Modification of Swab Applicators Providing Full Compliance with NCCLS Standard M40-A

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ABSTRACT:

Background: Most swab/transport device manufacturers purchase swabs from subcontractors, thus have little control over the process or batch-to-batch variation. Vast improvements have been made in transport device media, packaging, and swab shelf life yet most commercial products do not fulfill all aspects of the NCCLS Performance Standard M40-A.

Methods: Copan, Brescia, Italy, has been focused on NCCLS compliance establishing control of applicator production by bringing all aspects of swab manufacturing in-house. A comprehensive review of swab literature consistently revealed hard data that the use of serum or albumin to coat swabs resulted in prolonged organism survival. This study compares a new Copan product, M40 Transystem Swab containing Amies medium (RED) with their standard Venturi Transystem Amies (BLU) and Starswab II with Amies (STR) Starplex Scientific Inc., Ontario, Can. M40-A procedures for swab vortexing of room temperature-stored swabs was employed using ATCC strains of Neisseria gonorrhoeae, Haemophilus influenzae, Streptococcus pyogenes, Fusobacterium nucleatum, and Peptostreptococcus anaerobius.

Results: The RED swab provided organism survival of all strains after 48h incubation at room temperature, thus, was in full compliance with NCCLS M40-A. The BLU device provided 48 h survival of all organisms except N. gonorrhoeae. STR produced < 1% recovery of N. gonorrhoeae @ 24h and failed to maintain viability of F. nucleatum, H. influenzae, or P. anaerobius @ 48h.

Conclusions: Pioneers of clinical microbiology proved that organism survival on swab applicators could be enhanced by coating them with bovine serum albumin. This is a poorly defined material with lot to lot variation but Copan has refined the protective material by using a simple vegetable protein admixed with specific amino acids as an additive to the glue used to wind swabs onto applicator sticks. Microbiology has simply been a boutique application for Q-tips, and these data suggest that controlled manufacturing incorporating historical performance enhancers has elevated the swab applicator to a medical device with defined performance characteristics.

INTRODUCTION:

Most swab transport manufacturers purchase the swab applicator portion of their respective devices from a subcontractor, thus losing control of how this critical element is manufactured. There is considerable batch to batch variation especially in the uptake of specimen volume and there have been some toxicity issues these authors and others have noted in past publications [1,2].

With the issuance of NCCLS Standard M40-A, swab transport devices have been hard-pressed to meet the Standard's demands of 48h survival for a broad range of bacteria of which some have diametrically opposing requirements for survival. M40-A furthermore requires not only freshly made devices to meet the criteria but those 1-2 years on the shelf. This is a tall order which has not been fully attained by most manufacturers.

A number of tactics have been advanced to improve the performance of swab transport devices. Vast improvements in media and in the sterilization process have eliminated some processing-induced media damage. Packaging has been altered to protect the transport medium and increase shelf life now demanded by the industry. These alterations in the basic swab transport device have pretty well exhausted avenues for improvement.

In light of the new challenge presented by M40-A, Copan has made an exhaustive review of historical scientific literature concerning the protective benefits of protein, albeit poorly defined in old literature, and brought it forward into the 21st century. Rather than using an undefined 'soup' such as bovine serum albumin or albumin, Copan has identified specific vegetable proteins and amino acids and has incorporated them into the standard winding process used to affix the swab fibers to the stick. This is a total in-house process with control over every manufacturing step.

The following data demonstrate, once again, the protective effects of specific proteins on organism survival and allows the clinical microbiology community to have at their disposal an excellent product for the collection and transport of a broad range of bacterial agents.....all in compliance with NCCLS Standard M40-A.

MATERIALS & METHODS:

Inoculum density was standardized using a $BaSO_4$ turbidity standard and a spectrophotometer for the list of organisms below in accordance with protocols specified by NCCLS M40-A. Inoculum preparation, dilutions, vortexing, plating, incubation, and quantitation followed M40-A guidelines Inoculated swabs were stored at 20 - 25°C (RT; room temperature). Storage at 4°C was not performed in this study because previous work had demonstrated superior survival with all swab systems/organisms at 4°C[3]. It is difficult to guarantee that samples have been correctly stored and shipped at 4°C. Ambient temperature represents a tougher and essentially realitic challenge for transport devices. Three swabs for each incubation duration (zero-time, 24h and 48h) were prepared for each device and organism (9 samples per microorganism/swab combination).

