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Comparison of Three Transport Systems for the Viability of Clinically Relevant Fastidious Organisms

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ABSTRACT

Background: The Copan Elution Swab (ESwab) (ES) Collection and Transport System (BD Diagnostics), BD BBL™ CultureSwab™ Plus (BD), and Remel BactiSwab® (REM) were compared for their ability to maintain viability of 10 clinically relevant isolates.

Methods: A 0.5 McFarland standard was created from which two dilutions of 10-3 and 10-4 were used to "seed" the three transport swab systems. Seeded swabs were held at two different temperatures (room temperature and 4°C) and were inoculated onto appropriate media at three time points (0, 24, and 48 h).

Results: All swabs used in this study supported the growth of the seeded organisms at the initial time point (0 h). Only the ES system supported the growth of all seeded organisms by the 48 h time point. *K. kingae* was not recovered using the BD or REM systems beyond the initial 0 h time point. The ES system had the highest recovery rate (70%) of organisms at the initial time point. With the exception of *B. distasonis*, the swabs held at 4°C had a better organism recovery rate. We observed that the seeded swabs held at room temperature continued to replicate for some organisms. The recovery rate of *E.coli* at 48 h was 71% ES, 45% BD, and 42% REM. *P. multocida* and *S. pyogenes* had > 100% recovery for all swabs tested. *Capnocytophaga* sp. had a recovery rate of 21% (ES), 5% (BD), and 22% (REM) at 48 h. *M. fortuitum* had a recovery rate of 71% (ES), 45% (BD), and 42% (REM) at 48 h. *C. perfringenes* had a recovery rate of 70% (ES), 22% (BD), and 0% (REM) at 48 h. *B. distasonis* had a recovery rate of 71% (ES), 50% (BD), and 24% (REM) at 48 h. As expected, the recovery rate for *H. influenzae* and *S. pneumoniae* was less than the other test organisms.

Conclusions: The data from this study illustrated that the ES transport system had a higher recovery rate than the BD and REM transport swabs and supported the growth of all organisms tested

INTRODUCTION

One of the most critical steps for the recovery of organisms in the laboratory is the collection and transport of specimens for the diagnosis of infections. Swabs are often used for the collection of these specimens to determine the bacterial etiology. Although not always the most optimal choice for specimen collection, swabs offer a convenient and easy way of collecting and transporting the specimens to the laboratory.

Collection and transport swabs have advanced from the first-used cotton swabs. Past improvements for collection swabs were the use of Amies agar with or without charcoal for the transport of specimens and the use of other synthetic fibers for the swab tip. Recent improvements for collection and transport swabs include the use of a flocked swab which is then placed in a small vial of liquid Amies transport medium.

Numerous studies have evaluated the efficacy of various swab collection/transport systems to maintain the viability of microorganisms (1-3). Until recently, the commercially available collection/transport swabs required manipulation in the laboratory if multiple media or Gram stain was needed. The ESwab System provides liquid Amies in addition to a flocked swab which would eliminate the need to manipulate the collection/transport swab once in the laboratory. This study was conducted to evaluate how the ESwab performed compared to other commercially available collection/transport swabs.

In this study, the viability of 10 clinically relevant organisms was compared using three commercially available swab collection and transport systems. The systems compared were the BactiSwab® (Remel, Lenexa, KS), CultureSwab™ Plus (BD Diagnostics, Sparks, MD), and Copan Elution swab (ESwab) (BD Diagnostics, Sparks, MD). Amies agar gel was used in both the BactiSwab® and CultureSwab™ Plus. The Elution swab used liquid Amies.

MATERIALS AND METHODS

Frozen stock cultures, either American Type Culture Collection (ATCC) or known reference organisms, were used in this study. All stock culture isolates, maintained at -70°C, were subcultured twice before being used in this study. The organisms tested are listed in TABLE 1.

Table 1. Organisms Tested

Capnocytophaga spp.	*Mycobacterium fortuitum*
Kingella kingae	*Streptococcus pneumoniae*
Pasteurella multocida	*Streptococcus pyogenes*
Escherichia coli	*Bacteroides distasonis*
Haemophilus influenzae	*Clostridium*

A fresh isolate of each organism was used to prepare a 0.5 McFarland. Serial dilutions of the 0.5 McFarland concentrations were performed to provide two working concentrations of 10-3 and 10-4. A 150 uL aliquot of the 10-3 and 10-4 dilutions were dispensed into sterile tubes and the collection/transport swabs were added to the tubes. Once the 150 ul aliquot was absorbed, no longer than 20 minutes, the swabs were placed in their respective transport system. The collection/transport swabs used in this study are listed in TABLE 2.

