C-365

Evaluation of ESwab a New Multipurpose Liquid Swab Transport System for Aerobic and Anaerobic Bacteriology

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REVISED ABSTRACT:

We receive a multiplicity of swab types including Liquid Stuart for aerobic culture and direct assays and Amies Agar Gel for aerobic and anaerobic culture. No single transport serves all our needs and we would like a more versatile multipurpose swab. We evaluated new ESwab (Copan Diagnostics) as a single replacement for all swab transports. ESwab (ES) was tested for its ability to maintain aerobes in comparison with Becton Dickinson CultureSwab (BDS) and Starplex StarSwab (STS) Liquid Stuarts and for anaerobes in comparison with Becton Dickinson CultureSwab Plus (BDA) and Starplex StarSwab (STA) Amies Gel. We also compared ES, BDS and STS using spiked cultures of S. pyogenes (SP) to evaluate suitability of using ES with Biostar Strep A OIA Max (BIOSTR) direct assay. Viability test were performed using *Roll-Plate Method* (CLSI M40-A) on swabs held at room temp (20 - 25°C) for 0, 24 & 48h time points. Test strains included *N. gonorrhoeae* (NG) ATCC 43069, *H. influenzae* (HI) ATCC 10211, SP ATCC 19615, *P. anaerobius* (PA) ATCC 27337 and *P. melaninogenica* (PM) ATCC 25845. Swabs from each device were inoculated with 100ul of dilutions (neat to 10^{-4}) made from 0.5 McFarland suspension of each organism. Swabs were inoculated in triplicate. Colony counts were compiled for each dilution & incubation time point and compared to 0hr counts to determine percent recovery. For the BIOSTR study ES, BDS & STS were inoculated with 10^7, 10^6 & 10^5 cfu of 4 strains of SP. ES maintained viability of all 5 ATCC strains at 24h with higher percent recovery than the other systems. At 24h NG recovery was 3.8% with ES, 3.7% BDS, 0.1% STS; HI 56.7% ES, 32.5% BDS, 0% STS; SP 115% ES, 85.5% BDS, 75.1% STS; PA 4.4% ES, 2.8% BDA, 0.0% STA; PM 23.4% ES, 6.6% BDA and 0% STA. At 48h recovery for HI was 35.2% with ES, 7.6% BDS, 0% STS; SP 124% ES, 65.8% BDS, 63% STS; PA 0.0% with ES & STA and 0.1% with BDA & STA. In BIOSTR study all 4 strains of SP were positive at 106 cfu conc. with ES and 2/4 positive with BDS & STS. ES is able to maintain aerobes and anaerobes for at least 24h and demonstrated a greater level of sensitivity in BIOSTR to other swabs. Further evaluation of ES with antigen and molecular assays is needed.

INTRODUCTION

The Network of The Chester County Hospital (CCH) is like many small community hospitals in this country; the core of CCH comprises a 238 bed hospital with satellite locations in West Chester, Downingtown, Exton, Kennett Square, and Lionville, Pennsylvania that include various out-patient labs and drawing stations that collect throat cultures and urine samples. In addition to this we service and collect samples from various physicians' offices. We receive a multiplicity of swab transport systems including Liquid Stuart for aerobic culture and rapid direct Strep A assays and Amies Agar Gel for routine aerobic and anaerobic culture. No single swab serves all our needs. Liquid Stuart is not suitable for maintaining anaerobic bacteria and Amies Agar Gel which works well for anaerobes but is not validated for use with many rapid strep antigen tests or other assays because of potential interference from the agar gel component. We decided to evaluate ESwab a new liquid based transport system from Copan Diagnostics Inc. (Corona, California) which supports aerobic and anaerobic bacteria. ESwab is unique and comprises a patented flocked nylon swab and 1ml of Liquid Amies Medium. The flocked swab consists of 50,000 – 60,000 short strands of nylon which are glued in an organized perpendicular fashion onto the surface of a molded applicator stick. The perpendicular strands of fiber provide an open structure that efficiently absorbs and elutes the patient's specimen by capillary hydraulics. As soon as the swab is placed into the liquid medium the entire patient sample is automatically eluted. With traditional swab transports the laboratory processes the swab applicator for culture or rapid assay but in the case of ESwab one or more aliquots of the liquid medium suspension can be removed for various microbiology analyses. We were attracted to evaluate this new swab because it has the potential for multipurpose use for aerobic and anaerobic culture and because it is liquid based it could allow us to perform our rapid Strep assays. Consequently ESwab might allow The Chester County Hospital network to standardize on one swab for all our current and future bacteriology needs.

