Performance Evaluation of Universal Transport Media Device using Clinically Relevant Bacteria

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Abstract

Swabs are routinely placed into non-propagating universal transport media (UTM) to preserve the viability and integrity of samples for clinical analysis. However, the variability in storage conditions as well as the transport medium itself creates inconsistent and unreliable results rendering this low cost alternative inefficient. The performance of a Puritan® Medical Products Company LLC (PMP) Universal Transport Medium (UTM) for Viruses, Chlamydia, Mycoplasma, and Ureaplasma was compared to a Universal Viral Transport System of Becton, Dickinson and Company (BD) manufactured by Copan Diagnostics of Italia using five bacterial strains (two *Mycoplasma* species, two *Chlamydiae* species and one *Ureaplasma* species.). These were tested at two dilutions, two temperatures (4°C and 22°C) and over the course of four time points (0, 24, 48, 72h) between inoculated UTM devices. To quantify the *Mycoplasma* and *Ureaplasma* viable bacterial load in the UTM post test conditions, 100 µL of the sample was inoculated onto appropriate agar plates. The plates were incubated at optimal conditions for an appropriate amount of time, after which colonies were enumerated and recorded using a standard light microscope for quantitative analysis. To quantify the *Chlamydiae* species, 100 µL of the sample was inoculated onto shell vials and incubated for 24 to 48h after which the cover slips were collected, mounted and stained with fluorescent antibodies. Using fluorescent microscopy, individual foci were enumerated and recorded for quantitative analysis. For both devices, bacteria were viable through 72h of storage at both temperatures. As determined by viability, no differences were noted between the two devices that could not be ascribed to normal microbiological variance. The PMP UTM device performed in an equivalent fashion to the BD UTM thus making it a valid system for the collection, storage, and transport of clinical specimens.

Detection of clinical pathogens requires specific equipment and training that are not always readily available. Swabs represent an alternative tool that require little training and equipment; however, recovery and detection of pathogens after collection has proven to be inconsistent. This derives the need for a transport medium that will preserve viability, inhibit propagation, and ensure quantitative detection via culture, PCR, immunostaining, etc.

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).

The objective of this study was to compare the performance of a PMP UTM to a BD UTM device. Comparison testing was accomplished through recovery, storage stability, and cytotoxicity studies.

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Materials and Methods

All culture stocks and cell lines were obtained from the American Type Culture Collection (ATCC) to establish traceability. Five bacterial strains were used for testing (two *Mycoplasma* species, two *Chlamydiae* species and one *Ureaplasma* species). The Mycoplasma strains chosen for testing were: *Mycoplasma hominis* and *Mycoplasma pneumoniae*. U*reaplasma urealyticum* served as the Ureaplasma species. The Chlamydia strains chosen for testing were: *Chlamydia pneumoniae* and *Chlamydia trachomatis.*

Quantitation of the five bacterial strains was tested in triplicate for two dilutions, two temperatures (room temperature (RT) [20-25°C] and refrigerated [4°C]), over the course of four time points (0, 24, 48, 72h) between a BD Copan manufactured and Puritan Medical Universal Transport Media (UTM) device (as seen in the flowchart presented in Figure 1).

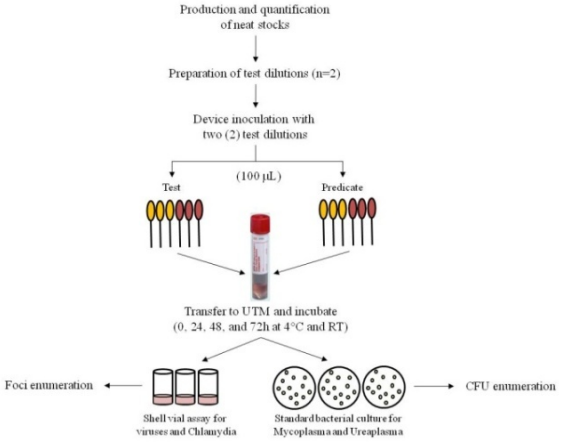

Figure 1. Flow chart for device comparison: virus and bacteria recovery testing.

