Performance Evaluation of Puritan© Opti-Swab® Medium in Detection of *Bordetella pertussis* Using Real Time Q-PCR.

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Abstract

Puritan Medical Products' Opti-Swab is a liquid Amies based medium intended for the collection and transport of clinical samples from the collection site to the laboratory for testing. The goal of this study was to assess the ability to detect pathogenic bacterial DNA following storage of bacterial cells in Opti-Swab medium. In this study we used a specific real-time quantitative PCR assay to measure relative concentrations of *Bordetella pertussis* stored for 24 and 48 hours at room temperature and 4ºC. *B. pertussis* strain ATCC 9797 was studied. ATCC 9797 was incubated for 72 hours on Regan-Lowe charcoal agar, and the resulting culture was used to prepare a suspension of 1.5 x 10^8 CFU/mL in 0.85% sterile saline. A series of five ten-fold dilutions of this suspension were made, resulting in final concentrations of 1.5×10^7, 1.5×10^6, 1.5×10^5, 1.5×10^4, and 1.5×10^3 CFU/mL, respectively. 100 µL aliquots of each dilution were then transferred into the wells of a 96-well microtiter plate. Sterile swabs were then immersed in the corresponding wells and allowed to absorb for approximately 15-20 seconds. Following absorption, the swabs were removed from the wells and immediately placed in vials containing Puritan Opti-Swab medium. The swabs were held in the transport medium for 0, 24, and 48 hours at both room temperature and 4ºC. DNA isolation was performed using the Promega Wizard Genomic DNA Purification kit as per the manufacturer's protocol. Quantitative real-time PCR (Qiagen Microbial DNA qPCR assay) was used to quantify *B. pertussis* concentrations as per the manufacturer's protocol using a BioRad CFX96™ PCR Detection System. Yields of DNA (mean 283 ng, N=43) were more than sufficient for multiple QPCR assays, even at the lowest concentration. QPCR detected a linear positive relationship between the initial sample concentration and detected relative copy number regardless of storage temperature or storage time. Neither storage temperature (4ºC vs room temperature) nor storage time (0, 24, or 48 hours) had a significant effect on the ability to detect cells (time: P=0.63; temperature: P=0.88). These results indicate that Puritan Opti-Swab medium does not have a negative effect on the ability to detect and, perhaps more importantly, quantify *B. pertussis.*

Methods

- *B. pertussis* bacterial suspension was prepared from fresh culture (72 hours). Concentration verified by 0.5 McFarland Standard to obtain 1.5 x 10^8CFU/mL suspensions.
- Five ten-fold dilutions were made resulting in final concentrations of 1.5×10^7, 1.5×10^6, 1.5×10^5, 1.5×10^4, and 1.5×10^3 CFU/ml.
- 100 µl aliquots of the dilutions were then transferred into the wells of a 96-well microtiter plate.
- Swabs included with Opti-Swab transport system were immersed in the corresponding wells and allowed to absorb for approximately 15-20 seconds and immediately placed in vials containing Puritan Opti-Swab medium.
- The swabs were held in the transport medium for 0, 24, and 48 h at RT and 4ºC.
- For each sample 100 µl was removed for PCR assay. DNA isolation was performed using the Promega Wizard Genomic DNA Purification kit.
- PCR (Qiagen Microbial DNA qPCR assay) was used to quantify *B. pertussis* concentrations as per the manufacturer's protocol using a BioRad CFX96™ PCR Detection System.
- Relative concentrations were calculated based upon Cq values for time 0 samples

Results

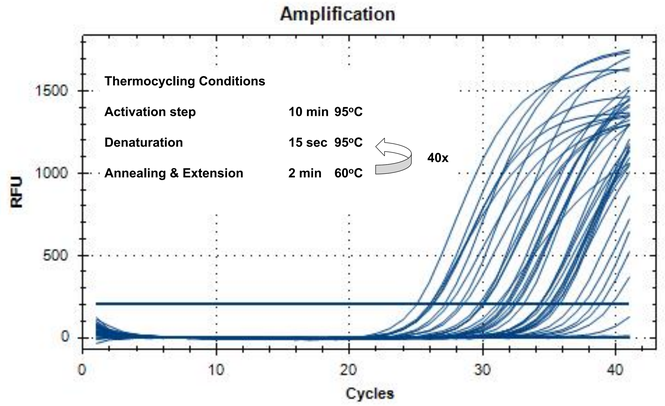

Figure 1. RT-PCR fluorescence data processing. Fluorescence readings were measured by BioRad CFX96™ PCR Detection System. The threshold for determining Cq value for each sample is indicated by the horizontal line. (RFU= relative fluorescence unit)