Organism	Strain	Duration of Incubation
Streptococcus pyogenes	ATCC 19615	48 hours
Haemophilus influenzae	ATCC 10211	48 hours
Peptostreptococcus anaerobius	ATCC 27337	48 hours
Fusobacterium nucleatum	ATCC 25586	48 hours
Neisseria gonorrhoeae	ATCC 43069	48 hours

SWAB DEVICES EVALUATED

Two popular, frequently used swab devices and one innovative new device were evaluated for compliance with M40-A criteria. Starswab II consisting of a single swab and clear Amies gel medium (STR; Starplex Scientific Inc., Etobicoke, Ontario, Canada), Copan's standard Amies Venturi Transystem (STD) and a new Copan product, M40 Transystem Swab containing Amies medium (M40; Copan Diagnostics Inc., Corona, CA).

VORTEX METHOD

After appropriate incubation times and holding temperatures, swabs were vortexed for 15s and serial dilutions made in saline from 10^{-1} - 10^{-3}. Duplicate platings were made in accordance with M40-A. For statistical accuracy, zero time plates had to have colony counts between 30 - 300 for testing to be valid. The final count was an average of the CFU of six plates (3 swabs with duplicate subcultures) from the dilution with 30 - 300 CFU.

RESULTS:

(See Table 1) Results of organism recovery following swab vortexing and room temperature storage (20-25°C). M40-A stipulates acceptable recovery or viability to be no more than a 3 log decline in CFU from zero time counts. Loss of viability was most significant with Neisseria gonorrhoeae. Starplex provided no survival after 24h and both Starplex and the Copan standard swabs fell out of the acceptable range after 48h with Neisseria gonorrhoeae. No Peptostreptococcus anaerobius survived after 24h with Starplex. The new Copan M40 swab was within acceptable limits for all organisms after the full 48h incubation at room temperature. Greater than a 1 log increase in counts occurred after 24h for both Streptococcus pyogenes and Haemophilus influenzae with the M40 swab. The NCCLS Standard specifies that overgrowth be accessed by storing swabs at 4°C. Previous work by many has demonstrated the effectiveness of preventing organism reproduction in swab transport devices when they are refrigerated. Refrigeration is not always possible nor practical so the real challenge for swab manufacturers is to maintain fastidious organism survival at room temperature. The potential for overgrowth of unwanted commensals is the downside and must be managed, when necessary, by the use of selective media and refrigeration when possible.

Swab Compliance with NCCLS M40-A

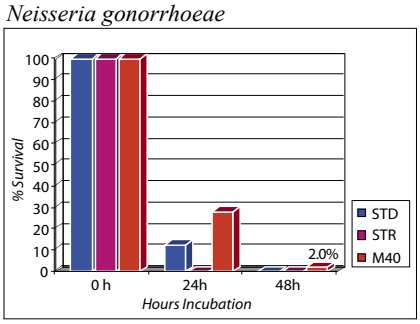
Vortex Method: 20-25°C Storage (Recovery Study)

Table 1

	Organism Tested	*Streptococcus pyogenes*			*Haemophilus influenzae*			*Neisseria gonorrhoeae*			*Fusobacterium nucleatum*			*Peptostreptococcus anerobius*		
Increase (CFU)	>1 $\log_{10}$		●	●		●	●									
	1 $\log_{10}$															
	CFU @ 0 Time	□★●	□★	□	□★●			□★●	●		□★●	●		□★●	●	
	1 $\log_{10}$			★		□★	□		□			□★	●		□	●
	2 $\log_{10}$									●						
	3 $\log_{10}$												□			□
Decrease	>3 $\log_{10}$						★		★	□★			★		★	★
	Incubation	0	24	48	0	24	48	0	24	48	0	24	48	0	24	48

