubation

2.6 Cytotoxicity Testing

The objective of this task was to determine if the test device exhibited cytotoxicity in an *in vitro* MRC-5 cell culture assay. Testing consisted of adding a known quantity of UTM to cell monolayers in a 96-well format followed by incubation and fixation of cellular proteins with trichloroacetic acid (TCA) and staining with sulforhodamine B. The amount of sulforhodamine B bound to cellular proteins following washing steps was measured colorimetrically to assess cellular density. Decreased cellular biomass as a result of decreased dye intensity was indicative of cellular toxicity. Results from this task served as the basis for comparing the toxicity of unexpired test UTM to several lots at expiration.

Cytotoxicity testing was performed according to Vichai and Kirtikara (2006) using a commercially available kit (In Vitro Toxicology Assay Kit, Sulforhodamine B Based, Product No. TOX-6, Sigma-Aldrich, Saint Louis, MO). In brief, MRC-5 cells were seeded in a 96-well tissue culture treated plate to ensure 60-80% confluency following 24 hours of incubation. Following successful monolayer formation, samples were added to the cells according to the plate map outlined in Figure 2:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		091115 (A, 75%)	091115 (B, 75%)	N	091018 (A, 75%)	091018 (B, 75%)	N	110202 (A, 75%)	110202 (B, 75%)	N	PC (TritonX)	
C		091115 (A, 75%)	091115 (B, 75%)	N	091018 (A, 75%)	091018 (B, 75%)	N	110202 (A, 75%)	110202 (B, 75%)	N	PC (TritonX)	
D		091115 (A, 75%)	091115 (B, 38%)	N	091018 (A, 75%)	091018 (B, 38%)	N	110202 (A, 75%)	110202 (B, 38%)	N	PC (TritonX)	
E		091115 (A, 38%)	091115 (B, 38%)	N	091018 (A, 38%)	091018 (B, 38%)	N	110202 (A, 38%)	110202 (B, 38%)	N	PBS control	
F		091115 (A, 38%)	091115 (B, 38%)	N	091018 (A, 38%)	091018 (B, 38%)	N	110202 (A, 38%)	110202 (B, 38%)	N	PBS control	
G		GC	GC	GC	GC	GC	GC	GC	GC	GC	PBS control	
H												

Figure 2. Plate map for cytotoxicity testing.

Positive control (PC) wells were inoculated with 200 µL of 0.3% Triton-X 100 in order to establish a positive cytotoxicity response. Growth control (GC) wells were inoculated with 200 µL of growth medium. UTM samples were tested in duplicate (labeled as A and B). Samples labeled as 75% were inoculated with 150 µL of UTM and 50 µL of growth medium. Samples labeled as 38% were inoculated with 75 µL of UTM, 75 µL of phosphate buffered saline (PBS) and 50 µL of growth medium. Negative solvent control (N) wells were inoculated with 150 µL of PBS and 50 µL of growth medium. PBS control wells were inoculated with 100 µL of 1X PBS. The outer wells of the 96-well plate contained growth medium only and no cells.

Following incubation at 37°C, 5% CO_2 for 24h, cells were fixed by gently layering 50 µL of cold 50% TCA on top of the growth medium. The plate was centrifuged at 5,000 *x g* for 20 minutes after which it was incubated under refrigerated conditions for 1h. Following the cold incubation, the plate was rinsed a minimum of three times with sterile water and allowed to air dry. To each well, 100 µL of Sulforhodamine B solution (0.4%) were added. Cells were allowed to stain for 20-30 minutes at room temperature after which the stain was removed via aspiration. Samples were then quickly rinsed with 1% acetic acid until all unincorporated dye was removed and air dried to completion. To each well, 200 µL of Sulforhodamine B solubilization solution (10 mM Tris) was added and allowed to incubate at room temperature for five minutes. The plate was shaken gently in a plate shaker to enhance dye mixture after which it was read spectrophotometrically at 565 nm (with 690 nm background reading). The normalized optical density (OD) values were calculated according to the following equation:

$$\text{Normalized OD} = \text{Mean OD of samples} \div (\text{Mean OD of GC} - \text{Mean OD of blanks})$$

2.7 Perform Storage Stability Testing

The objective of this task was to establish the stability of the test device within the shelf life window. This was accomplished by measuring the viability of CMV, HSV-2, RSV, *C. pneumoniae*, and *M. pneumoniae* in expired and unexpired devices. This task was conducted to provide evidence that the product performance was maintained up to and including the expiration date.

