

Comparative Performance Evaluation of Viral Stability with Universal Transport Medium Devices

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Abstract

Introduction: Detection of clinical viruses 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. We compared the performance of a non-propagating transport medium test device with a similar commercially manufactured device.

Methods: Quantitation of nine viruses (Human adenovirus 1, Cytomegalovirus [CMV], Echovirus Type 30, Herpes Simplex Virus Types I [HSV-1] and II [HSV-2], Influenza A, Parainfluenza 3, Respiratory Syncytial Virus [RSV], and Varicella Zoster Virus [VZV]) 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 (BD) and Puritan (PMP) Universal Transport System (UTM) device. Following UTM storage, samples were inoculated onto shell vials containing a confluent monolayer of permissive cells and incubated 24 to 48h. Shell vial cover slips were collected, mounted and stained with strain specific fluorescent antibodies and visualized with a fluorescent microscope. Individual foci were counted for quantitative analysis.

Results: For both devices, test viruses could be quantified through 72h of storage at two separate temperatures. In general, refrigerated storage resulted in higher test strain recoveries. Adenovirus, CMV, Echovirus 30, HSV-1, Parainfluenza 3, and RSV were all relatively stable during the 72h time course, while HSV-2, Influenza A, and VZV decreased in stability. One-way ANOVA demonstrated statistical differences (P<0.05) in the two devices under certain conditions, concluded to be indicative of normal microbiological variability and swab manufacturing inconsistency. Specifically, Adenovirus demonstrated significantly lower recovery for 48h at both temperatures for the BD device. Echovirus 30 demonstrated significantly higher PMP device counts from 24-72h at 4°C. The PMP device evaluating HSV-2 resulted in significantly lower counts in the 1:10 dilution at 48h and 20-25°C. Parainfluenza 3 analysis resulted in significantly lower counts at 4°C for both dilutions at the 48h time point. VZV PMP device results were significantly higher at 0h and 48h for both temperatures and both dilutions. No significant differences were found testing CMV, HSV-1, Influenza A, and RSV.

Conclusions: From a clinical perspective, no differences were noted between the two devices, the PMP device performed in an equivalent fashion to the BD device thus making it a valid system for the collection, storage, and transport of clinical specimens.

Materials and Methods

UTM Inoculation and Incubation: Replicate devices were inoculated with 100 µL of test suspension onto the device swab tip and immediately placed into the PMP or BD UTM followed by incubation at specified holding temperatures. This was performed in triplicate for each dilution at each holding time (0, 24, 48, and 72 hours) at RT [20-25°C] and refrigerated [4°C]. Viability was assessed via modified shell vial cultures.

Shell Vial Cultures Assay:

Cultures were prepared with optimal cell lines (listed in Table 1) affixed to 12 mm glass cover slips in 24-well cell culture plates until confluent. 200 µL of spiked UTM from each device were removed and inoculated into the cultures in duplicate. Plates were centrifuged at RT for 30 min at 500 to 700 x g, inoculum was aspirated and replaced with media. Monolayers were examined daily until the expected CPE was observed and then gently washed with 1 mL of phosphate buffered saline (PBS) and cover slip was carefully removed, gently placed onto a glass slide, and fixed with 1 mL of cold acetone.

Immunofluorescent Assay:

Primary antibody was added to each cover slip and incubated for a target of 30 min. Reagent was then aspirated and each cover slip was gently washed three times with 1 mL of PBS/Tween 20. Remaining PBS/Tween 20 was aspirated and the cover slip was gently transferred and mounted (cell side down) onto a glass slide. 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 (Figure 1).

Test Strain	Test Strain ATCC Cat. #	Host Cell Line
Adenovirus	VR-1	A549
Cytomegalovirus	VR-977	HEp-2
Echovirus Type 30	VR-692	A549
Herpes Simplex Type 1	VR-733	Vero-E6
Herpes Simplex Type 2	VR-734	Vero-E6
Influenza A	VR-1469	MDCK
Parainfluenza 3	VR-93	MDCK
Respiratory Syncytial Virus	VR-1540	HEP-2
Varicella Zoster Virus	VR-1367	MRC-5