We tested ESwab (ES) ability to maintain the viability of aerobic bacteria in comparison with Becton Dickinson Liquid Stuart CultureSwab (BDS) and Starplex StarSwab (STS) Liquid Stuarts and for viability of anaerobes in comparison with Becton Dickinson Amies Agar Gel CultureSwab Plus (BDA) and Starplex StarSwab (STA) Amies Gel. Viability tests were performed using the Roll-Plate Method (CLSI M40-A) on swabs held at room temp (20 - 25°C) for 0, 24 & 48h time points. We also compared ES, BDS and STS liquid transports using dilutions of cultures of S. pyogenes (SP) to evaluate suitability of using ES with Inverness (formally Biostar) Strep A OIA Max (BIOSTR) direct assay.

METHODS

COMPARATIVE VIABILITY STUDY

1. Materials:

Commercial Transport swabs:

- **ES** — **Liquid Amies ESwab** — Copan Diagnostics Inc. Corona, CA
- **BDS** — **Liquid Stuart CultureSwab** — Becton Dickinson, Baltimore, MD
- **STS** — **Liquid Stuart StarSwab** — Starplex Scientific Etobicoke, Ont, Canada
- **BDA** — **Amies Agar Gel without Charcoal CultureSwab Plus** — Becton Dickinson Baltimore, MD
- **STA** — **Amies Agar Gel without Charcoal StarSwab** — Starplex Scientific Etobicoke, Ont Canada

Test organisms:

Species	Abbreviation	Strain
Neisseria gonorrhoeae	NG	ATCC® 43069
Haemophilus influenzae	HI	ATCC® 10211
Streptococcus pneumoniae	SP	ATCC® 19615
Peptostreptococcus anaerobius	PA	ATCC® 27337
Prevotella melaninogenica	PM	ATCC® 25845

2. Test Protocol (CLSI Roll-Plate Method)

The protocol used for viability studies was based upon the Roll-Plate Method as described in CLSI (formerly NCCLS) standard Quality Control of Microbiology Transport Systems. M40-A Vol 23 No. 34, 2003. Initial inoculum used for each investigation was prepared by making a direct suspension in 0.85% physiological saline (pH 6.8 to 7.2) of isolated colonies selected from an 18 to 24 hrs agar plate, fastidious and anaerobic bacteria required up to 48 hrs of incubation. The initial bacterial suspension was prepared to a concentration of approximately 1.5 x 10^8 CFU (equivalent to 0.5 McFarland Standard).

Preparation of dilutions:

Seven dilutions (10^{-1}, $10^{-1.5}$, 10^{-2}, $10^{-2.5}$, 10^{-3}, $10^{-3.5}$ and 10^{-4}) were prepared.

a) To prepare four log dilutions (10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}), 2.5 mL of 0.5 McFarland suspension was added to 22.5 mL 0.85% physiological saline (pH 6.8 - 7.2). The suspension was mixed and 2.5 mL was transferred to a second tube containing 22.5 mL saline. This step was repeated to achieve a total of four log dilutions.

b) To prepare three ½ log dilutions ($10^{-1.5}$, $10^{-2.5}$, and $10^{-3.5}$), an initial dilution was made by adding 0.3 mL of the starting 0.5 McFarland suspension to 9.7 mL 0.85% physiological saline (pH 6.8 - 7.2). The suspension was mixed and 2.5 mL was transferred to a second tube containing 22.5 mL saline. This step was repeated to achieve a total of three ½ log dilutions.

This dilution protocol provides suspensions with concentrations of approximately 1.5 x 10^7 CFU/mL to 1.5 x 10^4 CFU/mL in half log increments.