Four lots of the test device (three expired, one unexpired) were used for the duration of this study. UTM inoculation was performed as described under Section 2.2.3. Enumeration of viruses and bacteria was conducted as specified in Sections 2.2.4 and 2.2.5.

2.8 Statistical Analyses

Immunofluorescent foci, bacterial CFU counts, and cytotoxicity optical density data were log-transformed (to establish normality) followed by one-way analysis of variance (ANOVA) and Tukey's Multiple Comparison Test using GraphPad Prism, Version 5.03 (GraphPad Software, Inc., La Jolla, CA). Significance was based on the difference of group means and was defined as a p-value less than 0.05.

3.0 QUALITY CONTROL STATEMENT

It is essential that all microbiological tests are quality controlled to guarantee that results are true and comparable to other studies conducted in a similar manner. Quality control (QC) assures meticulous performance of test organisms, kits, reagents, and culture media while also increasing the precision in reporting. For this reason, commercially available pre-poured media, reagents, and kits that passed necessary QC by the manufacturer were used throughout the duration of this study. All primary cultures were certified as pure and were prepared as specified by the ATCC. QC measures, including thorough documentation of all materials, methods, and procedures, were instituted by LRRI throughout the study to maintain the study quality. When applicable, standard operating procedures were followed.

4.0 RESULTS AND DISCUSSION

Results from the pilot study indicated adequate recovery and foci enumeration for the test virus strains and *C. trachomatis* following UTM inoculation and 72 hours of incubation under room temperature conditions. Similarly, *M. hominis* testing demonstrated sufficient bacterial recovery and colony forming unit enumeration following storage under the same conditions.

Figure 3 depicts representative images of Adenovirus following immunostaining and fluorescent microscopy. Foci presentation for the remaining viruses and the two Chlamydia strains was similar.

