Performance Evaluation of a Opti-Swab® Liquid Amies Based Medium in Quantitative Detection of Viral and Bacterial Pathogens Using Species Specific Q-PCR

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Introduction

Emerging PCR-based techniques enable the detection of pathogens with increased speed and specificity. Essential to the implementation of these quantitative PCR-based technologies is the reliable collection and storage of clinical samples in transport media that protects nucleic acids for later analyses. The goal of this study was to assess the ability of currently available transport media to protect clinical samples for PCR based detection of pathogenic viral RNA or bacterial DNA following storage for up to 7 days. In this study, we used species-specific real-time quantitative PCR assays to measure concentrations of the Influenza A virus and the bacterial pathogens, *Haemophilus influenzae* and *Neisseria gonorrheae*.

Puritan Medical Products' Opti-Swab© is a liquid amies based medium intended for the collection and transport of clinical samples prior to laboratory testing. This study assessed the use of real-time quantitative polymerase chain reaction (qPCR) in detection of pathogenic bacterial and viral DNA and RNA following storage in Opti-Swab© medium. We hypothesized that there would be no significant change in qPCR detection of genomic material from 24 hours to 1 week at either room temperature (RT, 20 °C) or 4°C, supporting the utility of this medium in storing and transporting samples for later pathogenic identification by qPCR assay.

Methods

- *Haemophilus influenzae* strain ATCC 10211 and *Neisseria gonorrhoeae* ATCC 43069 were incubated for 24 hours on Haemophilus Test Medium agar and GC II Agar with IsoVitaleX, respectively. Resultant cultures were used to prepare suspensions of 1.5 x 10^8 CFU/mL in 0.9% sterile saline. Serial ten-fold dilutions of each suspension were made from 1.5×10^7 to 1.5×10^4 CFU/mL.
- Influenza A virus ATCC VR-1469 was propagated on monolayers of Madin-Darby Canine Kidney Cells (MDCK). Resulting lysates were then clarified by low-speed centrifugation (5000xg/20min/4°C), followed by filtration (0.45µm), pelleted by ultracentrifugation (100,000xg/1hr/4°C) and resuspended in 3 mL Eagle's Minimum Essential Medium (EMEM) without fetal calf serum. Virus stocks were enumerated using the Reed-Muench method and reported as 50% tissue culture infectious dose per milliliter ($TCID_{50}$/mL). Serial ten-fold dilutions of virus stocks were made ranging from 1.5×10^7 to 1.5×10^3 $TCID_{50}$/mL.
- Aliquots (100µL) of each viral and bacterial dilution were transferred into a 96-well microtiter plate and sterile swabs were immersed in the corresponding wells and allowed to absorb for 15-20 seconds. Following absorption, the swabs were placed in vials containing Puritan Opti-Swab© medium and were held in the medium for 24, 48, and 168 hours at either RT or 4°C.
- DNA/RNA isolation was performed using Promega or Invitrogen purification kits as per the manufacturer's protocol.
- Q-PCR was used to quantify *H. influenzae* and *N. gonorrhoeae* (Qiagen Microbial DNA qPCR Assay), and Influenza A (Qiagen virotype Influenza A qRT-PCR assay) concentrations using a BioRad CFX96™ PCR Detection System. Copy numbers of pathogenic targets were quantified based on threshold cycle (Ct, the cycle at which the baseline-corrected normalized fluorescence is significantly above background signal).
- Mean differences in relative copy number were compared to initial concentrations to assess degradation with storage time and temperature using multivariate ANOVA.

Results

Yields of DNA and RNA from all samples were sufficient for multiple PCR assays, even at the lowest concentrations. For both bacterial and viral samples, qPCR detected a linear positive relationship between the initial sample concentration and quantified relative copy numbers across all concentrations. Neither storage time (24 hours to 1 week, p=0.98) nor storage temperature (4°C or RT, p=0.93) had a significant effect on the ability to detect and quantify pathogen concentrations.

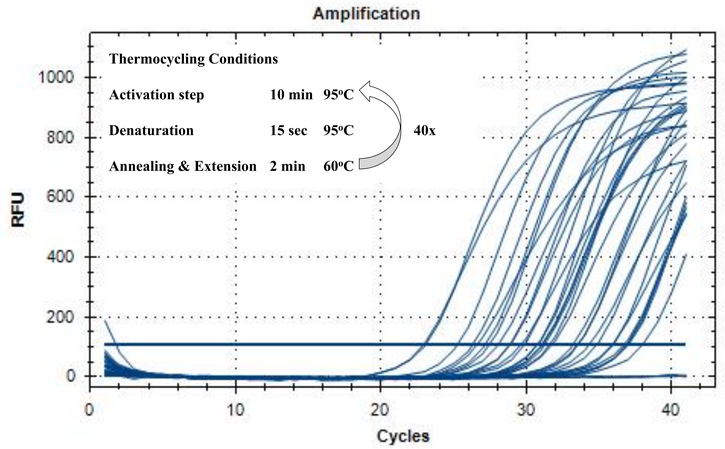

Figure 1. Quantitative PCR detection of *N. gonorrhoeae*. Fluorescence readings were measured by BioRad CFX96™ PCR Detection System. The threshold for determining Cq value for each sample is indicated by the horizontal line. (RFU= relative fluorescence unit)

