Comparison of Methodologies Described in NCCLS Document M40-P Quality Control of Microbiological Transport Devices

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Abstract:

Introduction: Prior to production of NCCLS document M40-P, Quality Control of Microbiological Transport Systems; Proposed Standard, there had not been a recognized procedure for determining the effectiveness of transport devices. Here we describe our experience using the new standard to evaluate 6 commercial swab transport systems.

Methods: ATCC strains of *Pseudomonas aeruginosa, Streptococcus pyogenes, Streptococcus pneumoniae, Haemophilus influenzae*, and *Neisseria gonorrhoeae* were inoculated to two different swab transport devices, (Amies without charcoal and liquid Stuart's), from each of three manufacturers, Copan Diagnostics, Starplex Scientific Inc., and Medical Wire & Equipment Co. Swabs were held at ambient (20-25°C) and refrigeration (4-8°C) temperatures and held for 0, 24, or 48 hours, and then plated on appropriate media (SBA or CHOC). Viable bacteria were counted following incubation. Growth recovery was determined for both a roll-plate and swab elution technique. Base-line counts were obtained from media inoculated at zero time. Performance characteristics were compared to acceptance criteria described in M40-P.

Results: Trends in growth recovery were similar using either the roll-plate or swab elution techniques, irrespective of the organism, holding time or temperature. Both the Amies and liquid Stuart's transport devices manufactured by Copan met or exceeded the acceptance criteria for all organisms. The Amies and liquid Stuart's transport devices manufactured by Starplex met or exceeded the acceptance criteria for all organisms, except *N.gonorrhoeae* and *H. influenzae* at ambient temperature. In addition, the liquid Stuart's failed to meet acceptance criteria for *S. pneumoniae* at ambient temperature. The Amies and modified liquid Stuart's transport devices manufactured by Medical Wire met the acceptance criteria only for *P.aeruginosa*. The modified liquid Stuart's media evaluated using the swab elution technique also met criteria for *S. pyogenes*.

Conclusion: We found the new proposed NCCLS standards provide an excellent means of evaluating swab transport systems. The roll-plate method described in the document is well suited for most laboratories, whereas, we found the swab elution technique to be costly and labour intensive without yielding additional information.

Introduction:

Proper collection and transport of microbiological specimens is essential for the successful isolation of pathogens. Although there are many swabs produced by various manufacturers, prior to the new NCCLS M40-Proposed Standard there were no standardized procedures for evaluating swab transport systems or other transport systems for microbiologic analysis. Because of this, manufacturers have had to rely on their own internal quality control protocols to evaluate their products. The new standard addresses this issue and provides methodologies and acceptance criteria for manufacturers and laboratorians to evaluate and compare products.

The purpose of this study was to evaluate 6 commercial swab transport systems using the methodologies and acceptance criteria outlined in M40-P, Quality Control of Microbiological Transport Systems; Proposed Standard, and to determine their appropriateness and use in evaluating and comparing swab transport systems. Aerobic, fastidious, and anaerobic organism test panels were used in evaluating the methodologies, however, only data for the aerobic and fastidious organism test panels are presented here.

Methods and Materials:

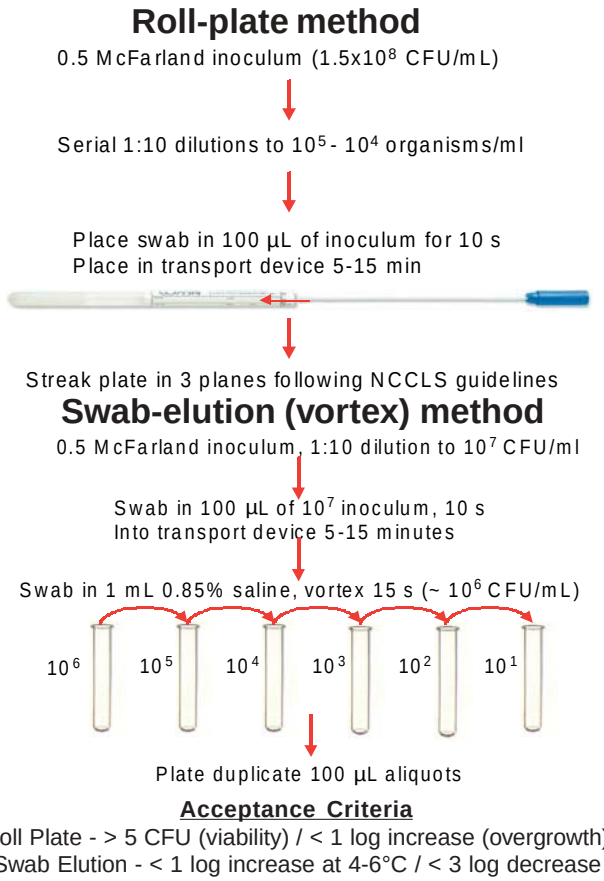
Viability Test Panel
- *Streptococcus pyogenes* (ATCC 19615)
- *Streptococcus pneumoniae* (ATCC 6305)
- *Haemophilus influenzae* (ATCC 10211)
- *Neisseria gonorrhoeae* (ATCC 43069)