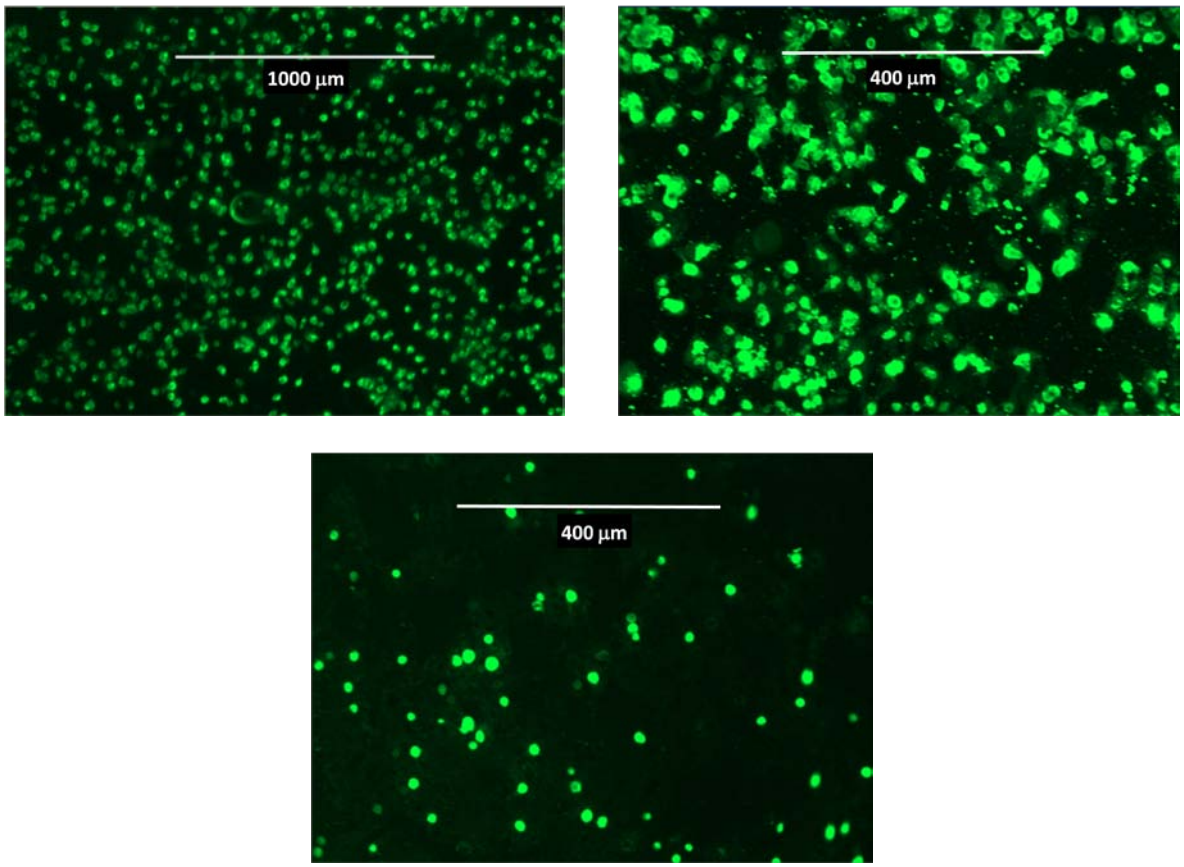

Figure 3. Representative immunostaining images: Adenovirus.

Top images: A549 cells post inoculation with Adenovirus/UTM suspension; left, 4X magnification; right, 10X magnification. Bottom image: LIGHT DIAGNOSTICS™ Adenovirus DFA Kit Positive Control Slide, 10X magnification. All images captured on the EVOS® Digital Fluorescent Microscope System.

4.1 Virus Quantification

Results for the recovery study conducted with viruses are presented below in Table 7.

Table 7. Virus Recovery and Foci Enumeration following Storage in Test and Predicate Devices