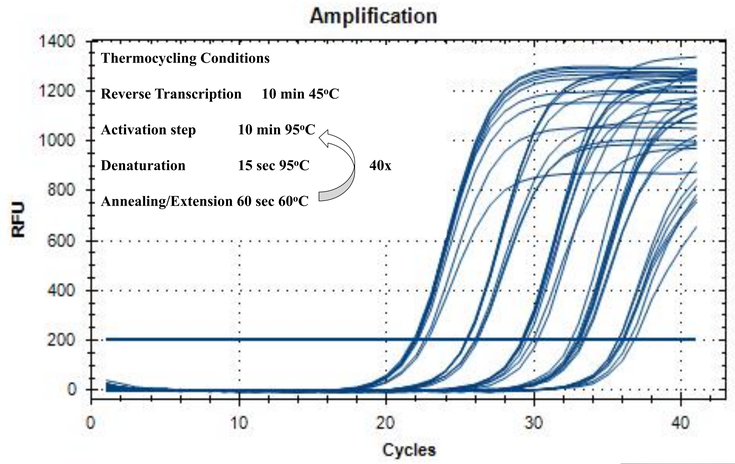

Figure 2. Quantitative RT-PCR detection of Influenza A virus RNA. Fluorescence readings were measured by BioRad CFX96™ PCR Detection System. The threshold for determining Cq value for each sample is indicated by the horizontal line. (RFU= relative fluorescence unit). Curves show samples for dilutions ranging from 1.5×10^7 to 1.5×10^3 $TCID_{50}$/mL.

Results (continued)

Organism	Effect	SS	Degr. of	MS	F	p
Influenza A	Time	0.75	2	0.37	1.7	0.209
	Temp.	0.03	1	0.03	0.1	0.705
Haemophilus influenza	Time	16.98	2	5.66	2.58	0.071
	Temp.	8.01	1	8.01	3.29	0.077
Neisseria gonorrhoeae	Time	0.82	2	0.41	0.11	0.898
	Temp.	2.10	1	2.10	0.55	0.464

Table 1. ANOVA Analysis of qPCR Fluorescence Data Across Time and Temperature Conditions

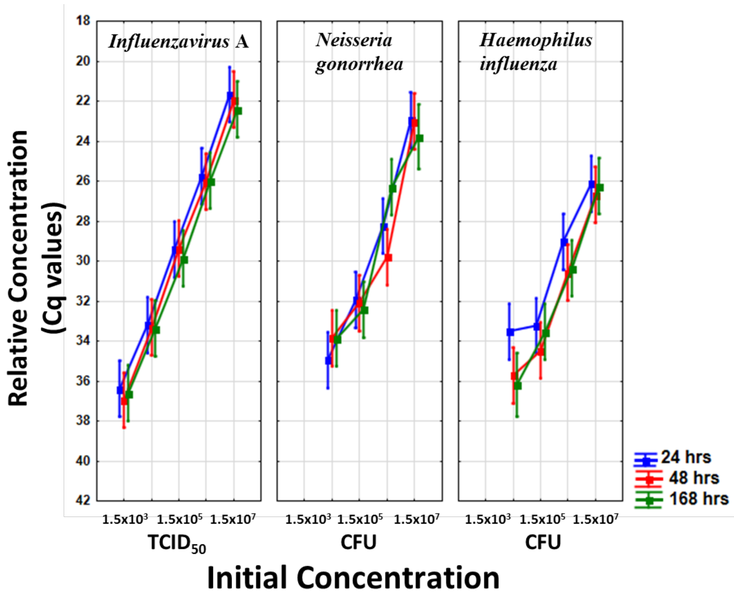

Figure 3. Quantitative PCR Results Expressed According to Relative Concentration. Quantitative RT-PCR detected a linear positive relationship between the initial sample concentration and detected relative copy number for all incubation periods. High Cq value denotes low initial copy number of DNA/RNA molecules.

Conclusions

Storage in Puritan Opti-Swab© medium does not have a negative effect on the ability to detect and accurately quantify samples, whether bacterial pathogens, *H. influenzae* and *N. gonorrhoeae,* or the viral pathogen, Influenza A. This data further supports the efficacy of this medium in collection and transport of nucleic acids from clinical specimens prior to qPCR assays.

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