Table 1. ATCC Virus Strains and Optimal Cell Lines Chosen for Testing

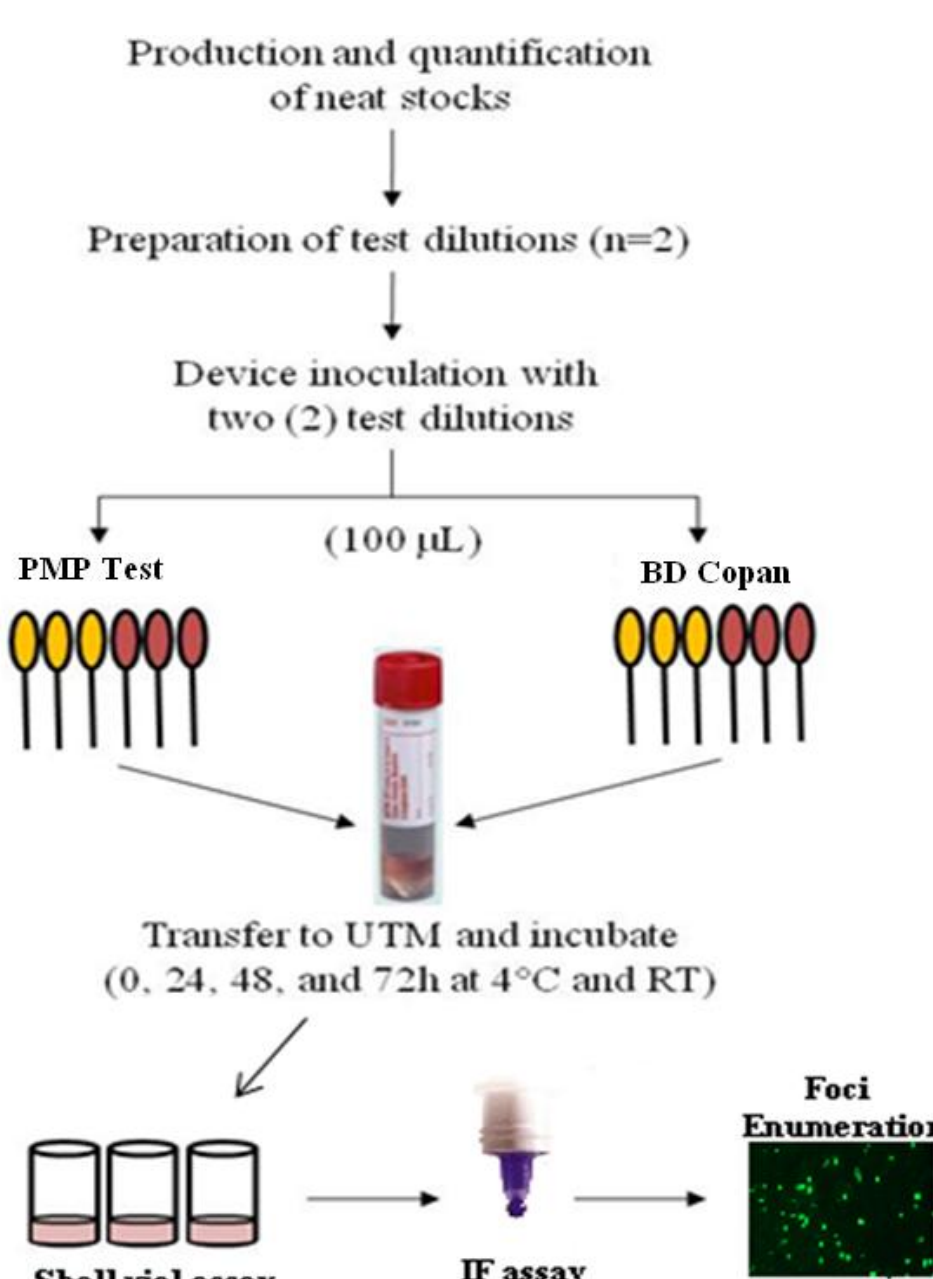
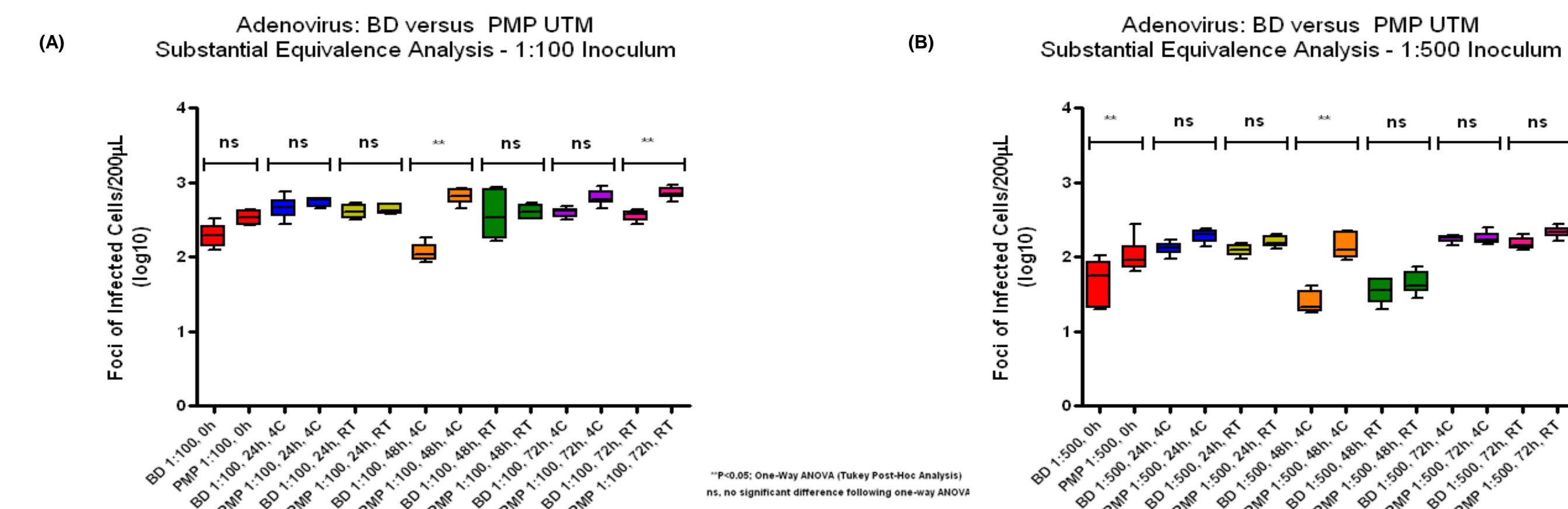