To quantify the *Mycoplasma* and *Ureaplasma* viable bacterial load in the UTM post test conditions, 100 µL of the sample was inoculated onto appropriate agar plates. The plates were incubated at optimal conditions for an appropriate amount of time, after which colonies were enumerated and recorded using a standard light microscope for quantitative analysis. To quantify the *Chlamydiae* species, 100 µL of the sample was inoculated onto shell vials and incubated for 24 to 48h after which the cover slips were collected, mounted and stained with fluorescent antibodies. Using fluorescent microscopy, individual foci were enumerated and recorded for quantitative analysis.

Prior to conducting the full length testing, a Pilot Study was conducted with 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 bacteria 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.

For each strain, neat stocks were prepared for use on the pilot and definitive studies. *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 *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 ATCC was resuspended as instructed and subcultured onto A8 agar (*Mycoplasma hominis* and *Ureaplasma urealyticum*) or into SP-4 broth (*Mycoplasma pneumoniae*). Bacteria were harvested following optimal incubation, resuspended in cryomedium, and stored frozen (-80°C) until needed.

For each device replicate, 100 L of test suspension was inoculated onto the device swab tip and immediately placed into both transport mediums 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.

Shell vial cultures were prepared and Cells were affixed to 12 mm glass cover slips in 24-well cell culture plates until proper confluency (at least 80%) was achieved. At the appropriate time points, 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 was observed or, in the case of poor CPE formation, the negative control wells began to present with cellular degradation. 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 commercially available kits. 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.

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 (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.

Results

Results from the Pilot Study (as seen in Table 1) indicated adequate recovery and foci enumeration for *C. trachomatis* following UTM inoculation and 72h incubation under room temperature conditions. Similarly, *M. hominis* testing demonstrated sufficient bacterial recovery and colony forming unit enumeration following storage under the same conditions.

Table 1. Results from Pilot Study.

Test Strain	Abbreviation[a]	UTM Test Dilution	Mean Count[b]	STDEV[a]
Chlamydia trachomatis	*C. trachomatis*	1:10	185	81
		1:100	40	20
Mycoplasma hominis	*M. hominis*	1:10	200	82
		1:100	33	12

[a]n/a, not applicable

[b]Average foci count per 200 mL following optimal incubation and immunofluorescent staining for viruses and Chlamydia; average CFU/mL following A8 agar inoculation and incubation for *M. hominis*; TNTC, too numerous to count

The *Chlamydia trachomatis* pilot study results demonstrated that after 72h of incubation in the UTM, viability was still high for this test strain. Results supported the capacity to proceed with the definitive study using test dilutions of 1:10 and 1:100 and 48 hours of infection in McCoy cells.

Recorded colony counts for *M. hominis* were lower than desired, but data did indicate that following 72h incubation in the UTM, viability was still high for this test strain. To address the low colony counts, it was concluded that higher initial bacterial concentrations would result in increased colony counts for the definitive study.

Results from the definitive study showed variability among all bacterial strains at all dilutions, temperatures and time points. The results for each microbial strain are broken out below:

C. pneumoniae

Results from the *Chlamydia pneumoniae* testing indicated that virus stability decreased slightly over time through 72h of storage at two temperatures (4°C and 22°C). Figure 2 demonstrates that countable foci (indicative of inclusion bodies) were present under each test condition, though a minimal dilutional effect between the two test dilutions (1:10 and 1:100) was observed. The variability noted in foci counts was likely due to inherent error (e.g., dilution error), inter-technician variability, and/or slight differences in the test inocula (i.e., normal microbiological variance). Microbiological variance was expected since virus inocula concentrations were relatively low, thereby allowing for greater error. Overall results support performance equivalence between the two devices and indicate the presence of detectable and quantifiable *Chlamydia pneumoniae* throughout the test period.

