Evaluation of ESwab against BD CultureSwab MaxV(+) and Medical Wire & Equipment Transwab for the Maintenance of Fastidious Aerobic Bacteria

Issa Siryani[1], Riyad Ghneim[1], Amal Abu-Rayan[2], Moein Kanaan[2], Musa Hindiyeh[1]

1. Caritas Baby Hospital, Bethlehem, PALESTINIAN TERRITORY, OCCUPIED 2. Bethlehem University, Bethlehem, PALESTINIAN TERRITORY, OCCUPIED

Abstract

Of utmost importance in evaluations of clinical samples for infectious agents is proper specimen collection and transport to the laboratory. Recently, Copan Diagnostics introduced ESwab, a new flocked swab with liquid based transport system designed to optimize specimen collection and to minimize specimen entrapment. In this study, we followed the CLSI M40-A Roll-Plate Method to compare the performance of the ESwabs [ES] against two semisolid gel swabs, the BD CultureSwab MaxV(+) [BD] and the Medical Wire & Equipment Transwab [MW]. The viability of 10 different *S. pneumoniae* serotypes (19F,9V,6A/B,23F,16F,19A,14,4,5,sg18),*H. influenzae* (ATCC 49766), *N. gonorrhoeae* (ATCC 49226), and *N. meningitidis* (ATCC 13090) was evaluated at 0, 6, 24, and 48hrs after 24˚C and 4˚C storage. Briefly, three dilutions of 0.5 McFarland standards were prepared and 100µl was inoculated on each of the swabs in triplicate. The 1:200 dilution which approximately gave 300 countable colonies on zero time was used to evaluate *S. pneumoniae*, *N. gonorrhoeae* and *N. meningitidis*, while the 1:1000 dilution was used for *H. influenzae.* The presence of ≥5 CFU was used to determine if a swab met the CLSI acceptance criteria. At 24˚C, all three swabs met the CLSI criteria for the maintenance of *S. pneumoniae* serotypes: 9V, 16F, 23F, 14, 5, and 4, viability for 48hrs. ESwabs satisfied the guidelines for the maintenance of *H. influenzae* and *N. gonorrhoeae* viability, for 48hrs and 24hrs, respectively. MW failed to maintain the viability of these two organisms past 6hrs. BD swabs maintained the viability of 19F, 19A serotypes and *N. meningitidis* for 48hrs; while ESwabs maintained the viability of 6A/B and sg18 serotypes for 48hrs. At 4˚C, all three swabs maintained the viability of 19A, 16F, 23F, 6A/B, 14, 5, 4, and *H. influenzae* for 48hrs. BD and ES maintained the viability of *N. gonorrhoeae* for 24hrs; while ES maintained sg18 and *N. meningitidis* for 48hrs. MW failed to maintain the viability of *N. gonorrhoeae* past 6hrs. Overall, BD performed the best after incubation at 24˚C while ES outperformed BD after incubation at 4˚C. MW swabs did not meet the CLSI standards for the majority of genuses tested.

Introduction

Diagnosis of bacterial pathogens in patient samples largely depends on sample collection and transport to the laboratory. Failure to collect an appropriate patients sample can lead to patient misdiagnosis and inappropriate treatment. It is well established that aspirates of fluids, and exudates from suspected infected sites are superior to samples collected on swabs (1, 2). However, because of the ease of using swabs, clinical microbiology laboratories continue to receive clinical samples on swabs. Copan Diagnostics introduced ESwab, a new flocked swab with liquid (1ml) based transport system designed to optimize specimen collection and to minimize specimen entrapment (Figure 1). In this study, we followed the CLSI M40-A Roll-Plate Method to compare the performance of the ESwabs [ES] against two semisolid gel swabs, the BD CultureSwab MaxV(+) [BD] and the Medical Wire & Equipment Transwab [MW] in maintaining the viability of fastidious aerobic microorganisms. BD CultureSwab MaxV(+) [BD] is manufactured by Copan and outside of the USA and is marketed under the name Copan M40 Transystem.