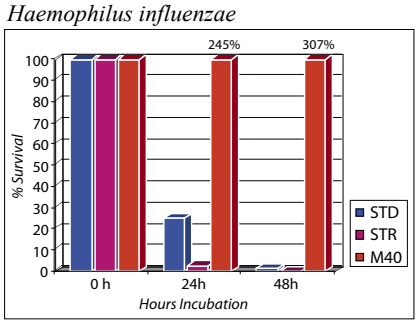
□ Copan Standard ★ Starplex Starswab II ● Copan M40 □★● Exceeds acceptable range

Neisseria gonorrhoeae



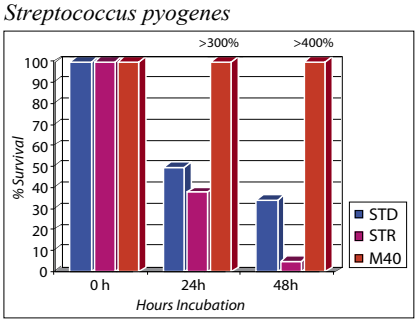
NCCLS M40-A sets the standard for Neisseria gonorrhoeae recovery for 24h. Data in this graph demonstrate the enhanced recovery of this pathogen at 24h as well as viable, recoverable organisms remaining at 48h thus exceeding the requirements of M40-A.

Haemophilus influenzae



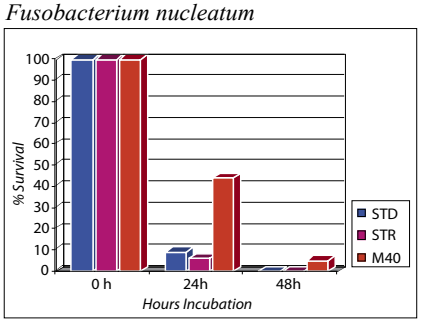
Similar results were noted with Haemophilus influenzae as with S. pyogenes. After 48h incubation, neither the Copan Standard swab nor Starplex provided much more than negligible recovery while Copan M40 apparently promoted organism multiplication under identical storage conditions.

Streptococcus pyogenes



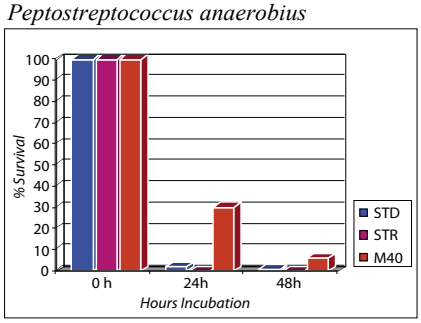
Percent survival from zero time through 48h incubation at room temperature with Streptococcus pyogenes. Copan Standard provided a higher count of viable organisms than Starplex. The new Copan M40 device promoted a 3 to 4 fold increase in S. pyogenes at room temperature.

Fusobacterium nucleatum



These data clearly demonstrate that Copan M40 was the only device tested to have achieved conformance with NCCLS M40-A requirements for the anaerobe Fusobacterium nucleatum.

Peptostreptococcus anaerobius



Peptostreptococcus anaerobius was recovered after 48h of room temperature incubation with Copan M40-A (6% of zero time counts). Copan Standard provided 2% of the original count at 24h and <1% at 48h while Starplex produced no recoverable organisms after 24h incubation.

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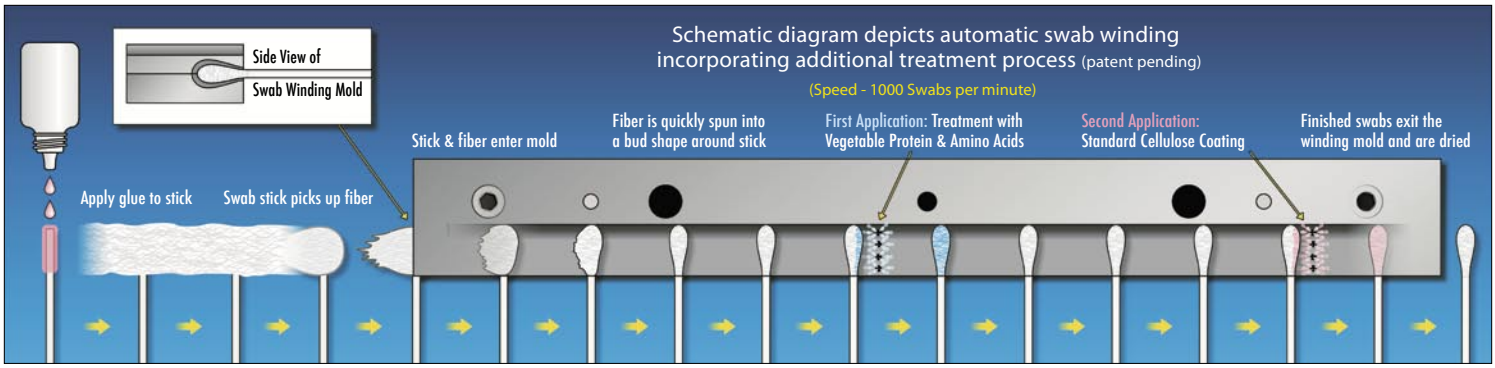

