Reproducibility of Swab Survival Studies Evaluated with the CLSI Method using Remel BactiSwab® and BBL™ CultureSwab™ Plus for Aerobic, Facultative and Obligate Anaerobic Bacteria.

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ABSTRACT

Background: The reproducibility of swab survival studies have been difficult to document. We compared replicate analysis testing using two swab systems; The Remel BactiSwab®, Amies without Charcoal Transport Media (Remel Inc., Lenexa KS) and the BD BBL™ CultureSwab™ Plus, Amies without Charcoal Transport Media. Organisms used to challenge the transport systems were chosen from the CLSI M40 document which included: Neisseria gonorrhoeae ATCC® 43809, Haemophilus influenzae ATCC® 10221, Streptococcus pneumoniae ATCC® 6305, Peptostreptococcus anaerobius ATCC® 27337 and Prevotella melaninogenica ATCC® 25845. Methods: Serial dilutions were performed for each organism beginning with a 0.5 MacFarland standard to yield a final concentration of organisms of approximately 10^6 cfu/ml (10^6 for S. pneumoniae and P. melaninogenica) into the culture swabs. Five swabs of each type were inoculated with organism suspensions of 0.1 ml of the aforementioned dilutions. The swabs were held at room temperature (RT) 20-25°C and 2-6°C and cultured at 0.24 hr (N. gonorrhoeae only) and 48 hr. The swabs were removed from the transport device, rinsed in 1ml of sterile saline and 0.1 ml was plated to duplicate agar plates. This dilution resulted in between 100 and 1000 cfu/ml on the culture plates. The entire process was repeated by a separate technologist. Culture plates were evaluated following 24 and 48 hrs incubation and colony counts were generated. Results: The results of the study show that the Remel swabs consistently out performed the BBL swabs. In particular, for NG and SP survival occurred for 48 hr at 4°C using Remel, however using the BBL swab, minimal growth was seen for NG at 24 hr. Counts ranged from 2.6 X 10^2 to 8.2 X 10^4 for NG and 5.0 X 10^2 to 1.56 X 10^2 for SP. Average counts showed spurious results throughout the study. Conclusions: Variation occurs using the standard method for comparing swab transports. Additional work is needed to determine whether such variations result in clinical significance.

INTRODUCTION

The collection and transport of clinical specimens to the microbiology laboratory are essential to dispensing good quality care. The evaluations of transport systems are essential since “not all swabs are created equal”. The CLSI have developed guidelines for evaluating transport systems. The purpose of this study was to compare the reproducibility of the CLSI procedure as well as tech to tech variation using this method

MATERIALS & METHODS

Swabs studied included the following:
1. Remel BactiSwab®, Amies without Charcoal Transport Media.
2. BD BBL™ CultureSwab™ Plus, Amies without Charcoal Transport Media

Testing protocol:
Each organism will be tested 10 times using (5 tested by each tech) different inoculums and dilutions each time. We determined which of the following dilutions would result in colonies that could be enumerated and then used that dilution for the study. Therefore each organisms would use either; 10^5, 10^6 and 10^7 dilutions (in duplicate).

Holding temperatures:
Room temperature (~20°C) and 4°C.

Holding times:
0 hour (baseline counts), 24 hours (GC only) and 48 hours.

Testing protocol:
Make a 0.5 McFarland standard (10^6) of each organism in saline. Serially dilute each organism from 10^5 to the target dilution. Aliquot 100 µL of the testing dilution (10^5, 10^6 or 10^7) into 12 microwells dip the swab into the microwell for 10 seconds and place the swab into the appropriate transport medium plate out the 0 hour swabs immediately by inoculate the plates using the saline wash protocol. These plates will be used as the baseline counts for each holding temperature (RT and 4°C) place the 24 (GC) and 48 hour swabs at the assigned holding temperature (RT and 4°C) for the appropriate incubation time (24 & 48 hours). The 24 & 48 hours swabs will be plated out in the same way at the appropriate times. Read and count the aerobic organisms after 24 hours of incubation and read the anaerobic plates after 48 hours of incubation (when counts were low, the GC plates were incubated and read for up to 5 days). Results will be reported according to the M-40 document in CFU’s.

RESULTS

Neisseria gonorrhoeae ATCC® 43809
Peptostreptococcus anaerobius ATCC® 27337
Haemophilus influenzae ATCC® 10221
Streptococcus pneumoniae ATCC® 6305
Prevotella melaninogenica ATCC® 25845

Fresh lyophilized cultures from ATCC will be used for this study. The organisms were passed twice to appropriate media prior to performing the study.

BIBLIOGRAPHY

7. CLSI M-40 SWAB REPRODUCIBILITY STUDY PROTOCOL.

CONCLUSION

The evaluations of swab samples using the CLSI document can be seen in the tables above. It is clear that variation occurs between techs and organisms. In particular for Haemophilus influenzae, Neisseria gonorrhoeae, Streptococcus pneumonia, and Prevotella melaninogenica replicate trials were performed by the technologist using the same procedure which resulted in different results. Care must be taken when evaluating data on the reliability of swab transport systems.