Evaluation of Three Flocked Swabs and Two Liquid Amies Transport Systems for the Recovery of Fastidious Bacteria

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\textbf{Abstract}

Objective: To compare the performance of Puritan’s liquid Amies transport medium with HydraFlock\textsuperscript{®} swab, (P) (Puritan Diagnostics LLC), with Copan’s ESwab\textsuperscript{™} transport system containing a treated flocked swab and ESwab\textsuperscript{™} transport medium with Copan’s standard FLOQSwab (C) (Copan Diagnostics Inc.). Method: Viability tests were performed using a modified Swab-Elution Method (CLSI M40-A) on swabs incubated at room temperature for 0, 24 and 48h prior to processing. All test plates were incubated in triplicate for more accurate average colony count results. Eleven strains were tested, including 8 Gram-negative (NG), N. meningitidis (ATCC 13077), (NNi), H. influenzae (ATCC 49247, ATCC 10121 clin #1, clin #2 and clin #3), (HIN), S. pneumoniae (ATCC 49619) (SPN) Bordetella bronchiseptica clin and Pasteurella multocida clin isolates. Nine swabs of each brand were inoculated with 100\(\mu\)l of \(\leq 1.0\times 10^9\) CFU/ml organism suspension and then placing them in their respective devices and kept for 0, 24 and 48h at room temperature. Subsequently, 100\(\mu\)l from each of these swabs or swabs from each of the three swab types was serially diluted 10-fold in 0.9ml sterile saline and four dilutions prepared. 100\(\mu\)l of each dilution was pipetted into chocolate agar or blood agar plates and incubated under optimal conditions for subsequent colony counts. After 48hrs incubation, countable colonies for each swab were inoculated at each time point was recorded. For each of the swabs used, two triplicate sets of swabs and dilution for each incubation period. Product performance was compared for each swab transport with the zero time counts at the dilution that produced 300 - 500 colonies.

\textbf{Results}

Average colony forming units (CFU)

\textbf{Conclusion}

1. Only the Puritan’s HydraFlock\textsuperscript{®} swabs completely absorbed the 100 \(\mu\)l inoculum.
2. Release/recovery function of all systems appears to be comparable at zero hour incubation but the recovery differed at 24h and 48h incubation.
3. In general based on this study, Puritan’s HydraFlock and the Copan’s ESwab systems are comparable in their ability to recovery fastidious bacteria after 24h and 48h at room temperature incubation.
4. Both Bordetella bronchiseptica and Pasteurella multocida increased logorhythmically at 24h and 48h from the zero hr counts on the E-Swab system. This may be a function of the E-Swabs being treated.
5. These results point to the fact that treating the swabs may not be beneficial for the recovery of all organisms.
6. The zero hr colony counts were lower for one of the five HIN (HIN ATCC 49247), as a result all systems failed to recover at 48h incubation.
7. Further studies will be performed and data presented using more fastidious organisms.

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\textbf{Method}

Protocol Tested (CLSI Swab Elution Quantitative Method): The protocol used for viability studies was modified based upon the Swab Elution Method as described in the CLSI standard Quality Control of Microbiology Transport System. M40-A Vol 23 No. 34, 2003.

Fastidious Bacteria tested:
- Neisseria gonorrhoeae ATCC 33609 (NG)
- Neisseria meningitidis ATCC 19424 (NNi)
- Haemophilus influenzae ATCC 49247 (HIN)
- Haemophilus influenzae ATCC 10211 (HIN)
- Streptococcus pneumoniae ATCC49619 (SPN)
- Bordetella bronchiseptica (Clin)
- Pasteurella multocida (Clin)

Transport swab system:
- HydraFlock\textsuperscript{®} Puritan Medical Products Company LLC (P)
- Modified Liquid Amies Medium. Puritan Medical Products Company LLC ESwab\textsuperscript{™} Transport System (Copan Diagnostics Inc.) (CT)
- FLOQSwab\textsuperscript{™} (Copan Diagnostics Inc.) (CB)

Preparation of growth control:
- Inoculum used for each investigation was prepared by making a direct suspension in sterile saline of isolated colonies selected from an 18 to 24h culture. The initial bacterial suspension was prepared to a concentration of approximately \(1.5 \times 10^{5}\) CFU/ml using a DenysCHEK\textsuperscript{®} (Biomerieux Vetec Inc).

Preparation of growth control:
- Six 10-fold dilutions were made using 33ul test suspension added to 3.0 ml saline. This dilution protocol provides suspensions with concentrations of approximately \(1.5 \times 10^\pm\) CFU/ml to \(1.5 \times 10^4\) CFU/ml.

For the experiments, each of the three tubes were plated out and colonies counted after 24 to 48 h incubation. Experimental design (in triplicate): For each organism, an aliquot of 100\(\mu\)l (\(\leq 1.0 \times 10^7\) CFU/ml) of the \(\leq 1.0 \times 10^5\) CFU/ml suspension (second tube) was transferred into 9 wells of a row of an 8-well microtiter plate (3 replicate time, 3 replica and 3 @ 48h) for each transport system under test.

One swab was placed in each of the wells and allowed to absorb the suspension for 30min.

The inoculated swabs were then placed in their respective devices containing 1.0 ml of liquid Amies broth.

At each of the time points, the swabs were removed from their devices and vortexed in 0.9 ml sterile saline for 30 seconds from which four 1.10 serial dilution were made.

100\(\mu\)l aliquot of each dilution was pipetted onto chocolates agar plates and or blood agar plate, streaked, and inoculated in CO\textsubscript{2} at 37\textdegree C for 24-48h. Bacterial recovery was determined by counting the colonies recovered in each of the dilutions.

The number of the organisms recovered is expressed as an average from triplicate samples and from triplicate plates (9 plates/dilution/organism). CLSI M40A recommends that the dilution to be used is that which has a zero time reading of \(\leq 100\) CFU/ml on a plate.

In this experiment the \(10^{-2}\) dilution (\(\leq 1.5 \times 10^5\) CFU/ml) was the one that most closely met these criteria.

\textbf{Introduction}

Organism viability, survival rate and subsequent release from the transport device are crucial components of the isolation of clinically significant organisms from liquid transport swabs.

Many factors play a role in the recovery of bacteria from clinical specimens. These factors range from the type of swabs and transport media used, to the length of time and temperature of transportation.

The new Flocked swab with liquid Amies is designed to improve the sensitivity obtained with the traditional transport swab systems. In this new design organisms are theoretically completely released into the 1 ml broth Amies liquid from which the inoculum is recovered after suspension up to a maximum of 10\(\mu\)l for culture plates. This potential advantage of this liquid based platform is that it can be used for either manual inoculation or with automated equipment.

We therefore decided to novel pending flocked swab from an exclusive swab manufacturer with their liquid Amies formulation and compare the results with two different flocked swabs from another manufacturer that are already on the market.