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Performance Evaluation of Puritan® Universal Transport System (UniTranz-RT™) for Preservation and Transport of Clinical Viruses

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The ability of a non-propagating microbial transport medium to maintain the viability of clinically relevant viruses was compared to a similar commercial medium to establish performance equivalence. Two dilutions of stock of test viruses, namely adenovirus (AdV), cytomegalovirus (CMV), echovirus Type 30 (EV), herpes simplex virus (HSV) types 1 and 2, influenza A, parainfluenza 3 (PIV), respiratory syncytial virus (RSV), and varicella zoster virus (VZV), were spiked into Puritan® Medical Products Company Universal Transport System (UniTranz-RT™) and BD™ Universal Viral Transport System (UVT) and incubated at 4°C and room temperature (RT) for up to 72 hr. Post incubation assessment of recovery of AdV, EV, HSV-2, PIV, and VZV from UniTranz-RT™ and UVT using shell vial assays followed by immunofluorescence staining demonstrated statistically significant differences between both transport media. In general, significantly higher recoveries of AdV, EV, and VZV were found from UniTranz-RT™ than UVT whereas HSV-2 and PIV were recovered better from UVT than UniTranz-RT™, under specific test conditions. The recovery of HSV-1, influenza A, PIV, and RSV showed no significant differences between transport media. Sulforhodamine B-based assay analysis of UniTranz-RT™ lots prior to and at expiration exhibited no cytotoxicity. The overall results of the study validate the full performance of UniTranz-RT™ as a viral transport medium and establish its effectiveness on par with the UVT. ***J. Med. Virol. 87:1796–1805, 2015***.

KEY WORDS: transport media; viruses; cytotoxicity; UniTranz-RT™; UVT; pre-analytics

INTRODUCTION

Specimen transport to the clinical laboratory is as important as specimen collection such that diagnosis of potential infectious disease and associated treatment may commence as early as possible and infected patients may be isolated to prevent nosocomial infections [Hayden, 2006]. For diagnosis of viral diseases, specimens such as tissue, aspirates, washed fluids, and swabs are routinely collected for diagnosis of the causative agent. Careful transportation of specimens from the site of sampling to a testing laboratory within an acceptable time frame is vital for proper diagnosis. The need for an effective transport medium may not be recognized when the transportation time between sampling and testing is short (<2 hr) [Baron et al., 2013]. The importance of transport medium arises when: (i) viability of the infectious agent must be maintained (for culturing), (ii) the time between sample collection and laboratory testing is >2 hr, (iii) there is need to limit or prevent the proliferation of commensal organisms for preserving reliable diagnostic test results, or (iv) there is a recognized need for maintaining integrity of viral antigens and nucleic acids [Johnson, 1990].

Viral infection is often diagnosed using a combination of clinical suspicion, physical examination, and detection of virus, although the presence of virus (e.g., CMV) does not necessarily imply active infection [Pechulis and Krachman, 2014]. Culture is considered as a gold standard in the diagnosis of etiologic agents. In addition, culture is used to isolate a wide variety of viruses, establish antiviral

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resistance patterns [Pechulis and Krachman, 2014], or perform serotyping, and epidemiological studies [Leland and Ginocchio, 2007]. Media for transport of infectious virus specimens such as swab-tube combinations (termed as device, e.g., Culturette and Virocult), and liquid media and filter entrapment have been reviewed [Johnson, 1990]. Several viral transport media such as Richards viral transport, sucrose-phosphate-glutamate, Virocult, HH medium, tryptose phosphate broth, cell culture medium, Bartel's viral transport, Universal Collection Medium®, Vira-Tans™, and other media [Daum et al., 2011; Barr et al., 2013] have been developed, evaluated, and used for maintaining viral stability during transport and storage of clinical samples over the past five decades [Johnson, 1990; Jensen and Johnson, 1994]. In comparison to viral transport devices, there have been several studies on quality control of bacterial transport devices and a small number of published studies compared viral recovery from different transport media using seeded cultures and clinical specimens [NCCLS, 2003].

Development of a Universal Transport Medium (UTM-RT) for transport of viruses, Chlamydia, Mycoplasma, and Ureaplasma [Castriciano et al., 2006] is an important contribution by Copan Italia S.p.A. (Brescia, Italy) in the past decade and the product was licensed for manufacturing by Becton Dickenson (BD) in North America as BD™ Universal Viral Transport System (UVT). Several culture and molecular-based studies have been conducted on Copan's UTM-RT to establish its performance to efficiently recover and transport viruses and bacteria for clinical diagnosis [Castriciano et al., 2006; Druce et al., 2012]. To meet the global market demand, Puritan Medical Products, LLC (Guildford, ME) developed a Universal Transport System (currently marketed as UniTranz-RT™) similar to Copan's UTM-RT and the chemical constituents of both transport media are similar to each other [FDA, 2012]. In order to establish performance equivalence, a systematic investigation of the viability of nine clinically important pathogenic viruses at 4 °C and room temperature (RT, 22 ± 1 °C) over 72 hr of incubation was conducted with both transport media and the results compared.

