Performance Evaluation of Puritan UniTranz-RT Universal Transport System for the Detection of Influenza A Virus

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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 by qRT-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-RT has on viral real-time 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)) were prepared . The titer of the virus was$1X10^5$ $TCID_{50}$. Samples were applied to swabs, extracted, and then evaluated using the ABI 7500 Real-Time PCR system.

Results

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 RT-PCR for the detection of additional influenza and respiratory viruses.

Methods

Dilutions

- Seven ten-fold dilutions(10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7}) of stock Influenza A were prepared.

Spikes

- 100µl from each dilution was applied directly to the Puritan UniTranz-RT swabs in duplicate
- 100µl from each dilution was pipetted directly into blank cell culture media in duplicate

Extraction

- Swabs were placed in the Puritan UniTranz-RT viral transport media and vortexed for one minute
- 200µl of each sample was removed and placed in MagNA Pure Total Nucleic Acid Isolation lysis buffer
- Extraction was performed using the MagNA Pure Total Nucleic Acid Isolation Kit on the MagNA Pure LC instrument following manufacturer's recommendations.

qRT-PCR

- RT-PCR was performed using Invitrogen Superscript III Platinum One-Step qRT-PCR Kit

Results

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

Figure 1. Results of Fisher's Exact Test indicate that there is no significant difference (p=0.1028) between Puritan UniTranz-RT and cell culture media when it comes to the detection of the Influenza A virus by qRT-PCR.

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

Figure 2. Results of Fisher's Exact Test indicate that there is no significant difference (p=0.1042) between Puritan UniTranz-RT 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.

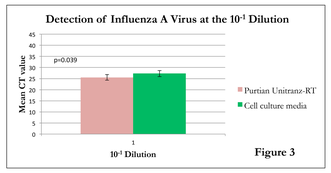
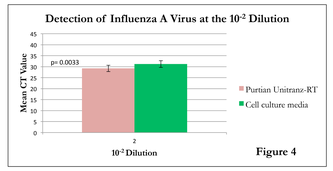


Figure 3



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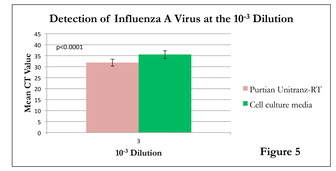

Figure 5

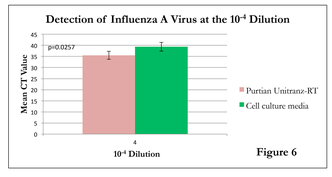

Figure 6

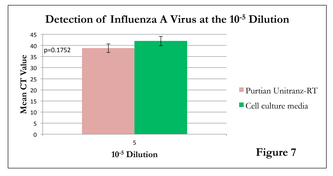

Figure 7

Figure 3-6. Results of Student T-test indicate that at each dilution, there is a significant statistical difference (P<0.05) between the CT values obtained from samples stored in Puritan UniTranz-RT and samples stored in cell culture media. Each dilution averages the CT value obtained at the 0, 72, and 192 hour time points. These results indicate that on average samples stored in Puritan UniTranz-RT typically yielded a lower CT value at each dilution.
Figure 7. Results of Student T-test indicate that for detection at 10^{-5} dilution there is no significant difference between the CT values obtained for samples stored in Puritan UniTranz-RT and samples stored in cell culture media.

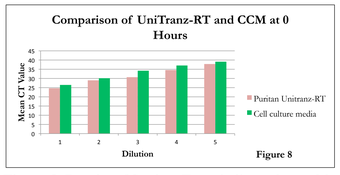

Figure 8

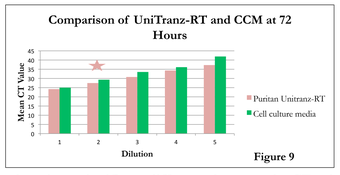

Figure 9

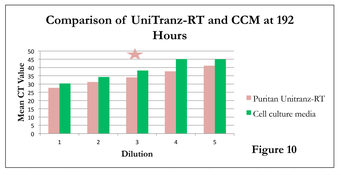

Figure 10

Figure 8. 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-RT swabs and samples stored in cell culture media.
Figure 9. 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-RT 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 10. 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-RT 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.

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-RT 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-RT 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. Based on the results of this pilot study, Puritan UniTranz-RT is beneficial for the storage and detection of Influenza A Virus by qRT-PCR. Puritan UniTranz-RT appears to support the virus 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 the virus.

Acknowledgements

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