Figure 1: Flow chart of processes from virus production to enumeration of fluorescent foci.

Results and Discussion



Figures 2 (A), (B). Substantial equivalence analysis of Adenovirus inoculated UTM devices. Results from testing indicate that virus was relatively stable over time through 72h of storage at 4°C and RT. One-way ANOVA analysis of the both dilutions demonstrated two instances of significance (P<0.05) between the BD and PMP devices for both dilutions. Following 48h of storage at 4°C with both dilutions, foci counts for the BD device were significantly lower than those in the PMP device. Following 72h of storage at RT for the 1:100 dilution, foci counts for the BD device were significantly lower than those in the PMP device (A). Overall results support performance equivalence between the two devices.

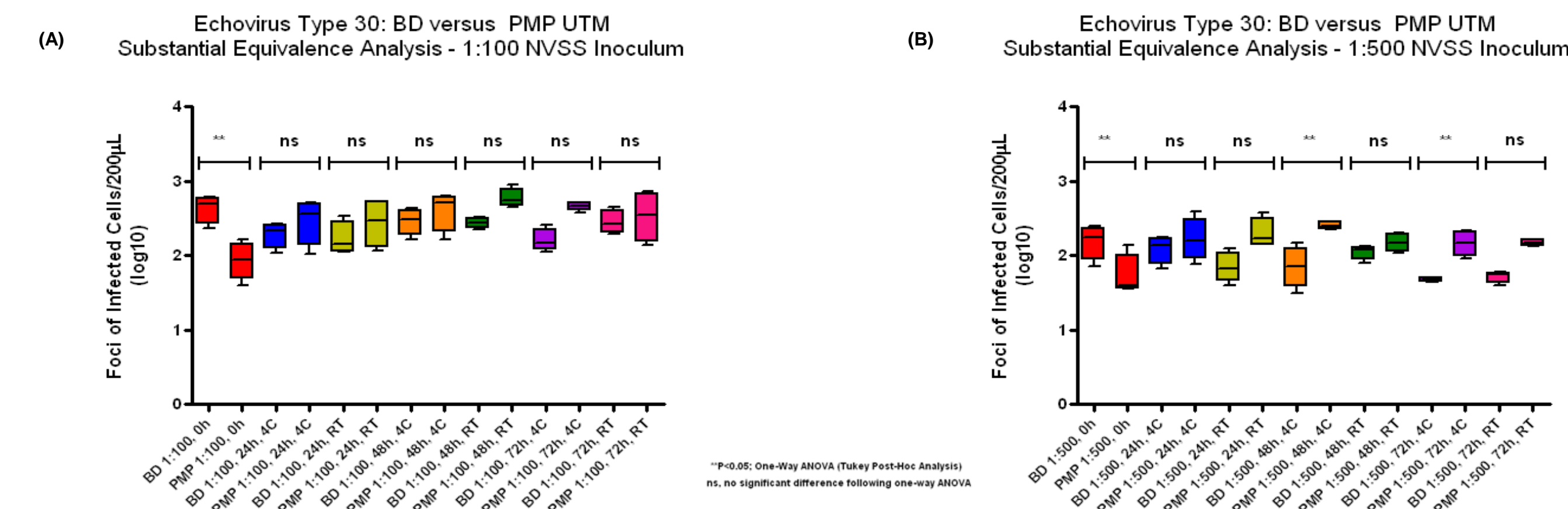


Figure 3 (A), (B). Substantial Equivalence Analysis of Echovirus Type 30 inoculated UTM devices. Results from testing indicate that virus was relatively stable over time through 72h of storage at 4°C and RT. One-way ANOVA analysis of the 1:100 test dilution data demonstrated one instance of significance (P<0.05) between the BD and PMP device for the 0h time point (A). The 1:500 test dilution data demonstrated three instances of significance (P<0.05) between the BD and PMP device (B). Foci counts for the 0h time for the PMP device were significantly lower than those in the BD device and following 48 to 72h of 4°C foci counts for the BD device were significantly lower than those observed in the PMP device.