MATERIALS AND METHODS

Virus culture stocks and host cell lines were obtained from the American Type Culture Collection (ATCC) (Manasas, VA). Table I shows viruses and their corresponding host cells used in the study. Immunodiagnostic reagents were obtained from EMD Millipore (LIGHT DIAGNOSTICS™ Adenovirus DFA kit, Parainfluenza 1, 2, and 3 DFA kit, CMV DFA kit, Echovirus 30 IFA kit, goat anti-mouse IgG antibody FITC reagent, Simulfluor® HSV/VZV kit, Influenza A & B DFA kit, Respiratory Syncytial Virus DFA kit, and echovirus 30 monoclonal antibody) (Billerica, MA). In vitro toxicity assay kit (sulforhodamine B-based) was from Sigma–Aldrich (St. Louis, MO). Cell culture media and related reagents were obtained from Life Technologies (Carlsbad, CA). All chemicals and reagents used were of reagent grade. UniTranz-RT™ Universal Transport System of Puritan containing polyester flocked swab (PurFlock®) was provided by Puritan Medical Products LLC (Guilford, ME) and BD™ Universal Viral Transport System (UVT) containing nylon flocked swab was purchased from BD. Selection of the host cell line for each virus was based on published literature [Landry and Hsiung, 2000] and ATCC recommendations. Cell lines used were fully characterized and authenticated by the ATCC demonstrating provenance and integrity, a major prerequisite for the study [European Collection of Cell Cultures, 2010]. Culture, propagation, and maintenance of cell cultures were done according to standard operating procedures [European Collection of Cell Cultures, 2010]. Quality control documents from reagents and culture media obtained from commercial sources were verified to maintain the study quality [Warford, 2000].

Preparation of Neat Stocks

Each host cell line was cultured under optimal conditions until 80–90% confluency was attained. The test virus (Table I) was then inoculated as described by Clarke [2010a,b]. Culture flasks were incubated with test virus for propagation until the cytopathic effect (80%) or cellular degradation was observed [Landry and Hsiung, 2000; Clarke, 2010a,b]. The amplified virus was harvested by rapid freeze-thaw cycles of host cells to release the virus, followed by centrifugation of the lysate and collection of the supernatant. Supernatants containing the virus (neat stocks) were stored at −80 °C until testing [Clarke, 2010b].

To determine the viability for each neat test virus stock, an end point dilution assay was used to quantify the amount of virus required to produce the cytopathic effect (CPE) in 50% of infected cells or kill 50% of infected cells ($TCID_{50}$). Percent infectivity was defined as the ratio of the number of immunofluorescent foci (from microscopic observations) to the number of viruses inoculated (based on $TCID_{50}$ values) multiplied by 100 after adjusting to host cell confluence [Azenabor et al., 2007]. Standard protocols were followed for infecting host cells with serial dilutions of virus stock [Clarke, 2010b]. In brief, neat virus stocks were serially diluted (1:2–1:10) and added to a confluent cell monolayer (as appropriate for each virus strain) in a 96-well format or in shell vials. Negative control wells lacked the addition of the test strain. Five replicate wells were tested for each dilution. Inoculated plates were allowed to incubate under optimal conditions for 4–21 days and were inspected daily for CPE. Upon initial CPE observation, incubation was ceased and the numbers of positive and negative wells were enumerated.

TABLE I. Cell Lines and Virus Strains Used in the Study

Test virus			
Name	Abbreviation	Strain	Host cell line
Human adenovirus 1 (ATCC® VR-1™)	AdV	Adenoid 71	Human epithelial cells, A549
Human parainfluenza virus 3 (ATCC® VR-93™)	PIV	C 243	Kidney epithelial cells, MDCK
Human cytomegalovirus (ATCC® VR-977™)	CMV	Towne	Human epithelial cells, HEP2
Human echovirus 30 (ATCC® VR-692™)	EV	HA antigen Frater	Human epithelial cells, A549
Human herpesvirus 1 (ATCC® VR-933™)	HSV-1	F	Kidney epithelial cells, Vero E6
Human herpesvirus 2 (ATCC® VR-933™)	HSV-2	G	Kidney epithelial cells, Vero E6
Influenza virus (H1N1)(ATCC® VR-1469™)	FLU-A	A/PR/8/34	Kidney epithelial cells, MDCK
Human respiratory syncytial virus (ATCC® VR-1540™)	RSV	A2	Human epithelial cells, HEP2
Varicella zoster virus (ATCC® VR-1367™)	VZV	Ellen	Human fibroblast cells, MRC-5

Distinction of virus-induced CPE from "nonspecific CPE" was made by following strict quality control measures of reagents and observing strict protocols plus additional precautions as described [Landry and Hsiung, 2000]. $TCID_{50}$ values were calculated as described by Reed and Muench [Reed and Muench, 1938] and others [Landry and Hsiung, 2000; Clarke, 2010b].