Organism	Dilution of Neat Stock[a]	Percent Infectivity of Host Cells	Storage Time	Incubation Time Prior to Reading	Mean Viability of Test Organism Using Test (Puritan {TransPRO™}) Device: Foci Counts[b]		Mean Viability of Test Organism Using Predicate (BD) Device: Foci Counts[b]	
		(% Infectivity)	(Hours)	(Hours)	4°C	RT	4°C	RT
Adenovirus	1:100	2%	0	24	n/a	343 ± 72	n/a	201 ± 72
			24		550 ± 77	434 ± 66	471 ± 163	409 ± 82
			48		652 ± 143	408 ± 89	118 ± 35	445 ± 303
			72		635 ± 157	729 ± 127	399 ± 56	358 ± 57
	1:500	3%	0	24	n/a	118 ± 78	n/a	57 ± 34
			24		192 ± 37	161 ± 28	132 ± 25	124 ± 22
			48		145 ± 57	47 ± 17	26 ± 9	37 ± 13
			72		180 ± 36	216 ± 37	175 ± 18	152 ± 29
Cytomegalovirus	1:10	100%	0	24	n/a	751 ± 71	n/a	731 ± 257
			24		209 ± 26	47 ± 3	163 ± 64	26 ± 3
			48		269 ± 58	319 ± 34	88 ± 32	243 ± 246
			72		29 ± 4	67 ± 55	22 ± 6	24 ± 16
	1:100	100%	0	24	n/a	242 ± 7	n/a	217 ± 36
			24		134 ± 13	47 ± 5	116 ± 7	45 ± 3
			48		86 ± 35	207 ± 110	50 ± 35	54 ± 40
			72		26 ± 12	36 ± 14	24 ± 21	17 ± 7
Echovirus Type 30	1:100	64%	0	24	n/a	95 ± 52	n/a	457 ± 167
			24		337 ± 178	332 ± 221	198 ± 67	184 ± 105
			48		454 ± 210	605 ± 194	300 ± 110	274 ± 41
			72		455 ± 56	415 ± 283	165 ± 62	295 ± 113
	1:500	100%	0	24	n/a	63 ± 48	n/a	169 ± 75
			24		194 ± 134	214 ± 108	129 ± 50	75 ± 35
			48		252 ± 31	151 ± 41	81 ± 48	114 ± 23
			72		154 ± 59	149 ± 16	47 ± 3	52 ± 9
Herpes Simplex Type 1	1:10	6%	0	24	n/a	207 ± 78	n/a	100 ± 44
			24		665 ± 189	325 ± 107	301 ± 151	249 ± 65
			48		609 ± 238	772 ± 243	645 ± 144	572 ± 266
			72		460[c]	710 ± 73	825 ± 85	489 ± 89
	1:100	48%	0	24	n/a	167 ± 101	n/a	87 ± 46
			24		89 ± 38	72 ± 17	82 ± 39	99 ± 46
			48		96 ± 14	107 ± 35	97 ± 27	45 ± 13
			72		117 ± 36	50 ± 18	63 ± 18	49 ± 10
Herpes Simplex Type 2	1:10	47%	0	24	n/a	126 ± 13	n/a	124 ± 14
			24		51 ± 21	85 ± 25	15 ± 3	94 ± 7
			48		108 ± 32	6 ± 3	53 ± 63	115 ± 54
			72		6 ± 1	0 ± 1	4 ± 1	0 ± 0
	1:100	97%	0	24	n/a	26 ± 6	n/a	26 ± 8
			24		25 ± 15	37 ± 13	12 ± 8	161 ± 198
			48		17 ± 6	8 ± 6	10 ± 2	4 ± 4
			72		2 ± 2	0 ± 0	0 ± 0	0 ± 0

[a]From each dilution, 100 µL were inoculated onto test or predicate swab tip followed by placement of the swab into the test or predicate device containing 3 mL of transport medium

[b]Average of triplicate tests performed on 200 µL of test or predicate device medium at each time point; n/a, not applicable as samples were processed shortly after spiking; RT, room temperature

[c]Standard deviation unavailable for this sample as remaining replicates were too numerous to count

Table 7, continued. Virus Recovery and Foci Enumeration following Storage in Test and Predicate Devices