Dosing Swabs and Holding/Incubation:

a) An aliquot of 100 µL of inoculum from each dilution was pipetted into each of 9 tubes (3 @ time zero, 3 @ 24 hours and 3 @ 48 hours) for each transport system under test.

b) One swab was placed into each of the tubes containing the microorganism suspension and allowed to absorb the suspension for 10 seconds.

c) Each swab was placed into the appropriate transport container in accordance with the Instructions for Use for the transport system under test.

d) Three swabs from each transport system were plated after 10 - 15 minutes to serve as the zero time controls.

e) All other inoculated swabs were held at 20°C - 25°C for 24 or 48 hrs (n=3 swabs for each holding temperature/time combination). *Neisseria gonorrhoeae* was assessed after a 24 hrs hold time only.

Cultivation of Swabs:

a) The swabs were removed from their transport containers (zero time controls and all swabs from each holding temperature/time combination).

b) Each swab was inoculated over the dried surface of an appropriate culture medium plate rotating the swab between the thumb and the index finger to ensure that all surfaces of the swab equally contacted the surface of the culture media. This was repeated by streaking the swab two more times, rotating the plate 60° each time to ensure an even distribution of the inoculum as outlined in NCCLS document M2, Performance Standards for Antimicrobial Disk Susceptibility Tests.

c) Streaked plates were incubated at 35°C - 37°C for 48 hr.

d) When the incubation period was complete, growth on each plate was counted and the average CFU was determined for cultures derived from three swabs for each holding temperature/time point.

e) The inoculum dilution yielding countable CFUs on zero-time plates closest to 300 CFU was selected.

COMPARATIVE PERFORMANCE A STREP A DIRECT ASSAY USING SWAB TRANSPORT SYSTEMS DOSED WITH VARIOUS STREPTOCOCCUS PYOGENES ISOLATES

A number of fresh patient isolates of Streptococcus pyogenes were collected for testing using STS, BDS and ES transports with the Inverness (formally Biostar) Strep A OIA MAX assay. Duplicate swabs provided with each transport system were dosed with 100 µL of a 0.5 McFarland suspension and 10^{-1} and10^{-2} dilutions of each streptococcus strain and then were tested in the assay. A detail description of the procedure follows.

1. Materials:

- STREP A OIA MAX 100 Test Kits Catalog No. 90003
- Phosphate buffered saline, micropipetters, sterile plastic pipette tips, nephelometer, centrifuge, timer and plastic disposable pipettes
- *Streptococcus pyogenes* clinical isolates
- STS, BDS and ES transport swabs.

2. Test Protocol

a) Prepare 0.5 McFarland suspensions of each of 13 clinical isolates of *Streptococcus pyogenes* checking the turbidity using a nephelometer. From each 0.5 McFarland organism suspension 10^{-1} and10^{-2} dilutions were prepared. The original McFarland suspension and each tube dilution was vortexed for 10 seconds before inoculating onto swabs provided with ES, BDS and STS commercial transport systems.

b) Two swabs from each system ES, BDS and STS were inoculated with 100 µL of neat 0.5 McFarland suspension and 10^{-1} and10^{-2} dilutions for each *Streptococcus pyogenes* strain then the swabs were placed into their respective transport tube devices.

c) **Each swab was held for 1 minute** in the transport device before proceeding to the next step.

Suspension & Loading	100 µL load on swab	Mixed with 1ml medium
Neat 0.5 McFarland	~ 1.5 x 10^7 cfu	~ 1.5 x 10^6 cfu
10^{-1} dilution	~ 1.5 x 10^6 cfu	~ 1.5 x 10^5 cfu
10^{-2} dilutions	~ 1.5 x 10^5 cfu	~ 1.5 x 10^4 cfu

Limits of detection of kit 1.2 x 10^4 stated in manufacturer's package insert.

