EVALUATION OF BACTERIAL SURVIVAL IN TWO SWAB TRANSPORT SYSTEMS INCUBATED AT TWO EXTREMES OF TEMPERATURE.

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ABSTRACT

Background: Collection and transport of bacterial specimens to the laboratory is a critical component in the success of the diagnostic process. This is under particularly scrutiny in Brazil because of the geographic size of the country and the latitude. To assess sample release characteristics, LEMC, a Brazilian reference microbiology laboratory, evaluated two of the most commonly used Amies agar transport swabs: Copan Transystem (Copan, USA) and Cral-plast (EuroMed, Italy), using protocols proposed by the NCCLS standard M40–P. **Methods:** The following isolates were evaluated for survival after incubation at room (25°C) and refrigerator (4°C) temperatures using both swabs systems: *N. gonorrhoeae* ATCC 43069, *H. influenzae* ATCC 10211, *S. pyogenes* ATCC 19615, *S. pneumoniae* ATCC 6305 and *N. meningitidis* ATCC 13090. Two protocols were performed using a roll-plate and a vortex elution methods. For both, a 0.5 McFarland suspension of each strain was performed in 0.85% physiological saline from an 18 hour growth of the organism. Various dilutions were prepared and the swabs were inoculated in triplicate with 100µl of each organism suspension. Swabs were held at 25°C and 4°C for 0, 6, 24 and 48 hours. Bacterial survival was evaluated after 24-48 hours incubation at 35°C. **Results:** Cultures from all swab dilutions were averaged. Bacterial recovery from swabs held for 0 hour were similar for both systems. However, after 6, 24, and 48 hour incubation, bacterial recovery rates from the Cral were dramatically lower compared to that from the Copan. Storage at 4°C demonstrated to be excellent to control overgrowth, although Cral swab showed lower recovery after incubation in either 4°C or 25°C. **Conclusion:** The recovery of test organisms did not appear to be method dependent. Only Copan swab was able to comply with all criteria for each organism's survival at all holding temperatures and time points as described in NCCLS M40–P. The proposed standard provides an important means for determining acceptable viability performance of transport swabs and assistance with the selection of a swab device for use under diverse temperature and prolonged transit time conditions.

INTRODUCTION

The transport of bacterial specimens to the laboratory is a critical component in the success of the tests, either for clinical or research purposes. Transport time and temperature is now a major concern as the original concept and design of swab transport devices is 30-40 years old and they were developed in a time when the patient was only minutes away from the laboratory. A good system of quality control of swabs is important as ambient transport temperature can vary dramatically depending on the time of the year and latitude. This problem is particularly true for Brazil because of the huge geographic size of the country and the latitude which means testing labs are often far from the patient and ambient temperatures are above 25°C for many months of the year. No performance standard currently exists for swabs and we at LEMC, a Brazilian reference microbiology laboratory, decided to evaluate two of the most commonly used Amies agar transport swabs in Brazil: Copan Transystem (Copan Diagnostics, California, USA) and Cral swab (EuroMed, Italy) using protocols described in a proposed NCCLS standard M40–P.

METHODS

The following bacterial strains were evaluated for survival after incubation at room (25°C) and refrigerator (4°C) temperatures using two transport swabs systems.

Bacterial strains:

1. *Neisseria gonorrhoeae* ATCC 43069
2. *Neisseria meningitidis* ATCC 13090
3. *Haemophilus influenzae* ATCC 10211
4. *Streptococcus pneumoniae* ATCC 6305
5. *Streptococcus pyogenes* ATCC 19615

Transport swab systems:

Copan Transystem (Copan, USA) and Cral-plast (EuroMed, Italy) were included in the evaluation. Both transport systems consist of a sterile peal pouch containing a rayon tip swab and Amies agar gel medium without charcoal.

Two protocols were performed using a roll-plate method and a vortex elution method.

Roll-Plate Method:

- A 0.5 McFarland suspension in saline was prepared, from an 18-24hr culture of each organism;
- From this suspension, four 1:10 serial dilutions (1:10, 1:100, 1:1000 and 1:10.000) were prepared;
- For all organisms, the 10-2, 10-3, and 10^{-4} dilutions were used to inoculate the swabs;
- 100µl of each organism suspension was transferred into wells of a microtiter plate using a volumetric pipette;
- Each swab type was rolled into the 100µl suspension (10 seconds) to completely absorb the inoculum and then placed into the transport device and held for the appropriate time/temperature (25°C and 4°C for 0, 6, 24 and 48 hours);
- For baseline counts (0hr), three swabs of each organism/dilution were removed from the transport device after 15 minutes and spread over the entire agar surface using the roll-plate technique;
- Swabs were plated out at the end of each time period. Plates were incubated at 35°C for 24-48 hours. Counts were then performed.