Organism	Dilution of Neat Stock[a]	Percent Infectivity of Host Cells	Storage Time	Incubation Time Prior to Reading	Mean Viability of Test Organism Using Test (Puritan {TransPRO™}) Device: Foci Counts[b]		Mean Viability of Test Organism Using Predicate (BD) Device: Foci Counts[b]	
		(% Infectivity)	(Hours)	(Hours)	4°C	RT	4°C	RT
Influenza A	1:50	10%	0	24	n/a	289 ± 86	n/a	463 ± 102
			24		470 ± 96	250 ± 89	205 ± 156	218 ± 38
			48		173 ± 95	93 ± 41	85 ± 49	78 ± 22
			72		96 ± 49	35 ± 16	79 ± 19	52 ± 37
	1:100	12%	0	24	n/a	186 ± 130	n/a	231 ± 118
			24		109 ± 56	181 ± 117	27 ± 2	106 ± 18
			48		82 ± 36	30 ± 13	60 ± 21	38 ± 27
			72		48 ± 13	13 ± 8	42 ± 16	12 ± 13
Parainfluenza A	1:10	3%	0	48	n/a	501 ± 116	n/a	322 ± 112
			24		30 ± 10	628 ± 208	151 ± 37	520 ± 122
			48		101 ± 26	107 ± 56	200 ± 34	216 ± 50
			72		358 ± 35	191 ± 42	187 ± 21	123 ± 59
	1:100	25%	0	48	n/a	358 ± 87	n/a	341 ± 172
			24		24 ± 10	292 ± 60	95 ± 23	293 ± 72
			48		47 ± 13	54 ± 23	91 ± 21	114 ± 50
			72		191 ± 36	94 ± 27	74 ± 8	77 ± 25
Respiratory Syncytial Virus	1:10	76%	0	24	n/a	140 ± 19	n/a	166 ± 13
			24		176 ± 20	170 ± 14	179 ± 77	147 ± 9
			48		78 ± 24	131 ± 26	60 ± 29	114 ± 19
			72		32 ± 9	153 ± 70	22 ± 16	59 ± 25
	1:100	100%	0	24	n/a	25 ± 6	n/a	29 ± 6
			24		74 ± 15	62 ± 5	71 ± 41	65 ± 6
			48		59 ± 19	74 ± 4	35 ± 17	62 ± 6
			72		19 ± 9	64 ± 16	22 ± 8	75 ± 65
Varicella-Zoster Virus	1:10	100%	0	24	n/a	325 ± 91	n/a	113 ± 144
			24		253 ± 51	212 ± 43	231 ± 69	211 ± 15
			48		33 ± 13	117 ± 47	23 ± 8	34 ± 16
			72		65 ± 33	77 ± 43	53 ± 12	71 ± 58
	1:100	100%	0	24	n/a	132 ± 45	n/a	124 ± 17
			24		97 ± 12	97 ± 3	67 ± 6	82 ± 15
			48		87 ± 69	94 ± 49	23 ± 7	19 ± 5
			72		44 ± 18	39 ± 25	40 ± 24	26 ± 19

[a]From each dilution, 100 µL were inoculated onto test or predicate swab tip followed by placement of the swab into the test or predicate device containing 3 mL of transport medium

[b]Average of triplicate tests performed on 200 µL of test or predicate device medium at each time point; n/a, not applicable as samples were processed shortly after spiking; RT, room temperature

4.2 Chlamydia Quantification

Results for Chlamydia testing are presented below in Table 8.

Table 8. Chlamydia Recovery and Foci Enumeration following Storage in Test and Predicate Devices

Organism	Dilution of Neat Stock[a]	Percent Infectivity of Host Cells	Storage Time	Incubation Time Prior to Reading	Mean Viability of Test Organism Using Test (Puritan {TransPRO™}) Device: Foci Counts[b]		Mean Viability of Test Organism Using Predicate (BD) Device: Foci Counts[b]	
		(% Infectivity)	(Hours)	(Hours)	4°C	RT	4°C	RT
C. pneumoniae	1:10	100%	0	48	n/a	169 ± 33	n/a	237 ± 177
			24		356 ± 70	456 ± 68	424 ± 75	601 ± 108
			48		301 ± 121	345 ± 66	299 ± 60	253 ± 54
			72		88 ± 29	197 ± 99	92 ± 29	125 ± 15
	1:100	100%	0	48	n/a	65 ± 6	n/a	52 ± 6
			24		163 ± 25	134 ± 35	235 ± 84	213 ± 73
			48		110 ± 24	131 ± 33	169 ± 27	115 ± 35
			72		127 ± 120	145 ± 31	66 ± 25	74 ± 39
C. trachomatis	1:10	100%	0	48	n/a	227 ± 63	n/a	273 ± 32
			24		204 ± 79	627 ± 197	159 ± 49	83 ± 26
			48		184 ± 62	234 ± 102	198 ± 65	217 ± 62
			72		167 ± 88	210 ± 200	606 ± 277	184 ± 57
	1:100	100%	0	48	n/a	73 ± 10	n/a	81 ± 11
			24		60 ± 12	138 ± 50	113 ± 20	31 ± 7
			48		57 ± 19	92 ± 32	64 ± 33	78 ± 43
			72		104 ± 44	95 ± 39	164 ± 93	91 ± 63

