



# A Comparison of Two Swab Transport Systems in the Recovery of Fastidious Organisms



Topic: C19 Specimen Collection, Transportation and Processing • S. L. Gamble; Maricopa Integrated Health System, Phoenix, AZ.

## ABSTRACT

**Background:** Evaluation of two commercial Amies without charcoal swab transport systems; BBL™ CultureSwab™ Plus, Becton Dickinson, MD (BD) and StarSwab II, Starplex Scientific, Ont., Canada (STR), for their ability to maintain fastidious bacteria up to 48 hrs. **Methods:** Two protocols, Roll Plate (RP) and Swab Elution (SE) methods, as described in NCCLS M40-A, were used to inoculate swabs with test organism and perform viability counts. For RP, four ten-fold dilutions were made from 0.5 McFarland standard solutions of bacteria, 100 ul volumes were removed from the 10<sup>-2</sup>, 10<sup>-3</sup>, and 10<sup>-4</sup> dilutions and placed into wells of a microplate. Three swabs for each transport device were placed into a well for 10 seconds, impregnating the swab with bacterial solution. Zero, 24 and 48 hr. time swabs, incubated at room temperature, were inoculated onto appropriate media by evenly swabbing the surface of the plates. For SE, a 10<sup>-1</sup> dilution was made and 100 ul placed in wells of a microplate, impregnating the swabs. At zero time each swab was placed in saline and vortexed for 15 secs. Three ten-fold dilutions were made. Vortexing was performed in triplicate for each swab device and time point and aliquots of the vortex suspension were plated for viability counts. The time points for the SE method were also zero, 24 and 48 hrs at room temperature. Organisms tested were *Haemophilus influenzae* (HI), *Streptococcus pyogenes* (SP) and *Neisseria gonorrhoeae* (NG).

**Results:** Percent decrease recovery, compared to time zero, at 24 and 48 hrs (both methods) is as follows; SP: BD (RP method): 0%, 0%; STR (RP method): 68%, 69%; BD (SE method): 2%, 91%; STR (SE method): 49%, 37%. HI: BD (RP method): 44%, 60%; STR (RP method): 77%, 96%; BD (SE method): 30%, 38%; STR (SE method): 100% (no growth), n/a. NG: Did not survive to 48 hrs on either swab with either method. BD (RP method): 20%; STR (RP method): 61%; BD (SE method) 0%; STR (SE method) 100% (no growth).

**Conclusion:** In our study BBL™ CultureSwab™ Plus demonstrated better recovery of fastidious organisms. Careful evaluation of swab devices is important to enable selecting appropriate devices if processing and plating of specimens is delayed for up to 48 hrs.

## INTRODUCTION

The transport of clinical specimens is a critical component for accurate microbiological analysis (1). Swab specimens represent a significant percentage of diagnostic samples processed in clinical microbiology labs. The primary objective of transport swabs is to maintain the specimen as near its original state as possible with minimal deterioration (2). Most reference manuals addressing transport indicate that prompt delivery of samples to the laboratory, in less than 2 hours, is most important (3,4,5). Prompt delivery ensures the survival and isolation of fastidious organisms and minimizes overgrowth of normal flora or commensal bacteria. Prompt delivery also provides a more accurate diagnosis of the infectious disease process (4). However, the increasing use of outpatient treatment and centralization of lab services are among many factors that have prolonged transport time and thus increased the importance of careful selection and use of transport swab devices (1). Previous studies have also highlighted the difference in the viability of bacteria when held or transported at Room Temperature (20 – 25°C) versus 4°C. As the necessity for local and distant transport of specimens to testing facilities increases it is important that transport swab devices maintain microbial viability. Until recently there were no published criteria or standards available to measure the performance of microbiology transport systems however in December 2003, NCCLS through its consensus building process developed and published such a standard, “Quality Control of Microbiological Transport Systems; Approved Standard”, M40-A. This group is committed to providing a global forum for the development of harmonized standards and guidelines that facilitate safety, best practices, and quality patient care in the world’s health care community (6). This standard provides criteria to the manufacturers and end users of the transport devices to systematically evaluate devices for performance effectiveness and allow for internal validation of product effectiveness (1). We used this approved guideline as a basis for our comparison study of two commercial Amies Agar Gel transport swabs produced by two different companies. We tested a new version of Starplex StarSwab II manufactured under the REMEL™ BactiSwab™ brand label and a new version of BBL™ CultureSwab™ Plus named BD CultureSwab™ MaxV(+). We used ATCC cultures of three clinically important aerobic bacteria *Streptococcus pyogenes*, *Haemophilus influenzae*, and *Neisseria gonorrhoeae*. Products

were tested using test protocols based upon the Swab Elution Method and Roll-Plate Method described in NCCLS M40-A. As the risk of elevated specimen transport temperatures is an important issue we face in our health region during most of year, we limited our study to Room Temperature storage and holding conditions. Based on previous studies elevated temperatures appear to represent a greater challenge for organism survival and swab transport capability (7, 8, 9, & 10).