Overgrowth Test Panel
Pseudomonas aeruginosa (ATCC BAA-427)

Swab Transport Devices
- Copan Amies gel, Lot # 2109 (**CA**)
- Copan Liquid Stuart's,Lot #2112 (**CL**)
- Medical Wire Amies gel, Lot #02C18 (**MA**)
- Medical Wire Liquid Amies, Lot #02C15 (**ML**)
- Starplex Amies gel, Lot #2B20A (**SA**)
- Starplex Liquid Stuart's, Lot #1M12A (**SL**)

Holding Temperatures
Viability studies - 4-8°C and 20-25 °C
Overgrowth studies - 4-8°C

Holding times
- Zero time (baseline)
- 24 hours
- 48 hours



Acceptance Criteria
Roll Plate - > 5 CFU (viability) / < 1 log increase (overgrowth)
Swab Elution - < 1 log increase at 4-6°C / < 3 log decrease

Statistical Analysis

Absolute colony counts were used for outcome measures with the roll-plate method. Change in numbers of organisms recovered, measured by +/- log10 change from absolute colony counts at zero time, was used with the swab elution method. Repeated measures analysis of variance was used to assess the impact of holding temperature and times. Logistic regression analysis was used to compare the rates of meeting acceptance criteria between the roll-plate and swab elution methods, for each swab type, at each time point, and simulated holding temperature.

Results:

Swabs varied in performance with Copan Amies and Stuart's meeting acceptance criteria most often, followed by Starplex Ames and Stuart's, followed by Medical Wire Amies and Stuart's. Differences in performance reached statistical significance ($p<0.001$). In addition, overall Amies gel performed better than Liquid Stuart's regardless of manufacturer, temperature or time ($p<0.001$).