[a]From each dilution, 100 µL were inoculated onto test or predicate swab tip followed by placement of the swab into the test or predicate device containing 3 mL of transport medium

[b]Average of triplicate tests performed on 200 µL of test or predicate device medium at each time point; n/a, not applicable as samples were processed shortly after spiking; RT, room temperature

4.3 Mycoplasma and Ureaplasma Quantification

Results for the Mycoplasma and Ureaplasma testing are presented below in Table 9.

Table 9. Mycoplasma and Ureaplasma Recovery and CFU Enumeration following Storage in Test and Predicate Devices

Organism	Dilution of Neat Stock[a]	Storage Time	Incubation Time Prior to Reading	Mean Viability of Test Organism Using Test (Puritan {TransPRO™}) Device: Foci Counts[b]		Mean Viability of Test Organism Using Predicate (BD) Device: CFU Counts[b]	
		(Hours)	(Days)	4°C	RT	4°C	RT
M. hominis	1:500	0	3	n/a	TNTC	n/a	TNTC
		24		TNTC	34 ± 5	TNTC	TNTC
		48		TNTC	75 ± 11	TNTC	TNTC
		72		TNTC	15 ± 3	TNTC	102 ± 14
	1:1000	0	3	n/a	171 ± 42	n/a	65 ± 13
		24		136 ± 9	28 ± 7	109 ± 13	59 ± 44
		48		160 ± 19	9 ± 5	109 ± 44	26 ± 6
		72		177 ± 29	1 ± 1	87 ± 25	6 ± 3
M. pneumoniae	Neat	0	6	n/a	TNTC	n/a	TNTC
		24		TNTC	TNTC	TNTC	TNTC
		48		TNTC	1116 ± 119	TNTC	TNTC
		72		1328 ± 362	481 ± 223	TNTC	917 ± 278
	1:10	0	6	n/a	887 ± 334	n/a	960 ± 336
		24		416 ± 177	275 ± 62	407 ± 257	547 ± 186
		48		600 ± 303	144 ± 53	709 ± 187	312 ± 92
		72		433 ± 64	16 ± 13	941 ± 253	63 ± 36
U. urealyticum	1:500	0	5	n/a	TNTC	n/a	TNTC
		24		TNTC	TNTC	TNTC	TNTC
		48		TNTC	TNTC	TNTC	TNTC
		72		TNTC	TNTC	TNTC	TNTC
	1:1000	0	5	n/a	811 ± 311	n/a	293 ± 80
		24		893 ± 486	775 ± 306	444 ± 142	425 ± 146
		48		611 ± 89	486 ± 134	674 ± 225	539 ± 318
		72		367 ± 40	352 ± 43	484 ± 153	1048 ± 113

[a]From each dilution, 100 µL were inoculated onto test or predicate swab tip followed by placement of the swab into the test or predicate device containing 3 mL of transport medium

[b]Average of triplicate tests performed on 100 µL of test or predicate device medium at each time point; n/a, not applicable as samples were processed shortly after spiking; RT, room temperature; TNTC, too numerous to count, defined as 1,000 CFU for *M. hominis* and 2,000 CFU for *M. pneumoniae* and *U. urealyticum*

4.4 Cytotoxicity Testing

Results from cytotoxicity analysis are summarized below in Table 10.

