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Comparative Evaluation of Puritan® and Copan Sputum Solution for Recovery of Respiratory Pathogens Using Automated Plating

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Revised Abstract

Background: Liquefaction and thorough mixing of sputum specimens ensures a representative sample for culture. Buffered phosphate solutions of dithiothreitol (DTT) are often used for this purpose. Tubes with DTT that are adaptable to automated plating systems are now available from Puritan Medical Products (P) and Copan Diagnostics (C).

Methods: P (1.0 mL/tube) and C (0.5 mL/tube) sputum solutions (SS) were inoculated with 100 uL of approx. 10^7 cfu/mL of ATCC and clinical strains of *H. influenza* (HI), *M. catarrhalis* (MC), *S. pneumoniae* (SPN), *S. pyogenes* (SPY), *P. aeruginosa* (PA), *S. aureus* (SA), *C. albicans* (CA), *K. pneumoniae* (KP), *B. cepacia* (BC), *S. maltophilia* (SM), *C. neoformans* (CN) and *M. abscessus* (MA). Tubes were plated using a BD Kiestra InoqulA delivering 10 uL of sample onto SBA or CHOC with zigzag streaking. Tubes were plated at 0, 2, 4, and 6 h. Additional studies were performed with ATCC HI plated at 0, 30, 60, 90 and 120 min. Changes in colony counts of 2 $\log_{10}$ or more as compared to time 0 h were considered significant. Sputum samples were also tested in SS. Using a transfer pipet, 100 uL of sputum was added to P and C SS and vortexed. Tubes were monitored for liquefaction every 15 minutes. Once liquefied, samples were plated onto SBA, CHOC and MAC using 30 uL with 4 quadrant streaking. Culture growth was graded 1+, 2+, 3+ and 4+ and compared to non-liquefied, manually plated cultures. Differences in growth of 2 grades or more were considered significant.

Results: Viability of PA, SA, CA, KP, BC, SM, CN and MA did not significantly change after exposure to P and C SS for up to 6 h. MC, SPN and SPY colony counts remained stable in P SS but 90%, 67% and 25% of strains demonstrated significant changes in colony count after 6 h in C SS, respectively. For some of these strains significant changes occurred as early as 2 hrs. None of the HI strains tested were viable after 6 hrs exposure to C SS. Significant decreases started at 2 hours for some strains of HI and with HI ATCC 10211, significant colony count reductions occurred at 1 hr. For sputum samples, the time required for adequate liquefaction ranged from 15 – 45 minutes. C SS treated samples remained more viscous than with P SS after the same exposure time with fibrin clot equipment errors. P and C SS treated samples yielded equivalent results as compared to non-treated manually plated samples and compared to each other. C and P SS cultures often yielded more confluent growth as compared to manually plated specimens.

Conclusions: P and C SS tubes hold potential for providing an efficient mechanism for liquefaction and automated plating of sputum samples. Care must be exercised with the length of time that samples are exposed to SS especially when using C SS. Sputum samples treated with P and C SS were comparable to each other and to non-liquefied manually plated samples. The lower volume of SS in C may cause mechanical issues with some automated plating systems due to higher viscosity as compared to P SS.

Introduction

Sputum samples are one of the most challenging specimen types for the clinical laboratory because of their viscosity and the heterogeneous distribution of bacteria. Liquefaction and thorough mixing of sputum specimens ensures a more representative sample for culture. A liquefied sample also allows for the use of automated specimen processing platforms. Buffered phosphate solutions of dithiothreitol (DTT) are often used for this purpose. Tubes with DTT that are adaptable to automated plating systems are now available: Puritan® Sputum Solution and Copan SLsolution. In order to determine the feasibility of using such a product on our automated system (BD Kiestra™ InoqulA™), a comparative evaluation of Puritan (P) and Copan (C) sputum solutions (SS) was performed. We present our findings from stability studies of commonly encountered respiratory pathogens and culture results of sputum samples submitted to the laboratory for bacterial culture.