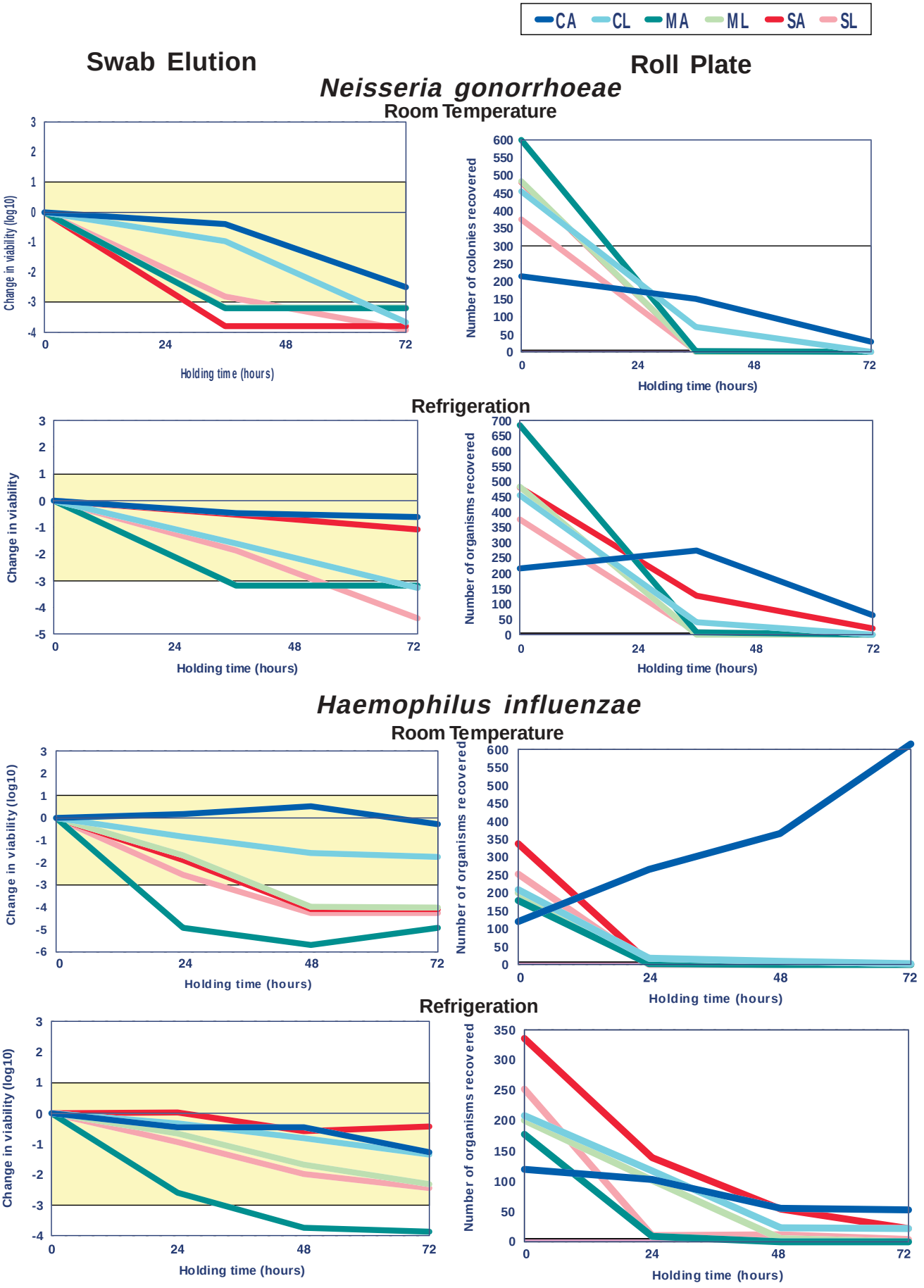
The Amies and Liquid Stuart's from Copan Diagnostics met acceptance criteria for all organisms held at both refrigerated and room temperatures.

Overgrowth, however, was encountered with *S.pyogenes* at room temperature.