For Copan ESwab:

We developed the following protocol to enable us to perform the Strep A OIA Max assays on Copan ESwab samples.

a) Vortex the ESwab tube with the swab inside then using a sterile tipped pipetter transfer 500 µL of the specimen/organism suspension to a small sterile conical bottom tube and label this tube A. Alternatively Copan can provide plain sterile empty ESwab size tubes.

b) Centrifuge tube A at 4,000 rpm for 10 minutes

c) Remove 450 µL using a sterile pipetter.

d) Reconstitute the extraction reagent in the Biostar Extraction Tube using Reagent 1 as per the manufacturer's package insert instructions. Transfer this entire hydrated reagent using a pipette into tube A.

e) Mix tube A well to re-suspend the centrifuge deposit and allow the reaction with the extraction reagent.

f) Wait for 1minute but no more than 3 minutes then add 3 drops of Reagent 2 into tube A.

g) Mix well and verify the color change from green to dark blue.

h) Using the pipette provided with the kit, transfer 1 – 2 drops of liquid from tube A to the reaction cassette.

i) Proceed as described in the manufacturer package inert instructions for further steps and interpretation of results

For BDS and STS Liquid Stuart

Reconstitute the Reagent 1 following the instructions of the OIA Max kit. Detach the swab from the cap of the BDS or STS transport system and follow the procedure as per the package insert for further steps and interpretation of results.

RESULTS

VIABILITY STUDY

ESwab was tested with aerobes in comparison with two traditional Liquid Stuart transport swabs and with anaerobes with two Amies Agar Gel transport swabs from Starplex and Becton Dickinson. At 24 hours Copan ESwab was able to maintain the viability of all organisms tested and demonstrated a higher percent recovery in each case. At 48 hours ESwab maintained the viability of 4/5 organisms tested. In contrast at 24 hours Starplex Liquid Stuart failed to recover *H. influenzae* and their Amies Agar Gel swab failed to recover both anaerobes *P. anaerobius* and *P. melaninogenica*. In most cases Becton Dickinson Liquid Stuart and Amies Agar gel swab transports performed similarly to Copan ESwab for aerobes and anaerobes respectively. We conducted these viability studies at Controlled Room Temperature (20°C - 25°C) only as we have found in previous studies that this shipping/holding temperature has a greater impact on the survival of aerobes and anaerobes. In our experience and based on other published work bacteria tend to survive better at 4°C but as our internal specimen transport system often exposes samples to prolonged periods at higher temperatures we wanted this evaluation to focus on this less favorable condition.

STREP A ASSAY

Two assay tests runs were performed comparing all three swab transports with the Inverness (Biostar) OIA MAX assay. After dosing the swabs in each transport kit with the streptococcus suspensions swabs were returned to their respective transport medium tubes as they would be during normal practice. Using the protocol we developed in-house for ESwab and the manufacturer's recommended procedure for STS and BDS swabs a positive Strep A assay was detected with all strains of *Streptococcus pyogenes* at the 10^{-1} dilution of the McFarland suspension and in many cases at the 10^{-2} dilution with ESwab. With 2/13 isolates the assay was positive at 10^{-1} but no assays were positive at the 10^{-2} dilution.

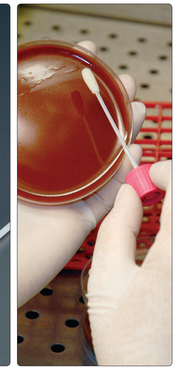
Initial load	Neat 0.5 McFarland	10^{-1} dilution	10^{-2} dilution
100 µL	~ 1.5 x 10^7	~ 1.5 x 10^6	~ 1.5 x 10^5
1st Run			
STS	4/4 Pos	2/2 Pos	All Neg
BDS	4/4 Pos	2/2 Pos	All Neg
ES	**4/4 Pos**	**4/4 Pos**	**1/4 Pos**
2nd Run			
STS	9/9 Pos	9/9 Pos	All Neg
BDS	9/9 Pos	9/9 Pos	All Neg
ES	**9/9 Pos**	**9/9 Pos**	**9/9 Pos**

STS = Starplex Liquid Stuart
BDS = BD Liquid Stuart
ES = **Copan ESwab LQ Amies**

When dosed swabs are returned to their transport medium tubes there is an opportunity for the transport medium to mix and dilute the bacteria dosed onto the swab. STS, BDS and ES all contain approximately 1ml of liquid transport medium which can result in an additional 10 fold dilution of the bacterial inoculum. The package insert for Inverness (Biostar) OIA Max assay claims a limit of detection of 1.2 x 10^4 CFU. Taking into consideration the potential dilution effect of the transport medium in our study in most cases (11/14) using ESwab we were able to detect Streptococcus pyogenes at the limits of detection of the assay. Using the centrifugation protocol develop by our laboratory for processing a 500 µL aliquot of ESwab medium we were able to demonstrate a greater level of sensitivity (1 log-10) with the Inverness (Biostar) Strep A assay then with traditional Liquid Stuart transport swabs.