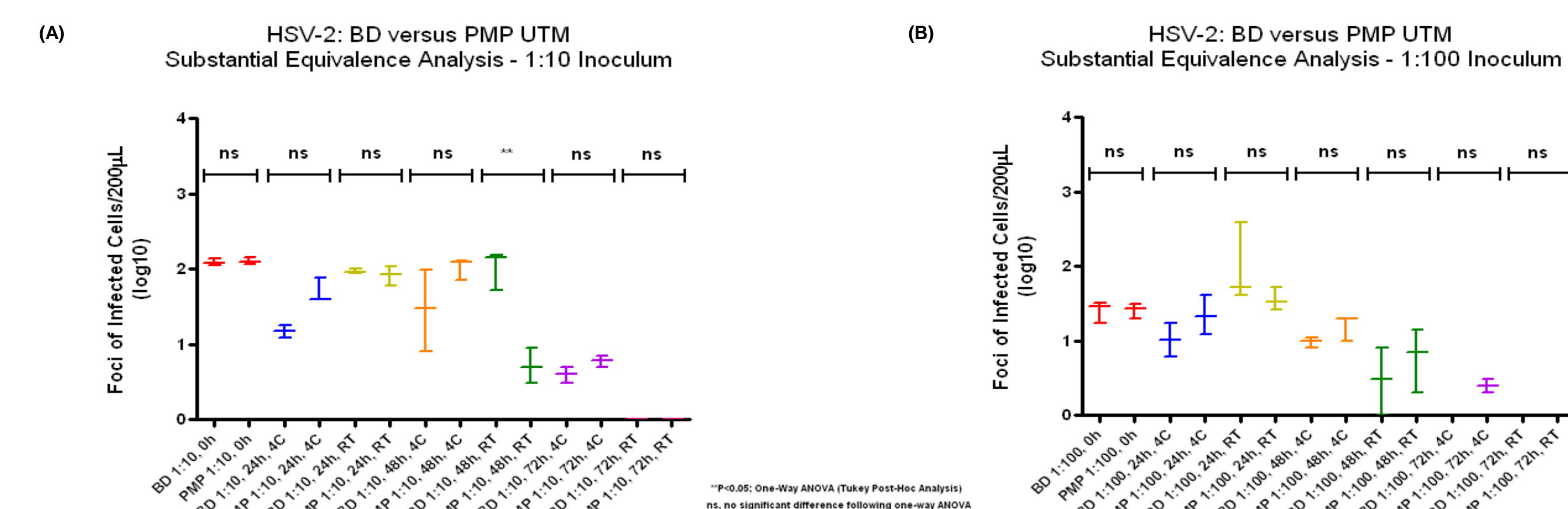


Figure 4 (A), (B). Substantial equivalence analysis of HSV-2 inoculated UTM devices. Virus declined slightly over time through 72h of storage at 4°C and RT. One-way ANOVA analysis of the HSV-2 1:10 test dilution data demonstrated one instance of significance (P<0.05) between the two devices. Following 48h of RT storage, foci counts for the PMP device were significantly lower than those in the BD device (A). The 1:100 test dilution data demonstrated no significant differences between the two devices under any of the tested conditions (B). Overall results support performance equivalence between the two devices and indicate the presence of detectable and quantifiable HSV-2 throughout the test period, though sub-optimal results were noted following 72h of storage.

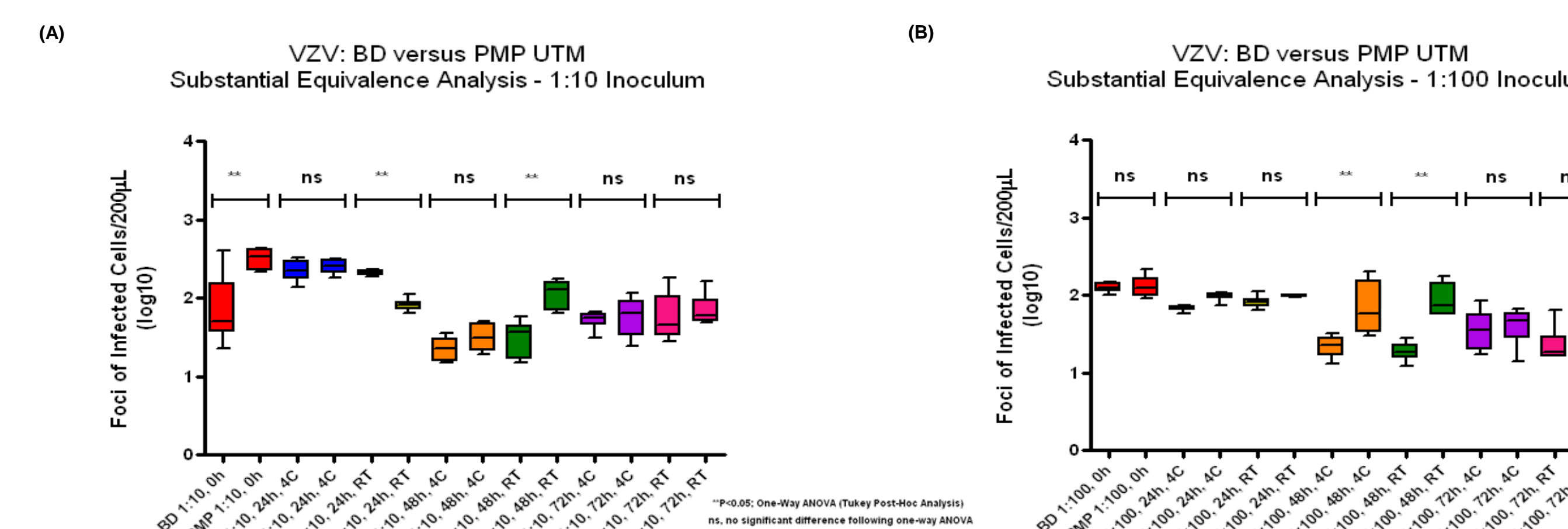


Figure 5 (A), (B). Substantial equivalence analysis of VZV inoculated UTM devices. Results from testing indicate that virus declined slightly over time through 72h of storage at 4°C and RT. One-way ANOVA analysis of the VZV 1:10 test dilution data demonstrated three instances of significance (P<0.05) between the two devices. Following 24h at RT conditions, foci counts for the PMP device were significantly lower than those in the BD device; at 48h counts for the PMP device were significantly higher than those observed in the BD device (A). The 1:100 test dilution data demonstrated two instances of significance (P<0.05) between the two devices. Following 48h of storage, foci counts for the BD device were significantly lower than those observed in the PMP device at both temperatures (B).