Table 10. Cytotoxicity Testing Results

Test Sample[a]	Lot[b]	Composition (µL each)[c]				Mean Norm. OD[d]	SD
		UTM	GM	PBS	0.3% T-X		
UTM	091115	150	50	-	-	3.423	0.044
UTM	091115	75	50	75	-	3.855	0.079
UTM	091018	150	50	-	-	2.998	0.112
UTM	091018	75	50	75	-	3.405	0.367
UTM	110202	150	50	-	-	2.874	0.215
UTM	110202	75	50	75	-	3.350	0.307
GM (no cells)	n/a	-	200	-	-	3.799	0.242
GC 1	n/a	-	200	-	-	OVRFLW	n/a
GC 2	n/a	-	200	-	-	OVRFLW	n/a
NC 1	n/a	-	50	150	-	3.426	0.289
NC 2	n/a	-	50	150	-	3.502	0.403
NC 3	n/a	-	50	150	-	3.574	0.384
PC 1	n/a	-	-	-	200	0.995	0.373
PC 2	n/a	-	-	200	-	0.287	0.058

[a]UTM, universal transport medium; GM, growth medium; GC, growth control; NC, negative solvent control; PC, positive assay control

[b]n/a, not applicable

[c]UTM, universal transport medium; GM, growth medium; PBS, phosphate buffered saline; T-X, Triton-X

[d]OD, optical density; OD_{565}-OD_{690}; OVRFLW, well measured as overflow (i.e., OD above threshold)

Sulforhodamine B assay results indicated no cytotoxicity associated with the test device as compared to controls. For this assay, toxicity increases proportionately with optical density (i.e., the higher the optical density, the more toxic the test sample). One-way ANOVA demonstrated no significant differences between the device lots. Positive control solutions were significantly ($P<0.05$) different from each UTM lot individually and from each other.

4.5 Storage Stability Testing

Results from the storage stability testing are presented by each strain below in Table 11.

Table 11. Test Organism Recovery and Enumeration following Storage Stability Processing in Separate Lots of Test Device

Lot	XP[a]	Organism	Dilution of Neat Stock[b]	Percent Infectivity of Host Cells	Incubation Time Prior to Reading	Storage Conditions and Mean Viability of Test Organism[c]							
						4°C				RT			
						0h	24h	48h	72h	0h	24h	48h	72h
091018	10/2010	Cytomegalovirus	1:10	100%	24 hours	n/a	135 ± 40	184 ± 32	79 ± 24	194 ± 4	105 ± 8	112 ± 72	100 ± 38
091026	10/2010					n/a	127 ± 13	247 ± 48	61 ± 4	132 ± 18	93 ± 25	265 ± 95	63 ± 18
091115	11/2010					n/a	139 ± 42	175 ± 111	41 ± 6	87 ± 7	79 ± 44	206 ± 125	89 ± 1
110518	5/2012					n/a	202 ± 1	210 ± 15	88 ± 6	137 ± 14	106 ± 8	245 ± 32	98 ± 16
091018	10/2010	Herpes Simplex Type 2	1:10	6%	24 hours	n/a	273 ± 167	198 ± 23	202 ± 4	368 ± 69	210 ± 59	42 ± 29	23 ± 4
091026	10/2010					n/a	380 ± 46	101 ± 37	51 ± 8	329 ± 234	118 ± 35	24 ± 7	131 ± 45
091115	11/2010					n/a	449 ± 54	305 ± 48	113 ± 8	333 ± 30	171 ± 11	16 ± 1	79 ± 19
110518	5/2012					n/a	376 ± 114	118 ± 25	155 ± 61	354 ± 188	171 ± 36	65 ± 21	30 ± 13
091018	10/2010	Respiratory Syncytial Virus	1:10	76%	24 hours	n/a	112 ± 22	153 ± 11	78 ± 12	153 ± 27	142 ± 13	117 ± 54	79 ± 4
091026	10/2010					n/a	101 ± 13	86 ± 23	96 ± 22	130 ± 1	132 ± 28	97 ± 16	87 ± 11
091115	11/2010					n/a	108 ± 4	101 ± 16	85 ± 16	157 ± 35	116 ± 18	106 ± 4	89 ± 0
110518	5/2012					n/a	107 ± 15	121 ± 26	102 ± 2	165 ± 11	102 ± 17	121 ± 26	86 ± 17
091018	10/2010	*C. pneumoniae*	1:10	100%	48 hours	n/a	135 ± 40	184 ± 32	79 ± 24	194 ± 4	105 ± 8	112 ± 72	100 ± 38
091026	10/2010					n/a	127 ± 13	247 ± 48	61 ± 4	132 ± 18	93 ± 25	265 ± 95	63 ± 18
091115	11/2010					n/a	139 ± 42	175 ± 111	41 ± 6	87 ± 7	79 ± 44	206 ± 125	89 ± 1
110518	5/2012					n/a	202 ± 1	210 ± 15	88 ± 6	137 ± 14	106 ± 8	245 ± 32	98 ± 16
091018	10/2010	*M. pneumoniae*	1:10	n/a	6 days	n/a	272 ± 170	1000 ± 113	912 ± 147	768 ± 260	428 ± 85	184 ± 11	24 ± 0
091026	10/2010					n/a	1028 ± 51	1156 ± 107	332 ± 28	836 ± 300	144 ± 79	68 ± 62	24 ± 11
091115	11/2010					n/a	664 ± 34	956 ± 322	668 ± 390	828 ± 28	252 ± 40	164 ± 51	24 ± 0
110518	5/2012					n/a	416 ± 177	600 ± 303	433 ± 64	887 ± 344	275 ± 62	144 ± 53	16 ± 13