Table 2. Collection and Transport Systems Tested

BactiSwab, Amies Clear	Remel, Lenexa, KS
CultureSwab Plus Amies Gel without Charcoal	BD Diagnostics, Sparks, MD
Copan Liquid Amies Elution Swab (ESwab)	BD Diagnostics, Sparks, MD

Two sets of the 10-3 and 10-4 were used to seed the transport/collection systems and were held at different temperatures, room temperature and 4°C. The seeded swabs were plated in triplicate at three time points (0, 24, and 48 h). The seeded collection/transport swabs were plated to either blood agar plates, chocolate agar plates, anaerobic blood agar plates, or Middlebrook 7H11 agar plates, depending on the organism tested. The collection/transport swabs were rolled in 3 directions on the agar plates. In addition to rolling the swab from the ESwab system, 100 uL of liquid was plated. Plates were then incubated at the appropriate temperature and atmospheric condition. After appropriate incubation time, colony counts were taken for each of the agar plates. These numbers were averaged and recorded for each dilution, time point, and temperature

RESULTS SUMMARY:

A summary of the results shown in Tables 3-6 is listed below.

- All collection/transport swabs in this study supported the growth of the seeded organisms at the initial time point (0 Time Point).
- *Kingella kingae* was not recovered beyond the initial time point using either the BD or REM collection/transport system.
- ESwab was the only collection/transport swab to support the growth of all seeded organisms at 48 h time point.
- ESwab had the highest recovery rate of 70% compared to the other collection/transport swabs.
- The recovery rate of seeded organisms was better when collection/transport swabs were held at 4°C compared to room temperature.
- Overgrowth at room temperature was observed for *P. multicida* with the ES and BD systems, *E. coli* with all three test systems, and *S. pyogenes* with the ES system only at 48 h.
- No growth at 24 h but growth at 48 h for a particular organism/swab combination was most likely due to sampling error.

RESULTS

TABLE 3. No. Colonies (10^{-4}) Recovered at RT (Recovery Rate %)

	ES 100 uL	ES Swab	BD	REM
Clostridium perfringenes				
0 Time Point	4	10	9	11
24 h Time Point	1 (25)	2 (20)	1 (11)	1 (9)
48 h Time Point	1 (25)	4 (40)	1 (11)	2 (18)
Bacteroides distasonis				
0 Time Point	70	5	100	127
24 h Time Point	30 (43)	1 (20)	0 (0)	4 (3)
48 h Time Point	0 (0)	0 (0)	16 (16)	40 (31)
Mycobacterium fortuitum				
0 Time Point	14	8	11	12
24 h Time Point	14 (100)	8 (100)	12 (>100)	10 (83)
48 h Time Point	10 (71)	3 (38)	6 (55)	13 (>100)
Pasteurella multocida				
0 Time Point	433	153	280	140
24 h Time Point	TNTC (*)	TNTC (*)	TNTC (*)	39 (28)
48 h Time Point	TNTC (*)	TNTC (*)	TNTC (*)	1 (< 1)
Kingella kingae				
0 Time Point	7	3	2	3
24 h Time Point	3 (43)	1 (33)	0 (0)	0 (0)
48 h Time Point	1 (14)	0 (0)	0 (0)	0 (0)
Capnocytophaga spp.				
0 Time Point	117	27	64	73
24 h Time Point	82 (70)	10 (37)	18 (28)	22 30)
48 h Time Point	1 (<1)	0 (0)	7 (10)	0 (0)
Escherichia coli				
0 Time Point	35	8	9	9
24 h Time Point	TNTC (*)	TNTC (*)	TNTC (*)	TNTC (*)
48 h Time Point	TNTC (*)	TNTC (*)	TNTC (*)	TNTC (*)
Haemophilus influenzae				
0 Time Point	TNTC	367	444	373
24 h Time Point	307 (*)	152 (41)	123 (28)	6 (1)
48 h Time Point	20 (*)	24 (7)	35 (8)	3 (<1)
Streptococcus pyogenes				
0 Time Point	53	42	99	61
24 h Time Point	125 (>100)	233 (>100)	34 (34)	31 (51)
48 h Time Point	TNTC (*)	TNTC (*)	40 (40)	70 (>100)
S. pneumoniae				
0 Time Point	TNTC	TNTC	720	70
24 h Time Point	3 (*)	0 (0)	140 (19)	267 (>100)
48 h Time Point	0 (0)	0 (0)	22 (3)	113 (>100)

TABLE 4. No. Colonies (10^{-4}) Recovered at 4°C (Recovery Rate %)