Transport Media Inoculation

A pilot study was conducted with AdV, EV, PIV, and VZV to assess the concentration of neat virus stocks so that sufficiently high number of viable viruses could be recovered from both transport media (UniTranz-RT™ and UVT) for comparison, upon incubation at both 4 °C and room temperature (RT, 22 ± 1 °C) for up to 72 hr. The results of the pilot study allowed us to assess two dilutions of neat stocks to be made for each virus for the full length study (Table II). Each dilution of the test virus (100 μl) was inoculated onto UniTranz-RT™ or UVT swab tips in triplicate. Following inoculation, each swab was transferred into UniTranz-RT™ or UVT transport medium followed by breaking the handle such that the swab would fit into the respective device. Inoculated samples were incubated at 4 °C or RT. At the end of the incubation period (0, 24, 48, or 72 hr), samples were mixed using a vortex mixer (Fig. 1). From each sample, an aliquot (200 μl) was drawn for shell vial assay as described below. Appropriate negative controls without virus were run in parallel.

TABLE II. Neat Stock Virus Quantity, Dilutions of Neat Stock, and Their Infectivity

Virus	Virions/ml of neat stock[a]	Neat stock dilutions and their infectivity[b]	
AdV	2.37×10^8	1:100 & 2%	1:500 & 3%
CMV	2.37×10^2	1:10 & 100%	1:100 & 100%
EV	6.81×10^6	1:100 & 64%	1:500 & 2.9%
FLU-A	4.22×10^7	1:50 & 10%	1:100 & 12%
HSV-1	4.22×10^6	1:10 & 6%	1:100 & 48%
HSV-2	4.22×10^5	1:10 & 47%	1:100 & 97%
PIV	2.37×10^7	1:10 & 3%	1:100 & 25%
RSV	3.16×10^5	1:10 & 76%	1:100 & 25%
VZV	2.70×10^3	1:10 & 100%	1:100 & 100%

[a]Based on $TCID_{50}$/ml determination.
[b]Percent infectivity corresponding to each dilution of virus neat stock.

Shell Vial Assay

Shell vial assays were performed as described by Landry and Hsiung [2000] and Clarke [2010a]. Briefly, host cells were cultured onto 12 mm coverslips in a 24-well culture plates until confluency. At appropriate intervals, 200 μl of spiked transport media were drawn from UniTranz-RT™ or UVT at the end of the incubation period and inoculated onto the 12-mm coverslip cell monolayers followed by centrifugation at 700×*g* for 30 min to allow for virus adhesion. Shell vial cultures were incubated under optimal conditions. Upon reaching appropriate CPE, the coverslip was removed from the shell vial, fixed with acetone, and mounted onto a standard glass microscope slide. Slides and associated coverslips were stained using immunofluorescent, virus-specific monoclonal antibodies contained in DFA kits described under Materials and Methods. Coverslips were 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). Schematic diagram for recovery testing from virus spiked transport media is shown in Figure 1. Prior to the enumeration of foci, the entire area of preparation was first examined and any extracellular staining or cell fragments showing fluorescence was regarded as nonspecific staining. Positive control slides accompanying each immunofluorescence kit were viewed with each specimen batch to ensure proper function of the reagent and