DISCUSSION:

Many of the most basic beliefs of the microbiology laboratory have undergone a transformation in recent years. New challenges have appeared such as cost cutting yet increased pressure to provide even faster turnaround times, dealing with emerging organism resistance, and adding tests based on molecular biology. These requirements have promoted review of time-honored laboratory procedures and policies by non-laboratorians, and in many cases, these procedures and policies have been modified or discarded.

Likewise, centralized group purchasing based on contract bidding has decreased laboratory costs but has often resulted in the necessity to use media and test kits from alternative suppliers with unfamiliar performance characteristics. Expendables are put on bid without concern for proven quality or performance. Swab transport devices are a prime example of how this purchasing practice often results in a 'low-bidder' device that has been assembled from sub-contracted components at the lowest possible cost. Often, microbiologists have no input into the selection of these consumables and they are provided in much the same manner as institutional facial tissue, toilet paper and hand towels.

NCCLS M40-A has presented a real challenge to swab transport manufacturers and status quo approaches are not options. A total re-design of the swab applicator is the remaining element to consider. For a number of reasons (cosmetic appearance, fear of undefined chemical additives, and a certain arrogance that historical work in microbiology is outdated for our high tech needs) the microbiology community has moved away from the known protective properties of charcoal, serum and albumin coating of swabs. Such products are still commercially available in Europe (charcoal-treated swabs from the State Serum Institute in Denmark) and are actually promoted for the recovery of N. gonorrhoeae by the World Health Organization (webmaster@whosea.org).

Microbiologists must re-familiarize themselves with the previously described benefits of serum, albumin, and charcoal coating of swabs to fully understand the importance of the Copan M40 swab[4-8]. Perusal of historical literature elucidated the notion that the swab applicator is not just an inanimate tool used to mechanically move specimen material/microorganisms. Apart from the mechanical and hydraulic importance of this tool for collecting and releasing the sample....it can also play an active role in maintenance and protection of live organisms.

Copan has brought the protective properties of protein into the modern age and rather than use undefined protein like serum or albumin, they have selected specific vegetable proteins and amino acids. Copan is one of the few companies having the total manufacturing process in-house so they have control over all facets of the operation and have incorporated these protein materials into the winding process that secures the swab fibers in place (note the graphic describing this entire innovative process). These specially prepared swabs have met criteria for biocompatibility and have passed the requirements for (1) irritation, (2) sensitization, and (3) cytotoxicity. Copan's M40 Transystem Swab is being introduced at this meeting as Becton Dickinson's product, BD CultureSwab™MaxV(+).

CONCLUSIONS:

While it is debatable whether swab/transport devices are appropriate for the collection and transport of clinical specimens, it remains a fact that such devices are used daily and often are transported from satellite facilities with a commensurate delay in processing. In light of NCCLS M40-A, if such devices are used, they should comply with the Standard and be of the highest quality. In the past, microbiology has been only one of a number of boutique applications for what are essentially 'Q-tips'. Ear wax removal and viability of Neisseria gonorrhoeae are at the far ends of the swab applicator spectrum. The data in this study suggested that controlled manufacturing of swabs and the incorporation of historical performance enhancers has elevated the swab applicator to a medical device with defined performance characteristics.

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