Methods

Bacterial strains: Based on manufacturer's quality control procedures, the following ATCC strains were selected for stability testing: *H. influenzae* (HI) ATCC 10211, *M. catarrhalis* (MC) ATCC 25238, *S. pneumoniae* (SPN) ATCC 6305, *S. pyogenes* (SPY) ATCC 19615, *P. aeruginosa* (PA) ATCC 27853, *S. aureus* (SA) ATCC 6538, and *C. albicans* (CA) ATCC 10231. In addition, clinical isolates of HI, MC, SPN, SPY, PA (including mucoid strains), *K. pneumoniae* (KP), *S. maltophilia* (SM), *B. cepacia* (BC), *C. neoformans* (CN) and *M. abscessus* (MA) were tested.

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Methods continued

Stability study: Freshly grown isolate suspensions were prepared in sterile demineralized water to a turbidity equivalent to approximately a 0.5 McFarland standard. A tenfold dilution was made of each isolate suspension. P SS (1.0 mL) and C SS (0.5 mL) tubes were inoculated with 100 uL of the diluted suspension of each test organism. Tubes were vortexed, maintained at room temperature and subcultured at time 0, 2, 4 and 6 hrs. Subculturing was performed utilizing the fully automated mode on the BD Kiestra InoqulA delivering 10 uL onto appropriate plated media using a zigzag streaking pattern. In an additional study, sputum solutions containing a diluted suspension of HI ATCC 10211 were subcultured at 0, 30, 60, 90 and 120 mins. All subculture plates were incubated at 35-37°C in 5% CO_2 for 48 hours (5 days for MA). Following incubation, colonies were counted to the nearest $\log_{10}$. Colony counts at 0 hr were compared to counts at subsequent time periods. A decrease in colony count of ≥ 2 $\log_{10}$ was considered significant.

Clinical specimen study: The residuals of sputum samples sent to the laboratory for routine bacterial culture were tested in the two sputum solutions. A transfer pipet was used to inoculate 100 uL of a representative portion of sputum into each brand of sputum solution. Inoculated tubes were vortexed and maintained at room temperature for at least 15 minutes as per recommendation of both manufacturers. After 15 minutes, tubes were assessed for liquefaction. If not completely liquefied, the tubes were vortexed and reassessed every 15 minutes. Once deemed liquefied, the solutions were subcultured to trypticase soy agar with 5% sheep blood, chocolate agar and MacConkey agar with the BD Kiestra InoqulA fully automated mode delivering 30uL to each plate and streaking in a four quadrant pattern. Samples yielding clot detection errors were re-run in fully automated mode. If fully automated plating was unsuccessful on the second attempt, the liquefied samples were plated using the semi-automated mode with manual inoculation of 30 uL onto each plate followed by automated four quadrant streaking. Plated media were incubated at 35-37°C in 5% CO_2 for a total of 48 hours. After incubation, culture growth was graded as 1+, 2+, 3+ or 4+ and compared to non-liquefied manually plated culture plates. In addition, culture growth from P and C SS were compared to each other. Decreases of 2 grades or more were considered significant.

Table 1: Organism stability after 6 hours in sputum solution[a]

	Puritan	Copan
	Number with stable colony count (%)	Number with stable colony count (%)
***Haemophilus influenzae* (10)**	10 (100)	0 (0)
***Moraxella catarrhalis* (10)**	10 (100)	1 (10)
***Streptococcus pneumoniae* (6)**	6 (100)	2 (33)
***Streptococcus pyogenes* (4)**	4 (100)	3 (75)
***Klebsiella pneumoniae* (4)**	4 (100)	4 (100)
***Pseudomonas aeruginosa* (5)**	5 (100)	5 (100)
***Stenotrophomonas maltophilia* (4)**	4 (100)	4 (100)
***Burkholderia cepacia* (3)**	3 (100)	3 (100)
***Staphylococcus aureus* (1)**	1 (100)	1 (100)
***Candida albicans* (1)**	1 (100)	1 (100)
***Cryptococcus neoformans* (1)**	1 (100)	1 (100)
***Mycobacterium abscessus* (1)**	1 (100)	1 (100)

[a] Stable colony count defined as no decrease or less than 2 log10 colony count decrease at 6 hours as compared to time 0 hour.