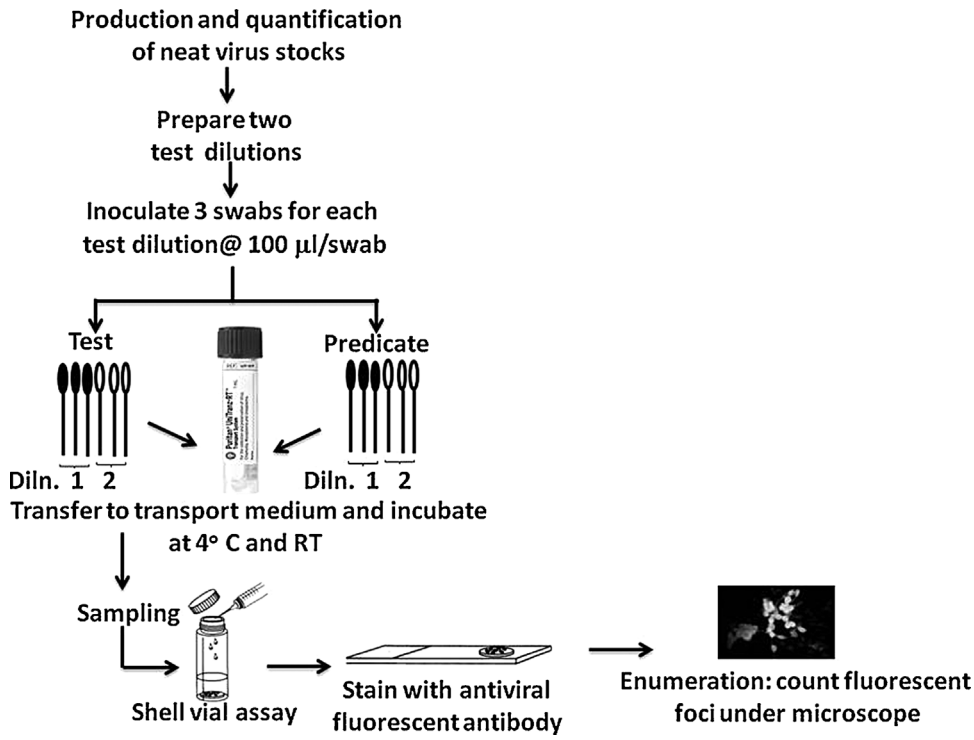

Fig. 1. Schematic diagram for recovery testing from UniTranz-RT™ (Test) and UVT (Predicate) spiked with pathogenic viruses. RT denotes room temperature (22 ± 1 °C).

proper staining technique. Appropriate negative controls without virus were run in parallel.

Cytotoxicity Testing

The cytotoxic effect of UniTranz-RT™ was assessed in MRC-5 cells using a commercially available sulforhodamine B-based in vitro toxicity assay kit [Vichai and Kirtikara, 2006]. In brief, MRC-5 cells were seeded in a 96-well tissue culture plate until 60–80% confluency was reached. For each sample well, 200 µl of cell culture media (control) or cell culture media containing various concentrations of UniTranz-RT™ (75% and 38% v/v) was added. Negative control wells were inoculated with 200 µl of cell culture medium containing 100 µl of phosphate buffer saline. Positive control wells were inoculated with 200 µl of 0.3% Triton X-100 to exhibit cytotoxicity to cell monolayers. Cells were incubated at 37 °C, 5% CO_2 for 24 hr to determine toxicity after which cellular proteins were fixed with trichloroacetic acid (TCA) and stained with sulforhodamine B according to manufacturer's instructions. Following washing steps, the amount of sulforhodamine B bound to cellular proteins was measured colorimetrically to assess the cellular density [Vichai and Kirtikara, 2006]. Decreased cellular biomass as a result of decreased dye intensity was indicative of cellular toxicity. Results from this assay served as the basis for comparing the toxicity of unexpired UniTranz-RT™ to expired lots.

Statistical Analyses

Culture and cytotoxicity data were log-transformed to establish normality prior to conducting one-way analysis of variance (ANOVA) and Tukey's Multiple Comparison Test using GraphPad Prism, Version 5.03 (GraphPad Software, Inc., La Jolla, CA). The null hypothesis (H_0) was that viruses are recovered at the same rate irrespective of the virus type and no difference in viability would be found for a given virus between UniTranz-RT™ and UVT transport media. The *P*-values were computed by ANOVA and then the test of significance was applied ($\alpha = 0.05$) by Tukey–Kramer Honestly Significant Difference test [Sall et al., 2007].

RESULTS

Virus Recovery

Two dilutions of the neat stock for each virus were prepared and their infectivity was determined prior to recovery testing (Table II). Dilutions of each neat virus stock used in the study and their infectivity expressed as $TCID_{50}$ are shown in Table II.

Significant differences in viability of AdV was observed between UniTranz-RT™ and UVT at the 1:100 dilution and tested at the 48 hr (4 °C) and 72 hr (22 °C) sampling time points (Fig. 2). Transport media spiked with 1:500 dilution of virus demonstrated significant differences in viability at 0 and 48 hr (4 °C) between UniTranz-RT™ and UVT. At a given stock virus dilution and incubation time, the viability of AdV was equal or slightly higher in UniTranz-RT™ than the UVT.

In comparison to initial (0 hr) virus foci counts, recovery of CMV showed a decrease upon incubation of spiked UniTranz-RT™ and UVT at 4 °C and RT for 72 hr at both dilutions. The viability of the virus declined steeply in both UniTranz-RT™ and UVT spiked with 1:10 diluted virus stock than transport media spiked with 1:100 diluted stocks, upon incubation at either temperature through the storage period (data not shown). Viable CMV foci recovered from UniTranz-RT™ and UVT spiked with 1:100 diluted virus stock decreased at 24 hr and the foci counts remained stable up to 72 hr of incubation at 4 °C and RT. One-way ANOVA analysis of EV recovery from UniTranz-RT™ and UVT spiked with 1:100 diluted stock virus and incubated at both 4 °C and RT demonstrated no significant differences (Fig. 2). On the other hand, EV recovery from UniTranz-RT™ and UVT spiked with 1:500 diluted virus showed significant differences at 0 hr, 48 hr (4 °C), and 72 hr (4 °C) of incubation (Fig. 2). The median viability of EV from the UVT was slightly higher than the same from UniTranz-RT™ at both dilutions. Additionally, the overall trend of EV viability at both dilutions showed a stable pattern with no dramatic declines.