Materials and Methods

Bacterial Stains:

The following bacterial strains were used:

- ***Streptococcus pneumoniae* (ATCC 49619) Serotype 19F**
- ***Streptococcus pneumoniae* (Clinical isolate) Serotype 19A**
- ***Streptococcus pneumoniae* (Clinical isolate) Serotype 9V**
- ***Streptococcus pneumoniae* (Clinical isolate) Serotype 16F**
- ***Streptococcus pneumoniae* (Clinical isolate) Serotype 6A/B**
- ***Streptococcus pneumoniae* (Clinical isolate) Serotype 14**
- ***Streptococcus pneumoniae* (Clinical isolate) Serotype 5**
- ***Streptococcus pneumoniae* (Clinical isolate) Serotype 23F**
- ***Streptococcus pneumoniae* (Clinical isolate) Serotype 4**
- ***Streptococcus pneumoniae* (Clinical isolate) Serotype sg18**
- ***Haemophilus influenzae* (ATCC 49766)**
- ***Neisseria gonorrhoeae* (ATCC 49226)**
- ***Neisseria meningitidis* (ATCC 13090)**

Transport Swab Systems:

- **ESwabs [ES]**
- **BD CultureSwab MaxV(+) [BD] outside USA Copan M40 Transystem**
- **Medical Wire & Equipment Transwab [MW]**

Bacterial Identification:

Conformation of the *S. pneumoniae* serotypes was performed according to Pai et al (3).

Agar Plates:

5% Sheep Blood Agar plates (Cat # Hy-PD 049) and Chocolate Agar (Cat # PD-013) were purchased from Hy.Labs (Park Tamara, Rehovot, Israel).

Bacterial Strain Cultures:

Bacterial strains were cultured on the appropriate media (5% Sheep Blood Agar or Chocolate Agar) for 18-24 hours at 37˚C. Well isolated colonies were used for sample preparation. Some fastidious organisms required 48 hours incubation before sample preparation.

Experimental Design:

The CLSI procedure M40-A (Roll-Plate Method) was followed to evaluate the different transport swabs (4). Briefly, a 0.5 McFarland (1.5 x 10^8 CFU/ml) standards of each organism freshly grown at 37˚C for 18-24hrs was prepared using BioMerieux Vitek Inc. colorimeter in 0.85% saline. A 1:10, 1:200 and 1:500 dilutions of this inoculum were performed in saline solution to provide final working concentrations ranging from 1.5 x 10^1 to 3 x 10^3 CFU/mL. For *S. pneumoniae* sg18 (1:500) dilution was used, while for *H. influenzae* a 1:10, 1:100 and 1:1000 dilution was used. In triplicate, an aliquot of 100 µL of each of these dilutions was used to inoculate swabs by absorbing the inoculum for 5 s from a sterile eppendorff tube. The survival of the organisms at 20-25˚C and 8˚C was determined at 0, 6, 24, and 48hrs. This concentration range was confirmed by quantitative colony counts on agar plates. Recovery of viable organisms was determined by sub-culturing the swabs at different scheduled times by rolling over the entire surface of the swab on the selected agar plate. For ESwab, an additional 100ul were inoculated directly on a second set of plates and the inoculum was spread by a plate spreader. The inoculum dilution yielding zero-time colony count closely approaching 300 CFU was used to complete viability studies. Colonies were counted after incubation of plates for 24–48hrs at 35–37˚C in 5% CO_2.