Results and Discussion (Continued)

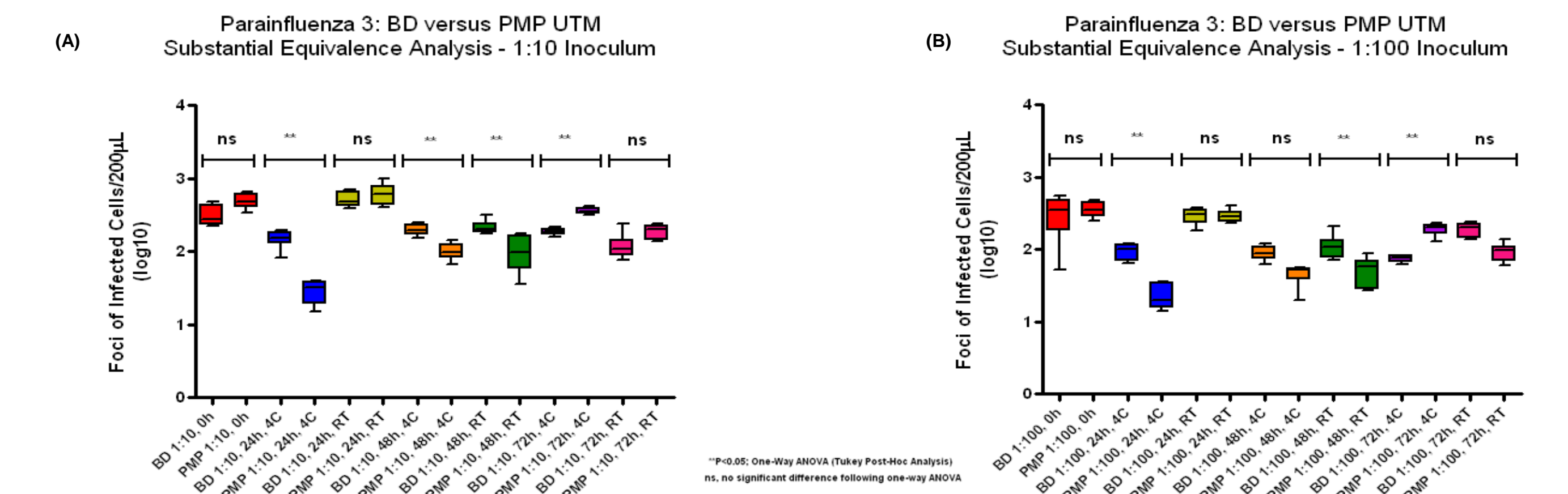


Figure 5 (A), (B). Substantial equivalence analysis of Parainfluenza 3 inoculated UTM devices. Virus was relatively stable over time through 72h of storage at 4°C and RT. One-way ANOVA analysis of the Parainfluenza 3 1:10 test dilution data demonstrated four instances of significance (P<0.05) between the two devices (A). Following 24 and 48h of storage at 4°C and 48h at RT, foci counts for the PMP device were significantly lower than those in the BD device. Foci counts at 72h and 4°C for the BD device were significantly lower than those in the PMP device. The 1:100 test dilution data demonstrated three instances of significance (P<0.05) between the two devices (B). Following 24h at 4°C, the PMP device counts were significantly lower than those in the BD device. Similar results were observed after 48h at RT. Following 72h of storage at 4°C, foci counts for the BD device were significantly lower than those observed in the PMP device.