[a]XP, expiration date

[b]From each dilution, 100 µL were inoculated onto test or predicate swab tip followed by placement of the swab into the test or predicate device containing 3 mL of transport medium

[c]Average of triplicate tests (± standard deviation) performed on 200 µL of test or predicate device medium at each time point; n/a, not applicable as samples were processed shortly after spiking; RT, room temperature

5.0 SUMMARY AND CONCLUSIONS

The objective of this study was to compare the performance of a non-propagating transport device with culture medium that comprises a UTM-RT with a similar commercial device. Based on statistical analyses, it can be concluded that the test device, the Puritan® Medical Products Company LLC (PMP) Universal Transport Medium (also branded as TransPRO™) for Viruses, Chlamydia, Mycoplasma, and Ureaplasma, performed equally to the predicate device, the Universal Viral Transport System of Becton, Dickinson and Company (BD).

For both transport systems, test viruses and bacteria could be quantified using two different spiking dilutions through 48 hours at two storage temperatures. In general, refrigerated storage resulted in higher test strain recoveries. One-way ANOVA demonstrated statistical differences ($P<0.05$) between the two devices under certain conditions that were concluded to be the result of normal microbiological variability and thus insignificant from the clinical stand point, particularly since qualitative (i.e., not quantitative) results are most often the critical endpoint in the clinical setting.

Storage stability testing on all four test device lots at the two storage temperatures demonstrated quantification of the viruses and bacteria through 48 hours. As observed in the recovery study, refrigerated storage was optimal. Statistical analysis demonstrated differences ($P<0.05$) between the lots under certain storage conditions, primarily between refrigerated and room temperature storage; this was concluded to be insignificant from the clinical stand point. Storage stability testing indicated maintenance of the test device performance up to and including the expiration date.

Cytotoxicity analysis demonstrated no cellular toxicity associated with the test device when statistically compared to negative controls. This is a critical finding in that viable analyses following sample collection, storage, and transfer may be performed using standard methods (specifically, cell culture) without false negatives imparted by the universal transport medium.

Microbiological results and statistical analyses demonstrated performance equivalence between the test and predicate devices through 72h of storage at two temperatures.

Overall, statistical analyses conducted on results obtained following recovery, stability and cytotoxicity testing demonstrated performance equivalence between the test and predicate devices through 72 hours of storage at two temperatures. From a clinical perspective, no differences were noted between the two devices. In certain cases, the test device performed in a superior fashion to the predicate thus making it a valid system for the collection, storage, and transport of clinical specimens.

6.0 **REFERENCES**

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