The HSV-1 foci counts of aliquots drawn from UniTranz-RT™ and UVT spiked with either virus dilution demonstrated stable counts over 72 hr of incubation at 4 °C and RT. Statistical analysis of the recovery of HSV-1 from spiked UniTranz-RT™ and UVT at both dilutions demonstrated no significant differences upon incubation at 4 °C and RT throughout the storage period (data not shown). On the contrary, the viability of HSV-2 spiked into UniTranz-RT™ and UVT with 1:10 diluted stock was stable up to 48 hr and declined later, with no measurable counts at 72 hr and RT storage (data not shown). Statistical analysis of the recovery of HSV-2 from spiked UniTranz-RT™ and UVT at 1:10 dilution demonstrated no significant differences upon incubation at 4 °C and RT throughout the storage period. Significantly higher foci of HSV-2 were enumerated from the UVT than UniTranz-RT™ spiked with 1:10 diluted stock and after storage at 4 °C for 72 hr. Viability of HSV-2 spiked with 1:100 diluted stock showed significantly lower viability compared to the viability of the same virus spiked with 1:10 diluted stock in both transport media throughout the storage period. At 72 hr of storage (RT), HSV-2 viability was completely (Fig. 2) lost in UniTranz-RT™ and UVT spiked with 1:10 and 1:100 diluted stocks.

The overall trend of FLU-A viability in both transport media demonstrated a gradual decrease as a function of incubation time at either temperatures. In general, the viability of FLU-A in both transport media were higher at 4 °C than the same at RT (Fig. 2). There were measurable foci at the end of the incubation period throughout the study. Both transport media demonstrated ability to support the viability of FLU-A and differences throughout the test period were statistically insignificant between them. Results of PIV recovery from both transport media spiked with either dilution showed fluctuation of foci counts upon incubation at either temperature, throughout the storage period (Fig. 2). One way ANOVA analysis of PIV counts demonstrated significant differences between UniTranz-RT™ and UVT spiked with 1:10 diluted stock incubated at 24 hr (4 °C), 48 hr (4 °C and RT), and 72 hr (4 °C). PIV viability of samples drawn from UniTranz-RT™ and UVT spiked with 1:100 diluted viruses also demonstrated significant differences at 24 hr (4 °C), 48 hr (RT), and 72 hr (4 °C) of incubation. UVT supported significantly higher counts of PIV over UniTranz-RT™ at 24 and 48 hr of incubation; however, UniTranz-RT™ demonstrated significantly higher counts of PIV than UVT at 72 hr of incubation (Fig. 2).

A small decrease of RSV foci (~0.7 log) in UniTranz-RT™ and UVT spiked with 1:10 diluted stock upon incubation through 72 hr was observed, whereas such differences were less profound in UniTranz-RT™ and UVT spiked with 1:100 diluted virus stocks (data not shown). The viability of RSV followed the trend of FLU-A which showed statistically insignificant differences between the UniTranz-RT™ and UVT spiked with either dilutions of virus stock and incubation temperatures throughout the storage period. Determination of VZV viability in UniTranz-RT™ and UVT spiked with 1:10 diluted virus stock demonstrated significant differences at 0 hr, 24 hr (RT), and 48 hr (RT) of incubation (Fig. 2). A dip in the viability trend was evidenced at 48 hr of storage of transport media spiked with 1:10 diluted virus stock. The overall viability of VZV gradually decreased through the storage period and countable foci were found throughout the test period in transport media spiked with 1:100 diluted virus stock. At 1:100 dilutions, significantly higher viable counts in UniTranz-RT™ as compared to UVT were found at 48 hr of incubation (4 °C and RT) and a slow but gradual decline in viability through the storage period was also found (Fig. 2).

