Evaluation of a New Viral Transport System for Culture Recovery of Influenza Viruses

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Abstract

There are many transport systems available for the collection and stabilization of respiratory virus specimens.1 These products are designed for maximum capture of specimen, stabilization during transport, and growth in culture.2 This project evaluated the performance of a new flocked swab and viral transport medium system, the Puritan Medical Products (*also branded as TransPRO™ CVM*) Universal Transport Medium (UTM-RT), for the recovery of influenza viruses in culture (Figure 1). The Puritan system was spiked with known concentrations of influenza A and B stock viruses and cultures were inoculated at time points 0, 24, and 48 hours post-spiking. Viral growth was determined by fluorescent antibody staining of the cultures after 48 hours and counting the fluorescing foci (Figure 2). The Puritan (*also branded as TransPRO™ CVM*) flocked swab and UTM-RT system was found to support the stabilization and viability of influenza virus for recovery in culture.

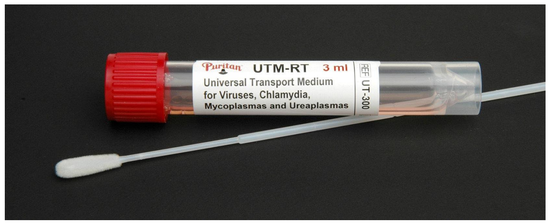

Figure 1. The Puritan Medical Products (also branded as TransPRO™ CVM) specimen collection and viral transport system.

Methodology

Puritan Medical Products (PMP) (*also branded as TransPRO™ CVM*) flocked swabs were inoculated with 330uL of influenza A virus and influenza B virus at previously established $TCID_{50}$ concentration. Inoculated swabs were incubated at room temperature for five minutes and then placed into UTM-RT and stored at 4-8 ºC (9 samples per virus). At 0, 24, and 48 hour time points, three swab/UTM-RT specimens for each virus were vortexed for two minutes and R-Mix shell vials were overlayed with 100uL of specimen. Cultures were incubated at 35 ºC for 48 hours and then stained with fluorescently-labeled monoclonal antibodies. Fluorescing foci in each culture were counted in three fields at 200x and 400x magnification by two technicians, for a total of six fields per culture. Density histograms and plots were used to examine frequency distributions and patterns over time. The recovery of virus from a very dilute specimen was also examined using a 1:1000 dilution of influenza A $TCID_{50}$ virus stock.

Acknowledgement: TransPRO™ is manufactured by Puritan Medical Products Company for Hardy Diagnostics.

Results

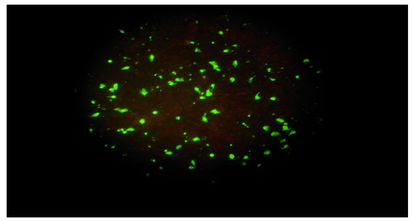

Figure 2. Influenza B virus-infected cells following 48 hour hold in PMP UTM-RT, (also branded as TransPRO™ CVM) 400x magnification.

All shell vials exhibited viral growth (Table 1) with no marked difference in viral recovery from the UTM-RT tubes held at 0, 24, or 48 hours before inoculation. Density plots at both 200x and 400x magnification were similarly distributed for all hold periods (Figure 3). Shell vials inoculated with a 1:1000 dilution of influenza A virus also demonstrated support of virus viability with similar growth across the three hold periods (Table 1).

	Average number of fluorescing foci per high power field						Average number of fluorescing foci per shell vial coverslip		
	$TCID_{50}$ Influenza A			**$TCID_{50}$ Influenza B**			**1:1000 $TCID_{50}$ Influenza A**		
	0hr	**24hr**	**48hr**	**0hr**	**24hr**	**48hr**	**0hr**	**24hr**	**48hr**
200x	93	80	74	52	65	61	3	1	2
400x	32	26	25	21	30	29			

Table 1. Average number of fluorescing foci per high power field for influenza A and B viruses at 200x and 400x after storage in Puritan Medical Products UTM-RT (also branded as TransPRO™ CVM) for 0, 24, and 48 hours.

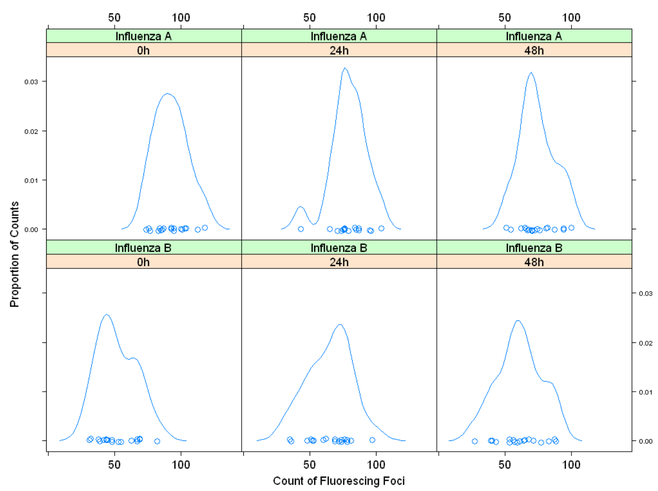

Figure 3. Density plots of influenza A and influenza B virus cultures at 200x magnification.

Conclusions

The Puritan Medical Products (*also branded as TransPRO™ CVM*) flocked swab and UTM-RT system supports the collection, stabilization, and robust recovery of influenza viruses at high concentrations, while also maintaining viral viability at low concentrations. No significant decrease in viral recovery was seen over 48 hours storage time at 2-8 ºC with either high or low concentrations of virus. Variation seen in virus recovery in this study is likely due to typical variation in microbe biological activity and is not significant for clinical purposes.

Limitations

This study can be further built upon by examining how specimen hold times of greater than 48 hours prior to shell vial inoculation affects viral recovery. In this study, the technicians each chose three fields at random when counting positive cells. Although hypothetically every cell in the monolayer had the same chance of infection, this cannot be proven in this study. Potential misclassification bias could be addressed in future studies by controlling for technician bias in microscope field selection.

Acknowledgments

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References

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2. Dunn, J.J., Billetdeaux, E., Skodack-Jones, L., & Carroll, K.C. (2003). Evaluation of three Copan viral transport systems for the recovery of cultivatable, clinical virus isolates. *Virology, 45*, 191-197.