Interpretation of Results

For viability studies to be considered acceptable, there shall be ≥5 CFU following the specified holding time from the specific dilution that yielded zero-time plate counts closest to 300 CFU

Results

(Table 1)

Recovery of *Streptococcus pneumoniae* Serotypes after Storage at Room Temperature

Serotype	Swab System	0 hrs	6 hrs	24 hrs	48 hrs
4	BD	580(100%)	430(74%)	240(41%)	**128(22%)**
	MW	803(100%)	567(71%)	280(35%)	**93(12%)**
	ESwab	727(100%)	623(86%)	280(39%)	**160(22%)**
	ESwab (100ul)	767(100%)	687(90%)	357(47%)	**195(25%)**
6A/B	BD	337(100%)	240(71%)	**8(2%)**	1(0%)
	MW	450(100%)	377(84%)	166(37%)	**19(4%)**
	ESwab	410(100%)	347(85%)	107(26%	**15(4%)**
	ESwab (100ul)	423(100%)	280(66%)	132(31%)	**16(4%)**
9V	BD	350(100%)	187(53%)	72(21%)	**76(22%)**
	MW	527(100%)	470(89%)	100(19%)	**19(4%)**
	ESwab	367(100%)	180(49%)	30(8%)	**9(2%)**
	ESwab (100ul)	373(100%)	197(53%)	41(11%)	**6(2%)**
14	BD	383(100%)	200(52%)	53(14%)	**21(6%)**
	MW	433(100%)	307(71%)	96(22%)	**15(3%)**
	ESwab	380(100%)	353(93%)	183(48%)	**29(8%)**
	ESwab (100ul)	387(100%)	347(90%)	117(30%)	**36(9%)**
19F	BD	183(100%)	80(44%)	60(33%)	**111(61%)**
	MW	290(100%)	147(51%)	**9(3%)**	0(0%)
	ESwab	340(100%)	143(42%)	36(11%)	**5(1%)**
	ESwab (100ul)	387(100%	155(40%)	47(12%)	**10(3%)**
23F	BD	277(100%)	260(94%)	179(65%)	**50(18%)**
	MW	517(100%)	333(65%)	131(25%)	**73(14%)**
	ESwab	540(100%)	360(67%)	253(47%)	**147(27%)**
	ESwab (100ul)	560(100%)	370(66%)	290(52%)	**227(40%)**
19A	BD	257(100%)	110(43%)	12(5%)	**68(26%)**
	MW	310(100%)	101(33%)	**13(4%)**	1(0.3%)
	ESwab	353(100%)	143(41%)	**20(6%)**	1(0.3%)
	ESwab (100ul)	333(100%)	143(43%)	**24(7%)**	2(1%)
16F	BD	213(100%)	220(103%)	132(62%)	**167(78%)**
	MW	363(100%)	310(85%)	207(57%)	**60(17%)**
	ESwab	365(100%)	235(64%)	92(25%)	**18(5%)**
	ESwab (100ul)	430(100%)	260(61%)	86(20%)	**29(7%)**
5	BD	433(100%)	247(57%)	158(37%)	**45(10%)**
	MW	500(100%)	423(85%)	317(63%)	**130(26%)**
	ESwab	517(100%)	380(74%)	330(64%)	**130(25%)**
	ESwab 100ul	500(100%)	443(89%)	307(61%)	**130(26%)**
sg18	BD	267(100%)	253(95%)	**32(12%)**	1(0.4%)
	MW	410(100%)	238(58%)	**69(17%)**	1(0.3%)
	ESwab	310(100%)	200(65%)	93(30%)	**8(3%)**
	ESwab 100ul	363(100%)	253(70%)	112(31%)	**18(5%)**

(Table 2)

Recovery of Fastidious Aerobic Bacteria after Storage at Room Temperature

H. influenzae	BD	457(100%)	293(64%)	94(21%)	**85(19%)**
	MW	507(100%)	313(62%)	**33(7%)**	0(0%)
	ESwab	517(100%)	553(107%)	307(59%)	**114(22%)**
	ESwab 100ul	517(100%)	530(103%)	333(64%)	**148(29%)**
N. meningitidis	BD	707(100%)	92(13%)	23(3%)	**15(2%)**
	MW	750(100%)	**99(13%)**	0(0%)	0(0%)
	ESwab	863(100%)	383(44%)	**52(6%)**	1(0.1%)
	ESwab (100ul)	917(100%)	447(49%)	**53(6%)**	1(0.1%)
N. gonorrhoeae	BD	242(100%)	**7(3%)**	2(1%)	
	MW	368(100%)	**5(1%)**	0(0%)	
	ESwab	453(100%)	111(25%)	**8(2%)**	
	ESwab 100ul	443(100%)	132(30%)	**9(2%)**	