Cytotoxicity

The sulforhodamine B assay results demonstrated no significant differences between UniTranz-RT™ lots prior to and after expiration, at 75% or 38% replacement levels. The protein levels from cells

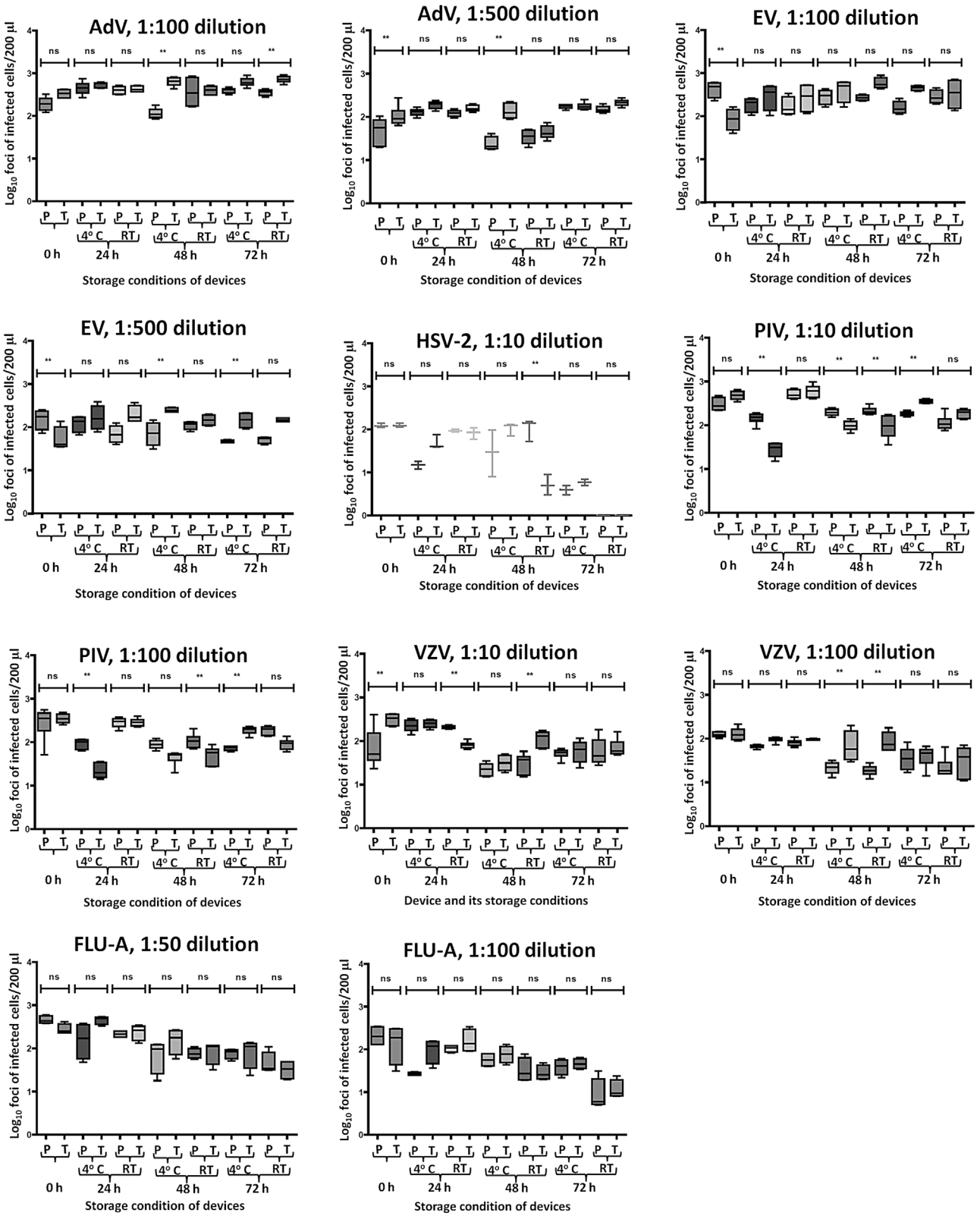

Fig. 2. Continued.

incubated in PBS containing growth media (negative control) were not significantly different from the protein levels from cells incubated in UniTranz-RT™. On the contrary, protein levels from cells exposed to Triton X-100 (positive control) decreased and were significantly different from the protein levels from cells incubated in UniTranz-RT™ or negative controls.

DISCUSSION

Specimens for microbiological testing require careful handling, transport, and storage to facilitate maximum recovery of pathogens and minimize proliferation of commensals [Garcia et al., 1998]. Depending on the clinical pathogen in question, transport of specimens to the laboratory often takes up to or beyond 48 hr after collection [Wilson, 1996]. Clinical specimens suspected of viral infections are transported in sterile containers, syringes, and/or as swab specimens in vials containing transport media. The collection medium depends on various factors, including the suspected type of virus, specimen nature, method of testing, and distance and time between sample collection and analysis [St. George, 2012]. A swab-transport media combination must be able to maintain viability of organisms plus maintain a relatively high proportion of initially obtained organisms [Tano and Melhus, 2011]. Careful evaluation of transport media-swab combination is critical to understand the product limitations for use with diagnostic specimens. The gross chemical constituents of UniTranz-RT™ and UVT transport media are the same [FDA, 2012]. Further, UTM-RT or UVT is widely used in diagnostic microbiology and has been used in several culture and molecular-based studies [Castriciano et al., 2006; Chernesky et al., 2006; Druce et al., 2012].