## METHODS AND MATERIALS

*Streptococcus pyogenes* ATCC 19615, *Haemophilus influenzae* ATCC 10211, and *Neisseria gonorrhoeae* ATCC 43069 were prepared from lyophilized cultures. They were passed twice before use on non-selective media. Two commercial Amies without charcoal swab transport systems were used:

<u>Swab product</u>	<u>Abbreviation</u>
BD CultureSwab™ MaxV(+)	(BD)
REMEL™ BactiSwab™	(STR)

Direct colony suspensions were made of isolated colonies from 18 to 24 hrs cultures (longer incubation times up to 48 hrs may be necessary for fastidious or anaerobic organisms) from non-selective media. A spectrophotometer was used to standardize the inoculum to obtain a 0.5 McFarland density (approx.  $1.5 \times 10^8$  CFU/mL).

Two methods, the Roll Plate (RP) and the Swab Elution (SE), were used to determine approximate CFU/mL.

**Roll Plate (RP):** After the 0.5 McFarland standard is prepared serial 1:10 dilutions of each organism is made to  $10^4$  CFUs/mL. 100 µL aliquots of inoculum were placed into a 96-well plate and the swabs allowed to absorb the inoculum for 10 seconds before being placed into the plastic carrier. 24 hrs and 48 hrs specimens were held at room temperature until plated. The 0 hr time was plated within twenty minutes onto appropriate media. The plates were streaked in three planes. Each dilution was plated in triplicate and incubated in appropriate atmosphere at 35°C.

**Swab Elution (SE):** After the 0.5 McFarland standard is prepared make a 1:10 dilution. Place 100µL of the 1:10 dilution into a 96-well plate and allow swabs to absorb the inoculum for 10 seconds. After incubation each swab is placed in 1 mL of 0.85% saline and vortexed for 15 seconds. Five tenfold serial dilutions are made. Vortex each dilution and add 100 µL to appropriate plate media. Perform in duplicate for each dilution. Spread inoculum evenly across the surface of the media. Incubate at 35°C in the appropriate atmosphere.

Plates were counted that had between 30 and 300 colonies. CFU counts for replicate plate cultures for each organism, time point and swab transport were averaged and recorded. The average number of colonies, times the dilution factor, determined the number of surviving viable cells.



BD



STR

## RESULTS

The results of Roll-Plate and Swab Elution studies are summarized in Table 1. The numbers reflect averaged scores from plates, with 30 to 300 colonies counted. In the original abstract viability was expressed in terms of percentage decline in growth compared to zero time counts with 100% reduction equivalent to a no growth score. Alternatively, for easier interpretation, in Table 1 viability is expressed as percent recovery compared to zero time counts. In this case 0% percent recovery would be equal to a no growth score. Table 2 has also been provided to show the decline in growth compared to zero time counts, as summarized in the background. In this case 100% decline in growth would be equal to a no growth score.

In the NCCLS M40-A standard criteria is described for interpretation of acceptable product performance using the Roll-Plate and Swab Elution methods. Using either methodology M40-A states that for an aerobic performance

claim, swab products should maintain the viability of *S. pyogenes* and *H. influenzae* for 48 hours and *N. gonorrhoeae* for 24 hours and demonstrate growth in accordance with specific acceptance criteria. In the Swab Elution method acceptable growth/performance means there should be no more than a  $3 \log_{10}$  ( $1 \times 10^3 \pm 10\%$ ) decline in CFU between the zero-time CFU count and the CFU of the swabs that were stored. In our Swab Elution study the BD swab was able to fulfill this performance requirement for 3/3 organisms tested whereas STR swab fulfilled acceptance criteria in 1/3 organisms. For the Roll-Plate method acceptable performance is interpreted as  $\geq 5$  CFU following the specified holding time from the specific dilution that yielded zero-time plate counts closest to 300 CFU. In our Roll-Plate study we recorded acceptable performance in 3/3 organisms with both BD and STR however, when we compared percent recovery from each system we noted a significant difference in the viability. In the Roll-Plate method we recorded significantly higher percentage recovery with BD compared to STR at all time points.

**Table 1:** Colony counts and percent recovery of three different organisms using two methods (Roll-Plate and Swab Elution), two swab types (BD and STR), and three time periods.