(Table 3)

Recovery of *Streptococcus Pneumoniae* Serotypes after Storage at 4°C

Serotype	Swab System	0 hrs	6 hrs	24 hrs	48 hrs
4	BD	580(100%)	287(49%)	166(29%)	**50(9%)**
	MW	803(100%)	785(98%)	417(52%)	**330(41%)**
	ESwab	727(100%)	650(89%)	393(54%)	**383(53%)**
	ESwab 100ul	767(100%)	707(92%)	437(57%)	**390(51%)**
6A/B	BD	337(100%)	205(61%)	137(41%)	**10(3%)**
	MW	450(100%)	400(89%)	357(79%)	**287(64%)**
	ESwab	410(100%)	437(107%)	273(67%)	**36(9%)**
	ESwab (100ul)	423(100%)	353(84%)	273(65%)	**46(11%)**
9V	BD	350(100%)	174(50%)	116(33%)	**13(4%)**
	MW	527(100%)	437(83%)	280(53%)	**287(54%)**
	ESwab	367(100%)	323(88%)	**98(27%)**	1(0.3%)
	ESwab (100ul)	373(100%)	312(84%)	**102(27%)**	1(0.3%)
14	BD	383(100%)	163(43%)	107(28%)	**38(10%)**
	MW	433(100%)	323(75%)	237(55%)	**137(32%)**
	ESwab	380(100%)	410(108%)	267(70%)	**173(46%)**
	ESwab (100ul)	387(100%)	387(100%)	293(76%)	**200(52%)**
19F	BD	183(100%)	**42(23%)**	1(1%)	0(0%)
	MW	290(100%)	188(65%)	44(15%)	**6(2%)**
	ESwab	340(100%)	103(30%)	10(3%)	**11(3%)**
	ESwab (100ul)	387(100%)	113(29%)	**11(3%)**	1(0.3%)
23F	BD	277(100%)	283(102%)	190(69%)	**148(53%)**
	MW	517(100%)	460(89%)	225(44%)	**136(26%)**
	ESwab	540(100%)	343(64%)	280(52%)	**293(54%)**
	ESwab (100ul)	560(100%)	360(64%)	333(60%)	**307(55%)**
19A	BD	257(100%)	104(40%)	68(26%)	**16(6%)**
	MW	310(100%)	115(37%)	59(19%)	**7(2%)**
	ESwab	353(100%)	140(40%)	94(27%)	**10(3%)**
	ESwab (100ul)	333(100%)	153(46%)	122(37%)	**6(2%)**
16F	BD	213(100%)	190(89%)	123(58%)	**89(42%)**
	MW	363(100%)	380(105%)	300(83%)	**230(63%)**
	ESwab	365(100%)	350(96%)	223(61%)	**87(24%)**
	ESwab (100ul)	430(100%)	300(70%)	225(52%)	**91(21%)**
5	BD	433(100%)	320(74%)	183(42%)	**65(15%)**
	MW	500(100%)	457(91%)	410(82%)	**333(67%)**
	ESwab	517(100%)	423(82%)	417(81%)	**267(52%)**
	ESwab 100ul	500(100%)	440(88%)	417(83%)	**233(47%)**
sg18	BD	267(100%)	142(53%)	**27(10%)**	1(0.4%)
	MW	410(100%)	233(57%)	213(52%)	**52(13%)**
	ESwab	310(100%)	180(58%)	140(45%)	**7(2%)**
	ESwab 100ul	363(100%)	225(62%)	160(44%)	**11(3%)**

(Table 4)