	ES 100 uL	ES Swab	BD	REM
Clostridium perfringenes				
0 Time Point	4	10	9	11
24 h Time Point	7 (>100)	8 (80)	4 (44)	4 (36)
48 h Time Point	0 (0)	7 (70)	2 (22)	0 (0)
Bacteroides distasonis				
0 Time Point	70	5	100	127
24 h Time Point	0 (0)	0 (0)	60 (60)	50 (39)
48 h Time Point	50 (71)	30 (>100)	50 (50)	30 (24)
Mycobacterium fortuitum				
0 Time Point	14	8	11	12
24 h Time Point	15 (>100)	15 (>100)	8 (73)	12 (100)
48 h Time Point	10 (71)	8 (100)	5 (45)	5 (42)
Pasteurella multocida				
0 Time Point	433	153	280	140
24 h Time Point	200 (46)	165 (>100)	TNTC (*)	175 (>100)
48 h Time Point	200 (46)	240 (>100)	280 (100)	240 (>100)
Kingella kingae				
0 Time Point	7	3	2	3
24 h Time Point	1 (14)	0 (0)	0 (0)	0 (0)
48 h Time Point	1 (14)	0 (0)	0 (0)	0 (0)
Capnocytophaga spp.				
0 Time Point	117	27	64	73
24 h Time Point	50 (43)	10 (37)	18 (28)	23 (32)
48 h Time Point	25 (21)	4 (15)	3 (5)	16 (22)
Escherichia coli				
0 Time Point	35	8	9	9
24 h Time Point	30 (86)	7 (88)	11 (>100)	5 (56)
48 h Time Point	48 (>100)	10 (>100)	6 (67)	5 (56)
Haemophilus influenzae				
0 Time Point	TNTC	367	444	373
24 h Time Point	TNTC (*)	110 (30)	213 (48)	30 (8)
48 h Time Point	127 (*)	25 (7)	30 (7)	50 (13)
Streptococcus pyogenes				
0 Time Point	53	42	99	61
24 h Time Point	51 (96)	45 (>100)	42 (42)	61 (100)
48 h Time Point	200 (>100)	250 (>100)	283 (>100)	447 (>100)
S. pneumoniae				
0 Time Point	TNTC	TNTC	720	70
24 h Time Point	TNTC (*)	240 (*)	TNTC (*)	473 (>100)
48 h Time Point	21 (*)	8 (*)	293 (41)	141 (<100)

TABLE 5. No. Colonies (10^{-3}) Recovered at RT (Recovery Rate %)

	ES 100 uL	ES Swab	BD	REM
Clostridium perfringenes				
0 Time Point	24	40	35	13
24 h Time Point	24 (100)	40 (100)	11 (31)	0 (0)
48 h Time Point	8 (33)	42 (>100)	11 (31)	1 (8)
Bacteroides distasonis				
0 Time Point	500	300	450	500
24 h Time Point	0 (0)	0 (0)	200 (44)	100 (20)
48 h Time Point	40 (8)	70 (23)	150 (33)	120 (24)
Mycobacterium fortuitum				
0 Time Point	150	120	184	170
24 h Time Point	150 (100)	95 (79)	200 (>100)	120 (71)
48 h Time Point	108 (72)	106 (88)	200 (>100)	67 (39)
Pasteurella multocida				
0 Time Point	TNTC	467	467	397
24 h Time Point	TNTC (*)	TNTC (*)	TNTC (*)	290 (73)
48 h Time Point	TNTC (*)	TNTC (*)	TNTC (*)	6 (2)
Kingella kingae				
0 Time Point	80	35	22	24
24 h Time Point	1 (1)	0 (0)	0 (0)	0 (0)
48 h Time Point	4 (5)	1 (3)	0 (0)	0 (0)
Capnocytophaga spp.				
0 Time Point	TNTC	TNTC	TNTC	TNTC
24 h Time Point	267 (*)	69 (*)	76 (*)	90 (*)
48 h Time Point	75 (*)	155 (*)	77 (*)	93 (*)
Escherichia coli				
0 Time Point	129	108	87	62
24 h Time Point	TNTC (*)	TNTC (*)	TNTC (*)	TNTC (*)
48 h Time Point	TNTC (*)	TNTC (*)	TNTC (*)	TNTC (*)
Haemophilus influenzae				
0 Time Point	TNTC	TNTC	TNTC	TNTC
24 h Time Point	TNTC (*)	440 (*)	480 (*)	49 (*)
48 h Time Point	58 (*)	52 (*)	240 (*)	2 (*)
Streptococcus pyogenes				
0 Time Point	187	317	356	244
24 h Time Point	TNTC (*)	TNTC (*)	333 (94)	387 (>100)
48 h Time Point	47 (25)	42 (13)	34 (10)	43 (18)
S. pneumoniae				
0 Time Point	TNTC	TNTC	TNTC	TNTC
24 h Time Point	287 (*)	193 (*)	143 (*)	313 (*)
48 h Time Point	0 (0)	0 (0)	42 (*)	78 (*)

TABLE 6. No. Colonies (10^{-3}) Recovered at 4°C (Recovery Rate %)

