Performance Evaluation of Puritan Unitranz-RT Universal Transport System for the Detection of Respiratory Viruses

De'Ashia Lee[1], Lea Heberlein-Larson[2], Alberto van Olphen[3], Cynthia Bucher[3], Andrew Cannons[2], and Susanne Crowe[4]

[1]APHL/CDC Emerging Infectious Disease Laboratory Fellow, [2]Florida Department of Health-Bureau of Public Health Laboratories, Tampa, FL, [3]University of South Florida, Center for Biological Defense, Tampa, FL, and [4]Florida Department of Health-Bureau of Public Health Laboratories, Jacksonville, FL

Summary

Introduction

Puritan Unitranz-RT is intended for the collection and transport of clinical samples containing viruses, chlamydiae, mycoplasmas, and ureaplasmas from collection site to the testing laboratory. In this study, we evaluated how storage in Puritan Unitranz-RT affects the ability to detect Influenza A virus, Adenovirus, and Rhinovirus by real-time RT-PCR, comparing results obtained from samples extracted directly from virus stocks and those stored in Puritan Unitranz-RT. This study reports for the first time the effect that Puritan Unitranz has on viral qRT-PCR detection on samples applied to swabs and stored in Puritan Unitranz-RT for more than 48 hours.

Methods Overview

Seven ten-fold dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7}) of stock Influenza A (A/California/04/2009(H1N1)) and Adenovirus were prepared. Eight ten-fold dilutions were prepared for Rhinovirus. The titers of the viruses were $1X10^5$, $1X10^7$, and $1X10^7$ $TCID_{50}$ respectively. Samples were extracted and then evaluated using the ABI 7500 Real-Time PCR system.

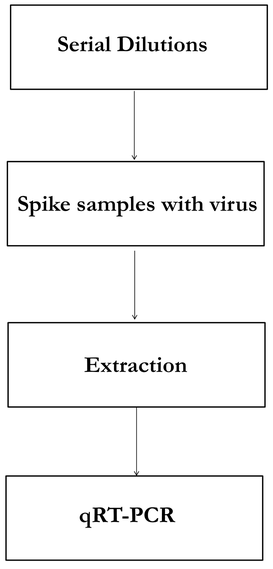
Results

For each virus, we found that there was a statistical difference between CT values for samples applied to swabs and samples processed directly from stock. Our results indicate that storage in Puritan Unitranz-RT is advantageous for qRT-PCR detection when compared to samples not stored in Puritan Unitranz-RT.

Conclusion

These results indicate that prolonged storage in Puritan Unitranz-RT does not have a negative effect on virus detection. Future work will consider qRT-PCR for the detection of additional influenza and respiratory viruses.

Methods



Results

Influenza A Virus

	Puritan Unitranz-RT	Cell Culture Media
Positive	27	21
Negative	3	9

Figure 1. Results of Fisher's Exact Test indicate that there is no significant difference (p=0.1042) between Puritan Unitranz and cell culture media when it comes to the reporting of clinical diagnostic results as positive or negative.
Positive: CT value<38, Negative: CT value≥38. *CT values labeled undetected did not report a specific CT value. For analysis purposes all undetected results were assigned a value of 45.

	Puritan Unitranz-RT	Cell Culture Media
Detected	29	24
Undetected	1	6

Figure 2. Results of Fisher's Exact Test indicate that there is no significant difference (p=0.1028) between Puritan Unitranz and cell culture media when it comes to the detection of the Influenza A virus by qRT-PCR.

Adenovirus

	Puritan Unitranz-RT	Cell Culture Media
Positive	40	27
Negative	2	15

Figure 3. Results of Fisher's Exact Test indicate that there is a significant difference (p=0.0007) between Puritan Unitranz and cell culture media when it comes to the reporting of clinical diagnostic results as positive or negative.
Positive: CT value<38, Negative: CT value≥38. *CT values labeled undetected did not report a specific CT value. For analysis purposes all undetected results were assigned a value of 45.