Figure 1 Figure 2 Figure 3

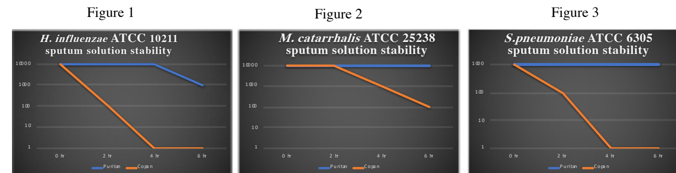
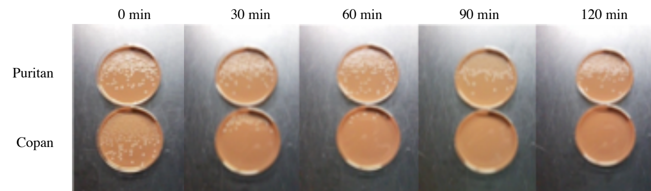


Figure 4 *H. Influenzae* ATCC 10211 stability in sputum solution



Results

Colony counts of all PA, SA, CA, KP, SM, BC, CN and MA remained stable after exposure to both P and C for up to 6 hrs (Table1). For all MC, SPN and SPY, colony counts remained stable in P, but only 10%, 33% and 75% of these strains were stable after 6 hrs in the Copan SS, respectively. For some MC, SPN and SPY strains, significant changes in colony count occurred as early as 2 hrs in C SS. None of the HI strains tested were viable after 6 hrs exposure to C SS, however, all HI strains were stable in P SS. HI, MC and SPN ATCC strain stabilities are depicted in Figures 1, 2 and 3). Significant decreases started at 2 hrs for some strains of HI in C SS and in an additional study with HI ATCC 10211, significant colony count reductions occurred at 1 hr in C SS (Figure 4).

For sputum samples, the time required for what was considered adequate liquefaction (visual elimination or reduction of clumps or strands of mucus) ranged from 15 – 45 minutes. Even after liquefaction, C SS treated samples remained more viscous than P SS samples after the same exposure time. The increased viscosity resulted in more fibrin clot detection errors in the fully automated testing mode. Twenty seven percent of C SS samples required semi-automated plating compared to 20% of P SS samples. P and C SS treated samples yielded cultures that were not significantly different from non-liquefied, manually plated samples as related to species recovered and graded growth although C and P SS cultures often yielded more confluent growth as compared to manually plated specimens (Figure 5). When P SS cultures were compared to C SS cultures there were no significant differences.

Conclusions

P and C SS tubes hold potential for providing an efficient mechanism for liquefaction and automated plating of sputum samples. Automated plating allows for standardized specimen inoculation on each plate and consistent streaking for isolation. Although the majority of specimens processed in the laboratory will require only the minimum 15 mins for liquefaction, some samples (e.g. cystic fibrosis samples) may potentially be exposed to SS for longer periods of time. Longer SS exposure times may also occur in busy laboratories where sputum sample processing may be prioritized behind more critical samples. Based on our studies, delays of up to 6 hours did not affect the viability of commonly isolated respiratory pathogens when in the presence of P SS. However, prolonged exposure could adversely impact culture outcomes for some pathogens if using C SS.

The final culture results of sputum samples treated with P and C SS were comparable to each other and to non-liquefied manually plated samples. Because C tubes contain only 0.5 mL of SS as compared to 1.0 mL in the P SS tubes, C SS cultures often yielded more confluent growth with fewer isolated colonies than P SS cultures. The lower volume of SS in C tubes also resulted in a more viscous end product as compared to P SS. This higher viscosity may cause mechanical issues with some automated plating systems. Both manufacturers suggest that best results are obtained with a sample to SS volumetric ratio of 1:1. In this study we determined that a 100 uL volume of sputum sample placed into SS using a transfer pipet provided a sample with a viscosity low enough to allow for use by an automated plating system. In addition, a SS sample aliquot of 30 uL onto each required culture media plate yielded cultures comparable to non-liquefied manually plated samples as related to species and graded growth. As with any new technique, each laboratory adopting regular use of a sputum solution will need to validate the use of manual or automated methods, the amount of specimen placed into the SS, the appropriate length of time for liquefaction, and the volume of liquefied specimen delivered onto culture media.

Figure 5. Sputum culture with *Moraxella catarrhalis*