To establish clinical relevance, representative pathogenic viruses commonly found in respiratory specimens, skin lesions, ocular specimens, urine, blood, bone marrow, and genital specimens were chosen for this comparative study [NCCLS, 2003]. Clinical guidelines emphasize the need for transport of specimens containing viruses at cold/refrigerated conditions based on the fact that stability of many pathogenic viruses is greater at 4 °C than at RT. Our study included RT as a testing variable since temperature fluctuations occur during transport [Sobey and Meschke, 2003]. Viability was tested up to 72 hr to account for possible delays in patient specimen transport.

For virus recovery studies, we chose a viable culture method (shell vial assays) as opposed to rapid techniques (i.e., molecular or immunological methods) since culture is still considered the gold standard for diagnosis of many viral infections such as congenital CMV [Stagno and Britt, 2006]. In addition, it is difficult to determine the viability of virus found in a clinical sample without culturing as well as to determine the clinical significance of a non-infectious virus particle. The shell vial assays employed in this study are routinely used in clinical virology settings and have the ability to detect >90% of viruses within 48 hr without compromising specificity thus providing results in an acceptable time frame for patient care management [Jayakeerthi et al., 2006].

The concentration of neat stocks for all viruses used in this study ranged from 10^3 to 10^7 $TCID_{50}$/ml, mimicking the upper range of clinically relevant virus titers [NCCLS, 2003]. The in vitro infectivity of viruses ranged from 2% to 100%. Among test viruses, AdV, FLU-A, CMV, and VZV exhibited similar infectivity at both test dilutions. Remaining test viruses except EV exhibited higher infectivity at higher dilutions. In addition to viability, the infectivity and amplification of virions against a cell line is dependent on the type and the virulence of a virus strain, availability of receptors for virus adhesion to initiate infection, cell culture media composition, cell culture conditions, and other related factors [Manning and Collins, 1979; Rodriguez et al., 2009].

A pattern of disproportionate recovery of test viruses relative to the dilution of stock virus was occasionally observed in the present study which may be related to a combination of factors, including normal microbiological variance, inter-technician variability, manufacturing variances of devices, and differences in physical properties of swabs between transport media. Differences in recovery at lower dilution of virus stock could be due to competition for virus binding sites on host cells, dilutional effects, ratio of host cells to infectious virus particles, and other related factors.

AdV recovery was consistent with UniTranz-RT™ and UVT except for a small dip of recovery observed

Fig. 2. Boxplot of virus recovery from UniTranz-RT™ and UVT spiked with pathogenic virus. Foci counts of viruses enumerated from 200 μl of aliquot from both transport media were $\log_{10}$ transformed and statistically analyzed using one-way ANOVA, for comparison under each storage condition. For each data set (≥6 data points), the minimum and maximum values are represented by whiskers below and above the grouped bars, respectively. The median is indicated as a horizontal bar within each data grouping. The upper, middle, and lower horizontal lines of the box indicate 75th percentile, median, and 25th percentile within each data grouping, respectively. Ns refers to not significant and ** refers to significant differences between UniTranz-RT™ and UVT ($P < 0.05$). The storage conditions (duration and temperature) of UVT (P) and UniTranz-RT™ (T) are shown on the X-axis. The dilution of virus stock for spiking the transport media is shown. RT denotes room temperature (22 ± 1 °C). The abbreviations used are as follows: AdV, adenovirus; EV, echovirus Type 30; Flu-A, Influenza A; HSV-2, herpes simplex virus type 2; PIV, parainfluenza 3 virus; VZV, varicella zoster virus.

at 48 hr of storage. This has been an expected result from non-enveloped viruses such as AdV that are more resistant to environmental changes [Sobey and Meschke, 2003]. These results were similar to those shown previously [Jensen and Johnson, 1994]. For example, Dunn et al. [2003] computed half-lives of AdV maintained at 4 and 22 °C in five different transport media to be 5.4–21 days and 5.4–13.6 days, respectively.

In comparison to AdV, decreased foci counts found in both transport media spiked with CMV demonstrated less stability of the virus over the storage period, though no significant differences were seen between the two transport media. Studies on CMV spiked onto vaginal tampons and stored in viral transport medium demonstrated quantitative recovery of viruses within 1 hr of inoculation and loss of infectivity of CMV upon further incubation at $\leq$4 °C but stable at −70 °C [Larew and Myers, 1982] suggesting virus lability. Lability of CMV and VZV among herpes viruses in transport media was also reviewed by Johnson [Johnson, 1990].