Roll-Plate	Time (Hours)	<i>S. pyogenes</i>		<i>H. influenzae</i>		<i>N. gonorrhoeae</i>	
		% Recovery	CFUs	% Recovery	CFUs	% Recovery	CFUs
BD	0	100%	$1 \times 10^6$	100%	$5.5 \times 10^5$	100%	$2 \times 10^5$
	24	>100%	$3 \times 10^6$	56.36%	$3.1 \times 10^5$	80%	$1.6 \times 10^5$
	48	>100%	$3 \times 10^6$	40%	$2.2 \times 10^5$		
STR	0	100%	$1.7 \times 10^6$	100%	$7.7 \times 10^5$	100%	$4.1 \times 10^5$
	24	32.35%	$5.5 \times 10^5$	23.37%	$1.8 \times 10^5$	39%	$1.6 \times 10^5$
	48	31.17%	$5.3 \times 10^5$	3.9%	$3 \times 10^4$		
Swab Elution		<i>S. pyogenes</i>		<i>H. influenzae</i>		<i>N. gonorrhoeae</i>	
BD	0	100%	$1.3 \times 10^6$	100%	$8 \times 10^4$	100%	$3.5 \times 10^4$
	24	100%	$1.3 \times 10^6$	70%	$5.6 \times 10^4$	>100%	$1 \times 10^5$
	48	9.2%	$1.2 \times 10^5$	62.5%	$5 \times 10^4$		
STR	0	100%	$5.4 \times 10^5$	100%	$1.3 \times 10^4$	100%	$3 \times 10^4$
	24	51.85%	$2.8 \times 10^5$	No growth	0	No growth	0
	48	62.96%	$3.4 \times 10^5$	No growth	0		

**Table 2:** Colony counts and percent decline in growth of three different organisms using two methods (Roll-Plate and Swab Elution), two swab types (BD and STR), and three time periods.

Roll-Plate	Time (Hours)	<i>S. pyogenes</i>		<i>H. influenzae</i>		<i>N. gonorrhoeae</i>	
		% Decline	CFUs	% Decline	CFUs	% Decline	CFUs
BD	0		$1 \times 10^6$	0%	$5.5 \times 10^5$	0%	$2 \times 10^5$
	24	0%	$3 \times 10^6$	44%	$3.1 \times 10^5$	20%	$1.6 \times 10^5$
	48	0%	$3 \times 10^6$	60%	$2.2 \times 10^5$		
STR	0		$1.7 \times 10^6$	0%	$7.7 \times 10^5$	0%	$4.1 \times 10^5$
	24	68%	$5.5 \times 10^5$	77%	$1.8 \times 10^5$	61%	$1.6 \times 10^5$
	48	69%	$5.3 \times 10^5$	96%	$3 \times 10^4$		
Swab Elution		<i>S. pyogenes</i>		<i>H. influenzae</i>		<i>N. gonorrhoeae</i>	
BD	0		$1.3 \times 10^6$	0%	$8 \times 10^4$	0%	$3.5 \times 10^4$
	24	2%	$1.3 \times 10^6$	30%	$5.6 \times 10^4$	0%	$1 \times 10^5$
	48	91%	$1.2 \times 10^5$	38%	$5 \times 10^4$		
STR	0		$5.4 \times 10^5$	0%	$1.3 \times 10^4$	0%	$3 \times 10^4$
	24	49%	$2.8 \times 10^5$	100%	No growth	100%	No growth
	48	37%	$3.4 \times 10^5$	100%	No growth		

## DISCUSSION

Swab transport systems are devices we generally take for granted that will always perform to a standard that is compliant with the changing needs and landscape of microbiology. We do not normally consider that these products could be a serious limiting factor in our quest to offer wider outreach services and more laboratory centralization. Swab studies in the past have compared one product with another but this approach does not ensure that either product will perform adequately. The new NCCLS M40-A standard provides test protocols which allow laboratories to make an independent assessment of swab performance against reasonable minimum performance criteria. This standard also provides manufacturers with performance targets that could help them improve their existing products or develop new products.

In our hands we found these M40-A test protocols easy to follow and while time consuming can be readily done in most labs to assess quality. We noted differences in performance between Roll-Plate and Swab Elution methodology but this may be a reflection of the fact that Roll-Plate is qualitative while Swab Elution is a quantitative procedure. The trend we recorded was in general the BD CultureSwab™ MaxV(+) was more likely to recover test organisms and in greater number when compared with REMEL™ BactiSwab™ manufactured by Starplex. Further testing is recommended to include other fastidious bacteria such as *Strep. pneumoniae* and anaerobic bacteria if a facility wishes to use Amies Agar Gel transport as a multipurpose bacteriology swab transport.

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