	Puritan Unitranz-RT	Cell Culture Media
Detected	41	34
Undetected	1	8

Figure 4. Results of Fisher's Exact Test indicate that there is a significant difference (p=0.0294) between Puritan Unitranz and cell culture media when it comes to the detection of Adenovirus by qRT-PCR.

Rhinovirus

	Puritan Unitranz-RT	Cell Culture Media
Positive	32	29
Negative	10	13

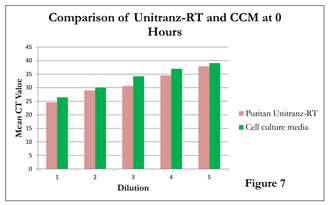
Figure 5. Results of Fisher's Exact Test indicate that there is no significant difference (p=0.6252) between Puritan Unitranz and cell culture media when it comes to the reporting of clinical diagnostic results as positive or negative.
Positive: CT value<38, Negative: CT value≥38. *CT values labeled undetected did not report a specific CT value. For analysis purposes all undetected results were assigned a value of 45.

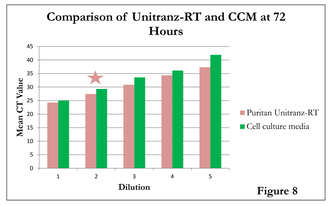
	Puritan Unitranz-RT	Cell Culture Media
Detected	39	31
Undetected	3	11

Figure 6. Results of Fisher's Exact Test indicate that there is a significant difference (p=0.0377) between Puritan Unitranz and cell culture media when it comes to the the detection of the Rhinovirus by qRT-PCR.

Results

Influenza A Virus





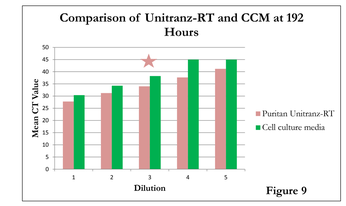
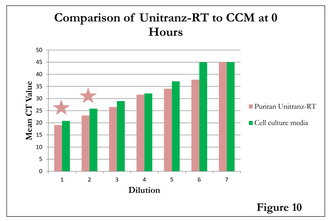
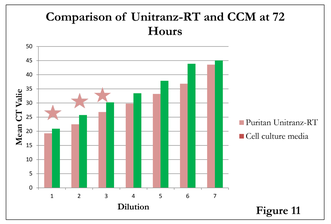


Figure 7. Results of Student T-test indicate that at 0 hours there is no significant difference between the CT values obtained for samples applied to Puritan Unitranz swabs and samples stored in cell culture media.
Figure 8. Results of Student T-test indicate that at 72 hours there is no significant difference between the CT values obtained for samples applied to Puritan Unitranz swabs and samples stored in cell culture media. However, p=0.0378 at the 10^{-2} dilution. Based on conventional criteria, this difference is considered to be statistically significant. Additional testing and increasing the sample size will provide a more accurate determination.
Figure 9. Results of Student T-test indicate that at 192 hours there is no significant difference between the CT values obtained for samples applied to Puritan Unitranz and samples stored in cell culture media. However, p=0.0220 at the 10^{-3} dilution. Based on conventional criteria, this is considered to be statistically significant. Additional testing and increasing the sample size will provide a more accurate determination.

Adenovirus





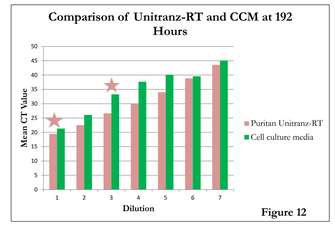
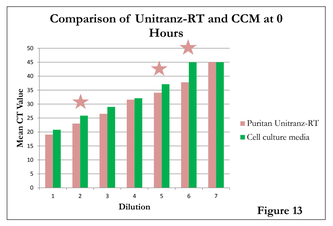
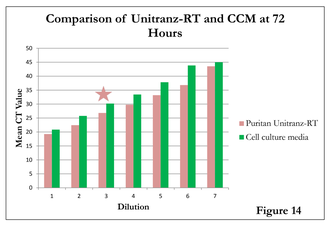