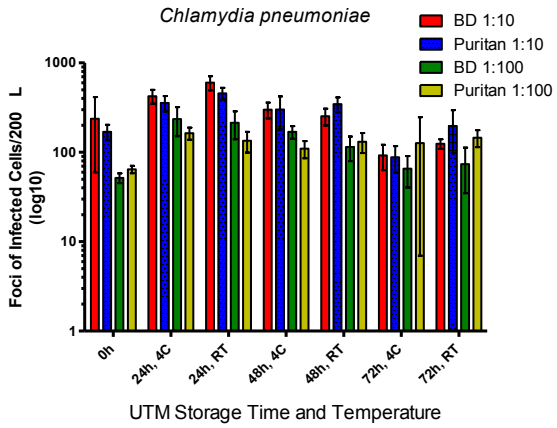

Figure 2. Foci of infected McCoy cells/200 µL inoculum of Chlamydia pneumoniae UTM suspension. Data (± standard deviation) are presented by UTM (BD or Puritan) as a function of storage time and temperature.

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C. trachomatis

Results from the *Chlamydia trachomatis* testing indicate that bacteria were relatively stable over time through 72h of storage at two temperatures (4°C and 22°C). Figure 3 demonstrates that countable foci (indicative of inclusion bodies) were present under each test condition. In addition, a slight dilutional effect between the two test dilutions (1:10 and 1:100) was evident. The variability noted in foci counts was likely due to inherent error (e.g., dilution error), inter-technician variability, and/or slight differences in the test inocula (i.e., normal microbiological variance). Microbiological variance was expected since virus inocula concentrations were relatively low, thereby allowing for greater error. Data trends indicated virus stability over time at two different storage temperatures. Overall results support performance equivalence between the two devices and indicate the presence of detectable and quantifiable *Chlamydiae trachomatis* throughout the test period.

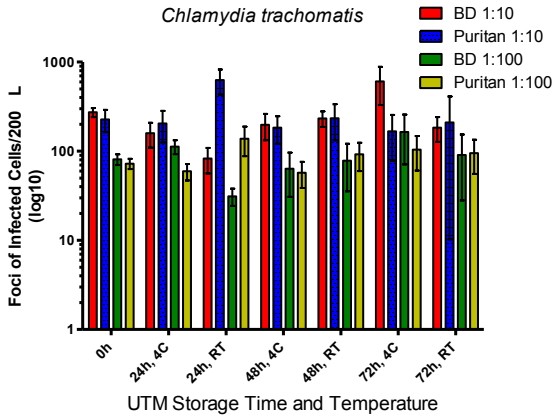

Figure 3. Foci of infected McCoy cells/200 µL inoculum of Chlamydia trachomatis UTM suspension. Data (± standard deviation) are presented by UTM (BD or Puritan) as a function of storage time and temperature.

M. hominis

Results from the *Mycoplasma hominis* testing indicated that bacterial stability/viability decreased steadily over time through 72h of storage at two temperatures (4°C and 22°C). Figure 4 demonstrates that countable CFU were present under each test condition. As expected for bacterial cultures, a dilutional effect between the two test dilutions (1:10 and 1:100) was observed. Unexpected variability noted in CFU counts was likely due to inherent error (e.g., dilution error), inter-technician variability, and/or slight differences in the test inocula (i.e., normal microbiological variance. Microbiological variance was expected since bacterial inocula concentrations were relatively low, thereby allowing for greater error. Data trends indicated bacterial stability over time at 4°C, but a decline in bacterial viability as time progressed at room temperature.

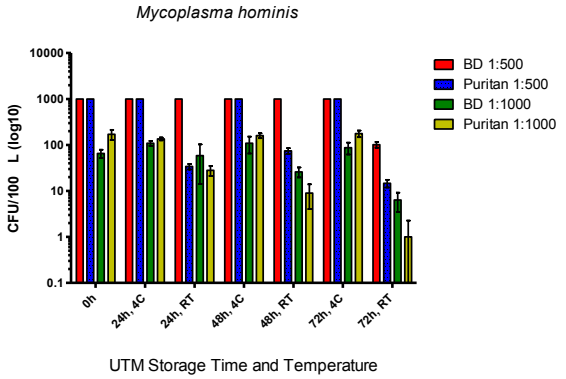

Figure 4. Mycoplasma hominis CFU/100 µL inoculum of UTM suspension. Data (± standard deviation) are presented by UTM (BD or Puritan) as a function of storage time and temperature.