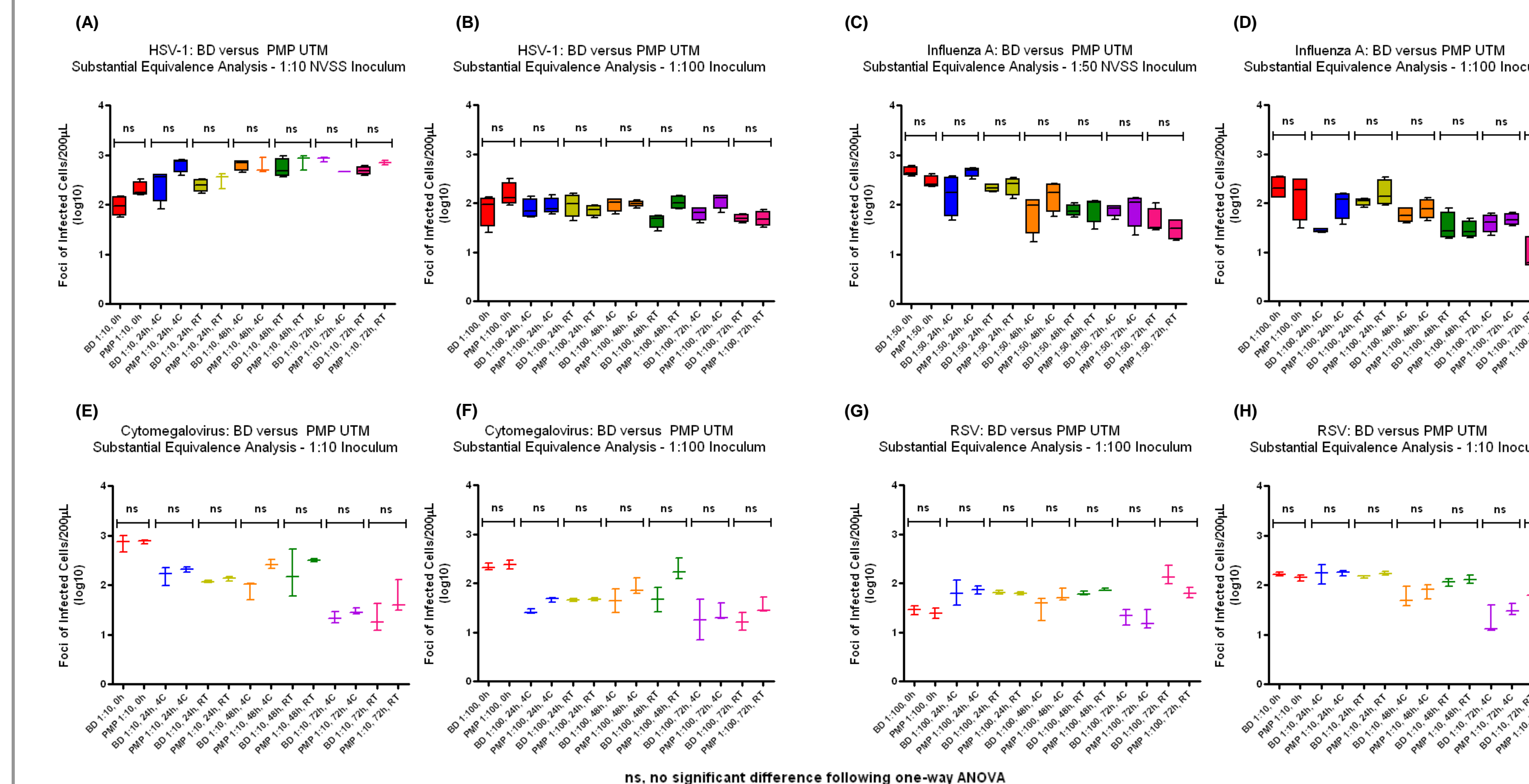


Figure 7 (A through H). Substantial equivalence analysis of various inoculated UTM devices. Results from HSV-1 testing indicate that virus was relatively stable over time through 72h of storage at 4°C and RT. One-way ANOVA analysis of the both dilutions demonstrated no instances of significance (P<0.05) between the BD and PMP device (A, B). Results from the Influenza A testing indicate that virus was relatively stable over time through 72h of storage at 4°C and RT. One-way ANOVA analysis of the Influenza A 1:50 and 1:100 test dilution data demonstrated no instances of significance (P<0.05) between the BD and PMP devices (C, D). Results from CMV testing indicate that virus declined slightly over time through 72h of storage at 4°C and RT. One-way ANOVA analysis of the CMV 1:10 and 1:100 test dilutions data demonstrated no significant differences between the BD and PMP devices under any of the tested conditions (E, F). Results from RSV testing indicate that virus was relatively stable over time through 72h of storage at 4°C and RT. One-way ANOVA analysis of the RSV 1:10 and 1:100 test dilution data demonstrated no significant differences between the BD and PMP devices under any of the tested conditions (G, H). Overall results support performance equivalence between the two devices and indicate the presence of detectable and quantifiable HSV-1, Influenza A, CMV and RSV throughout the test period.

Conclusions

For both transport systems, test viruses could be quantified between two different spiking dilutions and 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. From a clinical perspective, no differences were noted between the two devices, the PMP device performed in an equivalent fashion to the BD manufactured device thus making it a valid system for the collection, storage, and transport of clinical specimens.

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