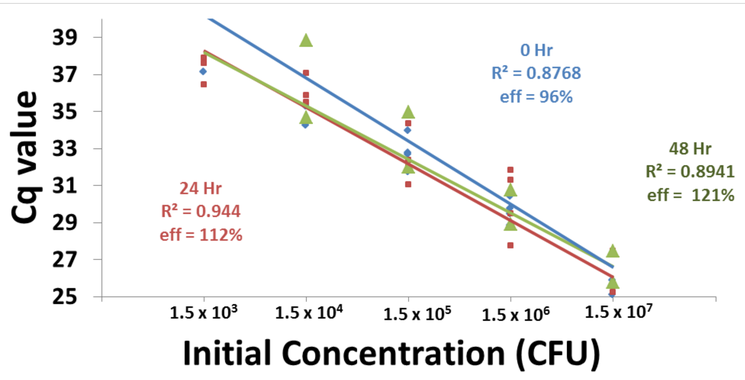

Figure 2. High Cq value denotes low initial copy number of DNA molecule. Quantitative RT-PCR detected a linear positive relationship between the initial sample concentration and detected relative copy number of *B. pertussis* for all incubation periods.

Sample Conc. CFU/ml	Storage Temp.	0 hr		24 hr		48 hr	
		Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
1.5 x 10^3	RT					0.8	0.0
	4º C			1.1	0.0	1.2	0.8
1.5 x 10^4	RT	3.2	4.0	8.0	0.0	2.7	0.0
	4º C			3.9	0.1	2.3	1.6
1.5 x 10^5	RT	21.8	23.9	23.1	1.1	52.6	31.5
	4º C			27.9	25.4	23.2	22.2
1.5 x 10^6	RT	210.8	170.0	228.6	159.9	393.7	494.6
	4º C			204.1	29.0	143.4	113.2
1.5 x 10^7	RT	1,920.2	1,450.9	6,903.2	0.0	4,191.9	0.0
	4º C			3,617.1	1,342.3	3,503.0	3,670.4

Table 1. Relative Concentration of DNA (x10^3) detected in Puritan Opti-Swab medium given different storage temperature (4ºC and room temperature), and storage time (0hr, 24hr, and 48 hr).

Results (continued)

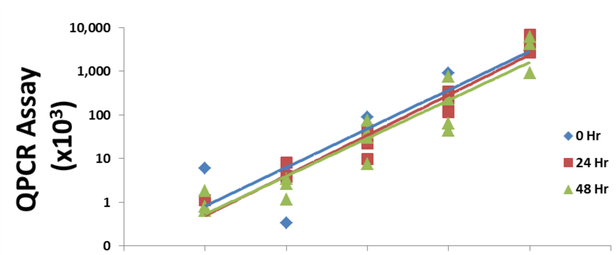

Figure 3. Mean relative concentrations versus initial sampled concentrations at 0, 24 and 48 hours. There were no significant differences in assayed concentrations with time.

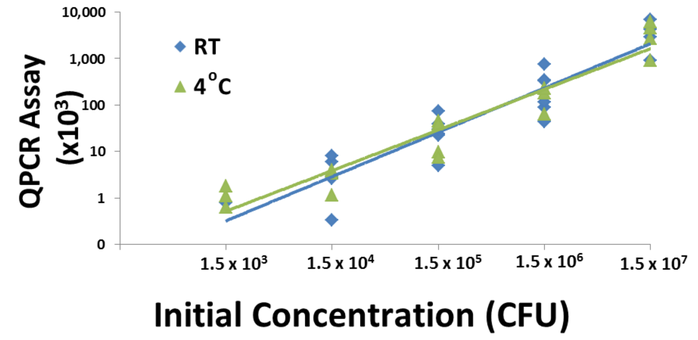

Figure 4. Mean relative concentrations versus initial sampled concentrations for samples stored at room temperature (RT) and 4ºC. There were no significant differences in assayed concentrations with storage temperature.

Conclusions

- Puritan Opti-Swab medium is a viable transport media for the storage and transport of *B. pertussis* samples allowing for the detection and quantitation of DNA suitable for genetic analysis.
- Yields of DNA from only 100 µl of media was sufficient for multiple QPCR assays, even at the lowest concentration.
- QPCR assays detected a linear positive relationship between the initial sample concentration and relative copy number.
- Storage temperature (4ºC or room temperature) did not have a significant effect on viability of genetic material (P=0.88).
- Storage time (0, 24, or 48 hours) did not have a significant effect on viability of genetic material (P=0.63).

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