	ES 100 uL	ES Swab	BD	REM
Clostridium perfringenes				
0 Time Point	24	40	35	13
24 h Time Point	30 (>100)	60 (>100)	50 (>100)	0 (0)
48 h Time Point	25 (>100)	35 (88)	12 (34)	40 (>100)
Bacteroides distasonis				
0 Time Point	500	300	450	500
24 h Time Point	200 (40)	0 (0)	0 (0)	0 (0)
48 h Time Point	250 (50)	300 (100)	200 (44)	250 (50)
Mycobacterium fortuitum				
0 Time Point	150	120	184	170
24 h Time Point	160 (>100)	140 (>100)	200 (>100)	200 (>100)
48 h Time Point	70 (47)	133 (>100)	263 (>100)	107 (63)
Pasteurella multocida				
0 Time Point	TNTC	467	467	397
24 h Time Point	TNTC (*)	TNTC (*)	TNTC (*)	TNTC (*)
48 h Time Point	TNTC (*)	TNTC (*)	TNTC (*)	TNTC (*)
Kingella kingae				
0 Time Point	80	35	22	24
24 h Time Point	1 (1)	1 (3)	0 (0)	0 (0)
48 h Time Point	1 (1)	1 (3)	0 (0)	0 (0)
Capnocytophaga spp.				
0 Time Point	TNTC	TNTC	TNTC	TNTC
24 h Time Point	300 (*)	16 (*)	115 (*)	93 (*)
48 h Time Point	22 (*)	7 (*)	28 (*)	2 (*)
Escherichia coli				
0 Time Point	129	108	87	62
24 h Time Point	242 (>100)	62 (57)	102 (>100)	107 (>100)
48 h Time Point	307 (>100)	60 (56)	79 (91)	66 (>100)
Haemophilus influenzae				
0 Time Point	TNTC	TNTC	TNTC	TNTC
24 h Time Point	TNTC (*)	TNTC (*)	TNTC (*)	TNTC (*)
48 h Time Point	393 (*)	213 (*)	260 (*)	227 (*)
Streptococcus pyogenes				
0 Time Point	187	317	356	244
24 h Time Point	267 (>100)	440 (>100)	333 (94)	145 (59)
48 h Time Point	TNTC (*)	TNTC (*)	347(97)	440 (>100)
S. pneumoniae				
0 Time Point	TNTC	TNTC	TNTC	TNTC
24 h Time Point	287 (*)	193 (*)	143 (*)	313 (*)
48 h Time Point	0 (0)	0 (0)	42 (*)	78 (*)

TNTC = Too Numerous to Count

* = Not Able to Determine Recovery Rate

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DISCUSSION

This study showed that all three transport systems were able to support the growth of anaerobic microorganisms. A comparison of the true recovery rate of these anaerobic organisms could not be obtained as a collection/transport system designed specifically for the recovery of anaerobes was not used in this evaluation. All three transport systems were able to maintain the viability of the rapid grower, *Myocbacterium fortuitum*.

One of the fastidious organisms, *Kingella kingae*, was not viable at 24 or 48 h with either the BD or REM systems, and the recovery rate was greatly reduced at 24 and 48 h with the ES system. This points out that certain organisms may be better recovered by another method. For example, samples such as joint fluid which may contain *K. kingae* may yield a higher recovery rate if the sample is directly injected into a blood culture bottle.

Recovery of *S. pneumoniae* and *H. influenzae* was reduced at 24 and 48 h with all three systems. This could potentially be a problem in the pediatric population where respiratory sample may only be collected by the use of a swab. This also illustrates the importance of specimens being sent to laboratory in a timely manner so that they can be plated in order to recover potential pathogens.

Overgrowth was seen with all three systems held at room temperatures. This could potentially affect patient care as certain organisms that are not necessarily the causative agent of an infectious process or the only cause of the infectious process may be overgrown. When this occurs, the results may not accurately reflect the predominant organism(s) causing the infection. Overgrowth with these systems has also previously been reported (3).

This study illustrated that all three swabs would be acceptable for use in the clinical microbiology laboratory. But, the ability to inoculate multiple media types and to be able to directly make smears for various stains makes the ESwab system a more practical choice for use in the laboratory.

REFERENCES

Farhat, S. E., M. Thibault, and R. Devlin. 2001. Efficacy of a swab transport system in maintaining viability of Neisseria gonorrhoeae and Streptococcus pneumoniae. J. Clin. Microbiol. 39:2958-2960.

Hindiyeh, M., V. Acevedo, and K. C. Carroll. 2001. 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. 39:377-380.

Van Horn, K. G., C. D. Audette, D. Sebeck, and K. A. Tucker. 2008. Comparison of the Copan ESwab System with two amies agar swab transport systems for maintenance of microorganism viability. J. Clin. Microbiol. 46:1655-1658.