M. pneumoniae

Results from the *Mycoplasma pneumoniae* testing indicated that bacterial stability/viability decreased steadily over time through 72h of storage at two temperatures (4°C and 22°C). Figure 5 demonstrates that countable CFU were present under each test condition. As expected for bacterial cultures, a dilutional effect between the two test dilutions (Neat and 1:10) was observed. Unexpected variability noted in CFU counts was likely due to inherent error (e.g., dilution error), inter-technician variability, and/or slight differences in the test inocula (i.e., normal microbiological variance). For the latter, it was noted that swabs were not 100% consistent with respect to volume uptake, particularly between the device manufacturers; BD swabs were unable to uptake the entire inoculum volume. Data trends for both devices indicated a decline in bacterial viability as time progressed.

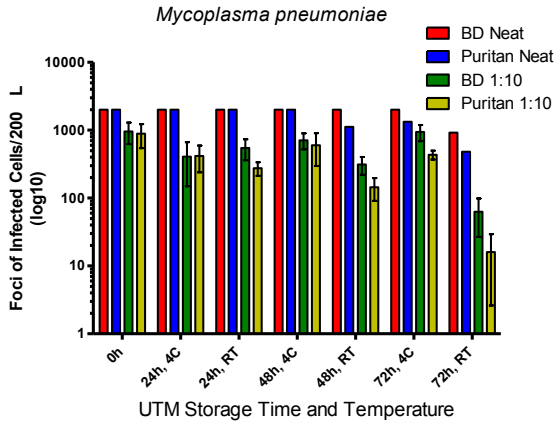

Figure 5. Mycoplasma pneumoniae CFU/100 µL inoculum of UTM suspension. Data (± standard deviation) are presented by UTM (BD or Puritan) as a function of storage time and temperature.

U. urealyticum

Results from the *Ureaplasma urealyticum* testing indicated that bacterial viability was relatively stable over time through 72h of storage at two temperatures (4°C and 22°C). Figure 6 demonstrates that countable CFU were present under each test condition. The above graph presents data only from the 1:1,000 test dilution as the 1:500 test dilution samples demonstrated bacterial concentrations at levels too numerous to count. Unexpected variability noted in CFU counts was likely due to inherent error (e.g., dilution error), inter-technician variability, slight differences in the test inocula (i.e., normal microbiological variance) and/or error associated with counting the microscopic colonies. Data trends for both devices indicated an overall stability in bacterial viability as time progressed.

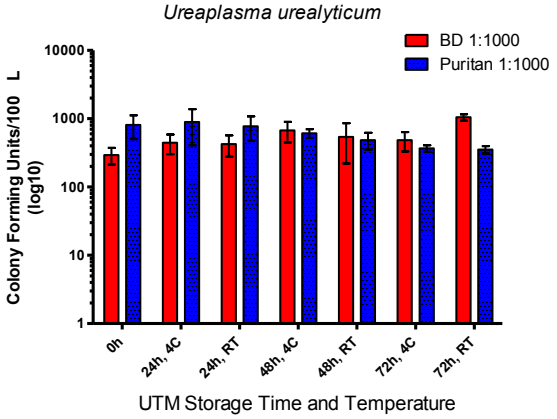

Figure 6. Ureaplasma urealyticum CFU/100 µL inoculum of UTM suspension. Data (± standard deviation) are presented by UTM (BD or Puritan) as a function of storage time and temperature.

Discussion

Based on statistical analyses, it can be concluded that the Puritan® Medical Products Company LLC (PMP) Universal Transport Medium for Viruses, Chlamydia, Mycoplasma, and Ureaplasma, performed equally to the Universal Viral Transport System of Becton, Dickinson and Company (BD).

For both transport systems, test bacteria could be quantified between two different spiking dilutions and two storage temperatures. In general, refrigerated storage resulted in higher test strain recoveries. From a clinical perspective, no differences were noted between the two devices, the PMP UTM device performed in an equivalent fashion to the BD Copan manufactured thus making it a valid system for the collection, storage, and transport of clinical specimens.

Literature Cited

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