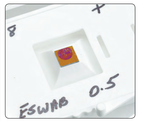
RESULTS SUMMARY

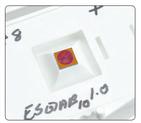
Aerobic Viability Study	Starplex STS Liquid Stuart		Becton BDS Liquid Stuart		Copan ES Liquid Amies + Flocked Swab	
	Av CFUs Dilution	% Recovery	Av CFUs Dilution	% Recovery	Av CFUs Dilution	% Recovery
S. pyogenes	10*3.5		10*3.5		10*3.5	
0hr	260	100%	230	100%	224	100%
24hr	195	75%	197	86%	259	116%
48hr	164	63%	151	66%	278	124%
H. influenzae	10*4		10*4		10*4	
0hr	190	100%	127	100%	110	100%
24hr	0	0%	41	33%	62	57%
48hr	0	0%	10	8%	39	35%
N. gonorrhoeae	10*1		10*1		10*2	
0hr	658	100%	319	100%	256	100%
24hr	0	0%	12	4%	10	4%

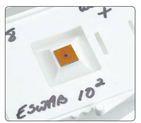


Open Flocked Fiber Structure

Tight Fiber Winded Structure







Anaerobic Viability Study	Starplex STA Amies Agar Gel		Becton BDA Amies Agar Gel		Copan ES Liquid Amies + Flocked Swab	
	Av CFUs Dilution	% Recovery	Av CFUs Dilution	% Recovery	Av CFUs Dilution	% Recovery
P. anaerobius	10*2		10*1		10*1.5	
0hr	463	100%	963	100%	429	100%
24hr	0	0%	27	3%	20	4%
48hr	0	0%	1	0%	0	0%
P. melaninogenica	10*3.5		10*3.5		10*3.5	
0hr	463	100%	407	100%	447	100%
24hr	0	0%	27	7%	105	23%
48hr	0	0%	0	0%	10	2%

10* = ten to the minus value of McFarland suspension

CONCLUSIONS

The purpose of this study was to evaluate ESwab, a new and novel swab transport system that might be suitable to replace the variety of contemporary bacteriology swab transport systems used by our network. We are in need of one multipurpose swab transport that maintains aerobic and anaerobic bacteria and is suitable for the expanding number of rapid direct assays we are required to perform. Our study showed that ESwab outperformed all other transports for maintenance of aerobes and anaerobes at 24 hours which is within the operational window of most of our specimen receipts. We developed and tested a procedure for processing an aliquot of the ESwab medium for our rapid direct Strep A assay. Using this procedure we were able to demonstrated greater sensitivity in our OIA MAX assay then with traditional transport swabs following the manufacturer's protocol in the package insert.

This initial work is very exciting as it opens up the possibility for multiple test analysis from one swab transport device. The flocked swab provided with ESwab has an open structure which automatically elutes the entire sample into the transport medium once it is inserted in the tube. In reality this provides 1 ml of sample suspension and our laboratory now has the opportunity of performing multiple tests by simply taking aliquots of the specimen suspension. When inoculating culture plates using the ESwab applicator as an inoculation wand, it transfers as much as 100 µL of sample onto each plate. We found that processing 500 µL of ESwab sample was adequate for our rapid Strep A assay. We are planning to develop additional protocols and validations of ESwab with our GeneOhm MRSA and Group B Strep assay as we recognize the potential for expanding capability and that the product provides a plentiful source of sample volume for these purposes. We are also investigating another potential advantage of ESwab for archiving specimens. Unlike traditional swabs ESwab is a liquid suspension and after processing it has the possibility to be archived for later retrieval of replicate aliquots of the original sample.

Further work and validation is necessary to realize the full potential of ESwab as a cost effective multipurpose transport for microbiology.