Figure 10. Results of Student T-test indicate than at 0 hours there is no significant difference between the CT values obtained for samples applied to Puritan Unitranz swabs and samples stored in cell culture media. However, p=0.0249 at the 10^{-1} dilution and p= 0.0466 at the 10^{-2} dilution. Based on conventional criteria, this difference is considered to be statistically significant. Additional testing and increasing the sample size will provide a more accurate determination.
Figure 11. Results of Student T-test indicate that at 72 hours there is no significant difference between the CT values obtained for samples applied to Puritan Unitranz and samples stored in cell culture media. However, p=0.0460 at the 10^{-1} dilution, p=0.0371 at the 10^{-2} dilution, and p=0.0251 at the 10^{-3} dilution. Based on conventional criteria, this is considered to be statistically significant. Additional testing and increasing the sample size will provide a more accurate determination.
Figure 12. Results of Student T-test indicate that at 192 hours there is no significant difference between the CT values obtained for samples applied to Puritan Unitranz and samples stored in cell culture media. However, p=0.0196 at the 10^{-1} dilution and p=0.0363 at the 10^{-3} dilution. Based on conventional criteria, this is considered to be statistically significant. Additional testing and increasing the sample size will provide a more accurate determination.

Rhinovirus





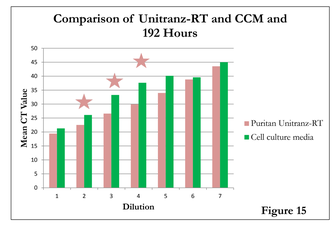


Figure 13. Results of Student T-test indicate than at 0 hours there is no significant difference between the CT values obtained for samples applied to Puritan Unitranz swabs and samples stored in cell culture media. However, p=0.0349 at the 10^{-2} dilution, p= 0.0447 at the 10^{-5} dilution, and p= 0.0331 at the 10^{-6} dilution. Based on conventional criteria, this difference is considered to be statistically significant. Additional testing and increasing the sample size will provide a more accurate determination.
Figure 14. Results of Student T-test indicate that at 72 hours there is no significant difference between the CT values obtained for samples applied to Puritan Unitranz swabs and samples stored in cell culture media. However, p=0.0208 at the 10^{-3} dilution. Based on conventional criteria, this is considered to be statistically significant. Additional testing and increasing the sample size will provide a more accurate determination.
Figure 15. Results of Student T-test indicate that at 192 hours there is no significant difference between the CT values obtained for samples applied to Puritan Unitranz swabs and samples stored in cell culture media. However, p=0.0196 at the 10^{-2} dilution, p=0.0382 at the 10^{-3} dilution, and p=0.0280 at the 10^{-4} dilution. Based on conventional criteria, this is considered to be statistically significant. Additional testing and increasing the sample size will provide a more accurate determination.

Discussion

We found that there was a significant difference between samples stored in Puritan Unitranz-RT and those stored in cell culture media. On average, the samples stored in Puritan Unitranz yielded a lower CT value at each dilution. At lower concentrations, a CT value was able to be detected for viruses stored in Puritan Unitranz more often than those stored in cell culture media. These results indicate that Puritan Unitranz-RT supports the virus in a way that allows it to be detected at lower concentrations over a longer period of time. Out of the three viruses, Puritan Unitranz-RT seems to be most advantageous for storing and transporting Adenovirus for detection by qRT-PCR. There was a significant statistical difference for the number of Adenovirus samples that were detected by qRT-PCR and that were reported as positive when compared to samples stored in cell culture media. Based on the results of this pilot study, Puritan Unitranz-RT is beneficial for the storage and detection of Influenza A Virus, Adenovirus, and Rhinovirus by qRT-PCR. Puritan Unitranz-RT appears to support the viruses for up to 192 hours for detection by qRT-PCR. This study can be further built upon by the inclusion of additional respiratory viruses, more interval time points, and increasing the sample size of each virus.

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