Enteroviruses are non-enveloped viruses that contain protein coats capable of withstanding harsh environments [Salo and Cliver, 1976]. The lability of echovirus type 9 spiked into three viral transport media of Copan, Micro Test M4, and BBL Viral Culturette determined by shell vial assays of aliquots sampled from media stored at 4°C or 22 °C for 72 hr also revealed no significant decline in titer from some test devices [Dunn et al., 2003] corroborating our observations of stability of EV in both transport media throughout the storage in this study.

Comparison of the stability of closely related alphaherpesvirinae subfamily members (HSV-1, HSV-2, and VZV) in both transport media demonstrated interesting differences under the experimental storage conditions. In this study, both transport media spiked with HSV-1 demonstrated stability at the tested dilutions and temperatures throughout the storage period. HSV-2 viability, however, drastically declined in both transport media through 72 hr of storage at 4 °C and RT. VZV foci counts were stable up to 48 hr and they showed a small decline at 72 hr of incubation under the experimental conditions. Recovery of HSV-1 and HSV-2 from Leibovitz charcoal viral transport medium and modified Leibovitz-Emory medium, incubated at ambient temperature revealed inactivation of both viruses within 2 hr and no virus recovery after 1 day of incubation at ambient temperature [Nahmias et al., 1971], suggesting the importance of transport media on virus recovery and storage temperature [Barnard et al., 1986; Jensen and Johnson, 1994]. Relatively poor stability of HSV-2 isolates over HSV-1 in both UniTranz-RT^{TM} and UVT observed by us is similar to the observations reported by others [Jensen and Johnson, 1994]. We found a general trend of gradual loss of VZV viability as a function of storage period in either transport media and others also reported similar results [Levin et al., 1984].

With respect to the respiratory viruses, namely FLU-A, RSV, and PIV, the storage stability pattern was similar between RSV and PIV, but not FLU-A. Higher stability of FLU-A virus was reported upon storage at refrigerated temperature than storage at RT for 24 hr, after which a gradual decline occurred irrespective of storage temperature [Barger et al., 2005]. These results are in contrast to our observations of the gradual decline of FLU-A viability in both transport media spiked with either dilution of virus and stored at 4 °C or RT through 72 hr which may be attributed to differences in assay methods and virus isolates used. Half-lives of PIV spiked into three Copan viral transport media and stored at 4 and 22 °C found no significant differences [Dunn et al., 2003]. Fluctuations in recovery PIV from both UniTranz-RT^{TM} and UVT were observed through the storage period in this study, which can be attributed to differences in detection methods and PIV isolates used.

The viability of RSV showed a similar stability pattern between UniTranz-RT^{TM} and UVT up to 48 hr after which differences were evident. Survival of RSV in various transport media also exhibited higher lability upon storage at 22 °C than at 4 °C [Dunn et al., 2003]. The viability of VZV at both tested dilutions showed a slow but gradual decline in both spiked transport media irrespective of storage temperature. Compilation of stability of different viruses in various transport media [Johnson, 1990] suggested stability of HSV and viability of labile viruses such as CMV, RSV, and VZV was found to be 1–3 days in various media.

Cytotoxicity testing of transport media was conducted to ensure that the transport medium allows for the viable enumeration and/or detection of viruses, in a diagnostic sample. In addition, this was conducted to evaluate if the transport media complied with an established specification for microbiological quality. Testing of cytotoxicity at product expiration was performed to ensure that the product is stable and to make sure that product aging and storage do not impact performance. Although many in vitro methods are available for cytotoxicity testing [Ehrich and Sharova, 2000], we chose the sulforhodamine B assay for sensitivity and specificity [Vichai and Kirtikara, 2006]. A recent report from a clinical laboratory group in Belgium pointing to the toxicity to cell cultures of UTM or VTM that had come into contact with the ESwab of Copan Diagnostics [Indevuyst et al., 2012] is a striking example of cytotoxicity of microbial transport media.

CONCLUSIONS

Our results demonstrated an overall equivalent performance of both transport media to support the viability of clinically relevant viruses at two different

temperatures of storage through 72 h. Although recovery of AdV, EV, HSV-2, PIV, and VZV demonstrated statistically significant differences between UniTranz-RT^{TM} and UVT, the results may not be of practical significance considering variability in patient samples in a clinical setting, thereby demonstrating their utility for transportation and preservation of specimens from the site of sampling to the laboratory for clinical diagnosis. Additionally, information on the survival rates of various pathogenic viruses as a function of storage temperature and virus concentration presented here is of clinical significance for laboratorians. Viral transport media tested in this study are excellent clinical microbiology tools for microbiologists and allows them to choose the product from multiple suppliers guaranteeing not only sustainable supply but also for product quality plus cost comparison. In a clinical microbiology setting, it is also important to emphasize that differentiation of viable from nonviable virus by culture is important for medical decision-making. Additionally, viral cultures are important for differentiating disease from latent infection, deciding the right time to implement, discontinue, or change antiviral therapy; or patient management.

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