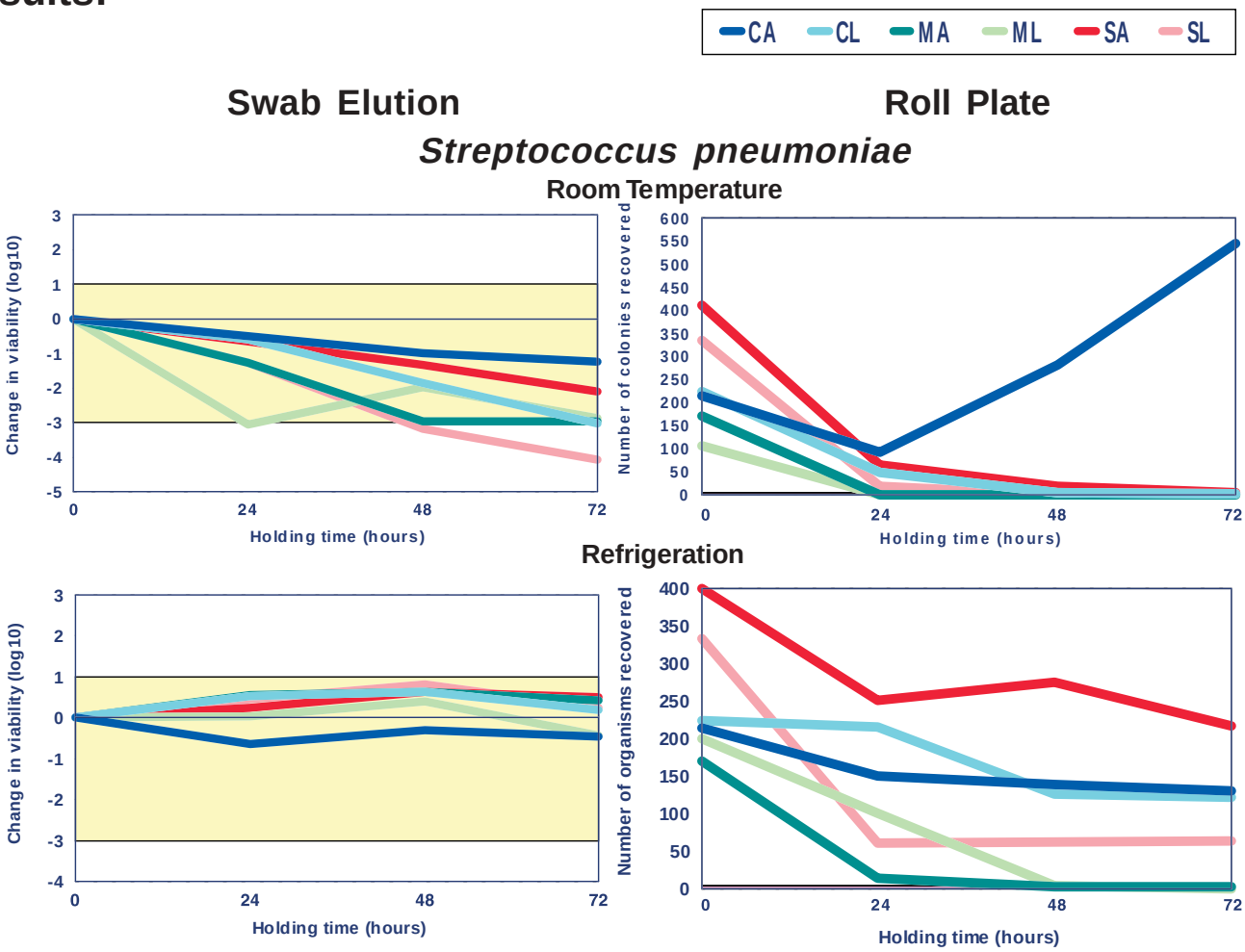
The Amies from Starplex Scientific met acceptance criteria for *S. pyogenes, S. pneumoniae,* and *P. aeruginosa* at both simulated transport temperatures, but failed to meet acceptance criteria for *N. gonorrhoeae* and *H. influenzae* at room temperature. Starplex Stuart's met acceptance criteria for *S. pyogenes* at both temperatures, but failed to meet acceptance criteria for *N.gonorrhoeae, S.pneumoniae,* and *H. influenzae* at room temperature.

Amies and Stuart's from Medical Wire performed poorly overall at room temperature, but the Amies met acceptance criteria for *N.gonorrhoeae, S. pyogenes,* and *S.pneumoniae* at refrigerated holding temperatures; while, Stuart's met acceptance criteria for *S. pyogenes* and *H. influenzae* only.

Results:


CA
CL
MA
ML
SA
SL
Swab Elution
Roll Plate
Neisseria gonorrhoeae
Room Temperature
Change in viability (log10)
Holding time (hours)
Number of colonies recovered
Refrigeration
Change in viability
Number of organisms recovered
Haemophilus influenzae
Room Temperature
Refrigeration

Results:



Discussion:

The proposed NCCLS standard M40 provides consistent, reproducible methodologies for manufacturers and laboratorians to validate and compare swab transport devices.

The aerobic organisms in the test panel provide an acceptable overview of product performance; however, the inclusion of *P.aeruginosa* for assessing viability on swab transport devices held at ambient temperature proved unnecessary. Strain ATCC BAA-427 is included in the test panel to assess overgrowth, because it is known to grow rapidly on swab transport devices held at room temperature, but not at refrigeration temperature - including this organism to assess loss in viability did not contribute additional information.

The two methodologies described in M40-P, roll-plate and swab elution, provided similar information. Although there were minor differences between results obtained with each method, variance did not meet statistical significance.

The swab elution method was more sensitive (able to detect decreases in viability equal to 3 logs), however, it was more labor-intensive, cumbersome, and expensive and did not contribute significantly to the information obtained using the roll-plate method. In comparison, the roll-plate method was much less labor-intensive and more suitable in the clinical lab setting. Although manufacturers may choose to perform both test methodologies, laboratories should choose the test method that most closely resembles their processing protocols.

Refrigeration of the samples was clearly superior for all organisms and all devices tested ($p<0.001$). Recommendations to hold swab transport devices at ambient temperatures during transport need to be re-addressed based on the superiority of refrigerated holding temperatures in preventing loss of viability.