Recovery of Fastidious Aerobic Bacteria after Storage at 4°C

H. influenzae	BD	457(100%)	413(91%)	123(27%)	**22(5%)**
	MW	507(100%)	427(84%)	272(54%)	**11(2%)**
	ESwab	517(100%)	527(102%)	410(79%)	**327(63%)**
	ESwab (100ul)	517(100%)	520(101%)	387(75%)	**330(64%)**
N. meningitidis	BD	707(100%)	142(20%)	**11(2%)**	2(0.3%)
	MW	750(100%)	160(21%)	**10(1%)**	0(0%)
	ESwab	863(100%)	530(61%)	397(46%)	**65(7%)**
	ESwab (100ul)	917(100%)	533(58%)	390(43%)	**73(8%)**
N. gonorrhoeae	BD	242(100%)	**13(5%)**	3(1%)	
	MW	368(100%)	**32(9%)**	0(0%)	
	ESwab	453(100%)	172(38%)	**32(7%)**	
	ESwab 100ul	443(100%)	130(29%)	**23(5%)**	

Conclusions

Room Temperature Studies

- **Copan ESwabs [ES] met the CLSI M40 guidelines for 11 of the 13 fastidious organisms tested.**
  ESwab did not meet the criteria for *S. pneumoniae* serotype 19A and *N. meningitides*.
- **BD CultureSwab MaxV(+) [BD] also know as Copan M40 Transystem outside USA met the CLSI M40 guidelines for 10 of the 13 fastidious organisms tested.**
  BD did not meet the criteria for *S. pneumoniae* serotypes 6A/B, sg18 and *N. gonorrhoeae*.
- **Medical Wire & Equipment Transwab [MW] met the CLSI M40 guidelines for 7 of the 13 fastidious organisms tested.**
  MW did not meet the criteria for *S. pneumoniae* serotypes 19A, 19F, sg18, *H. influenzae*, *N. meningitides* and *N. gonorrhoeae*.
- **Overall, ESwabs performance was the best followed by BD CultureSwab MaxV(+) and Medical Wire & Equipment Transwab .**
- **No difference was noted between using the ESwabs directly or using an aliquot (100ul) from the liquid medium to inoculate the plates.**
- **No overgrowth was noted for any of the fastidious bacteria tested at room temperature.**

4˚C Studies

- **Copan ESwabs met the CLSI guidelines for all four fastidious genuses tested with the exception to *S. pneunomiae* serotype 9V.**
- **Both BD CultureSwab MaxV(+) [BD] and Medical Wire & Equipment Transwab did not meet the CLSI guidelines for maintaining the viability of *N. meningitidis* and *N. gonorrhoeae*.**
- **No overgrowth was noted for any of the fastidious bacteria tested at 4˚C.**

Overall

- **The suspension of patient sample in the liquid medium of the ESwab is an important asset for the clinical laboratory since the remaining sample can be used for performing additional diagnostic assays (i.e molecular and antigen detection assay) on the patient sample.**
- **We rank Copan ESwabs as the best transport system followed by BD CultureSwab MaxV(+) and lastly, the Medical Wire & Equipment Transwab.**

References

- **Rishmawi N, Ghneim R, Kattan R, Ghneim R, Zoughbi M, Abu-Diab A, Turkuman S, Dauodi R, Shomali I, Issa Abdel-R, Siriani I, Marzouka H, Schmid J, Hindiyeh MY. Survival of fastidious and nonfastidious aerobic bacteria in three bacterial transport swab systems. J Clin Microbiol. 2007 Apr;45(4):1278-83**
- **Hindiyeh M, Acevedo V, Carroll KC. Comparison of three transport systems Starplex StarSwab II, the new Copan Vi-Pak Amies Agar Gel collection and transport swabs, and BBL Port-A-Cul) for maintenance of anaerobic and fastidious aerobic organisms. J Clin Microbiol. 2001 Jan;39(1):377-80**
- **Pai R, Gertz RE, Beall B. Sequential multiplex PCR approach for determining capsular serotypes of Streptococcus pneumoniae isolates. J Clin Microbiol. 2006 Jan;44(1):124-31.**
- **CLSI. 2003. Quality control of microbiological transport systems. Approved standard M40-A. CLSI, Wayne, Pa.**

Figure 1

creative.jad