Vortex Elution Method:

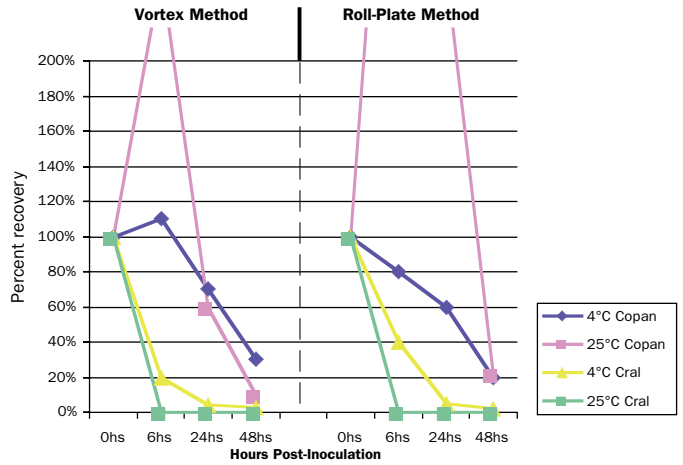
- A 0.5 McFarland suspension in saline was prepared, from an 18-24hr culture of each organism. The 0.5 suspension was diluted (1:10) in saline;
- 100µl of each organism suspension was transferred into wells of a microtiter plate using a volumetric pipette;
- Tests were performed in triplicate: 24 wells for each swab (time points 0, 6, 24 and 48 hours at both temperatures: 25°C and 4°C incubation);
- Each swab type was rolled into the 100µl suspension (10 seconds) to completely absorb the inoculum and then placed into the transport device and held for the appropriate time/temperature (25°C and 4°C for 0, 6, 24 and 48 hours);
- The zero hour swab were removed from the transport device within 15 minutes;
- After hold in appropriate time/temperature, all the swabs were placed in a tube with 1ml sterile saline and vortex for 15 seconds. Five 10-fold serial dilutions were prepared in saline (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} & 10^{-5});
- Each tube were vortexed. A 100µl of the suspension were plated in appropriate media and incubated at 35°C for 24-48hrs;
- Counts were then performed. Average counts for 6, 24 and 48 hrs are compared to the zero hour counts for the same dilution and organism.

CONCLUSION

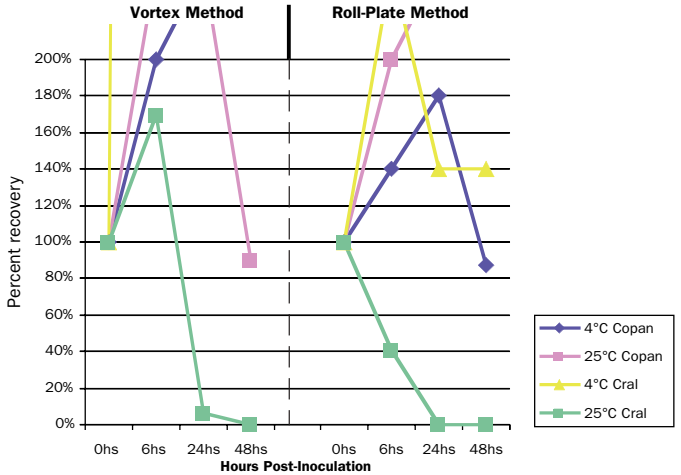
1. All the organisms tested showed rapid growth at 25°C of the original inoculum using the Copan Transport Swab;
2. Overgrowth of these organisms were controlled at 4°C;
3. Optimal recovery of the fastidious and non-fastidious organisms tested, was at 4°C, including *Neisseria meningitidis*;
4. Serious considerations should be given to recommend transport specimens for bacterial culture at 4°C instead of room temperature, including genital specimens cultured for *Neisseria gonorrhoeae*;
5. Two common methods were evaluated in this study and the recovery of test organisms did not appear to be method dependent;
6. Only Copan Transystem was able to comply with all criteria for each organism's survival at all holding temperatures and time points;
7. It is of crucial importance that institutions evaluate their specimen transport systems to ensure specimen integrity.

RESULTS:

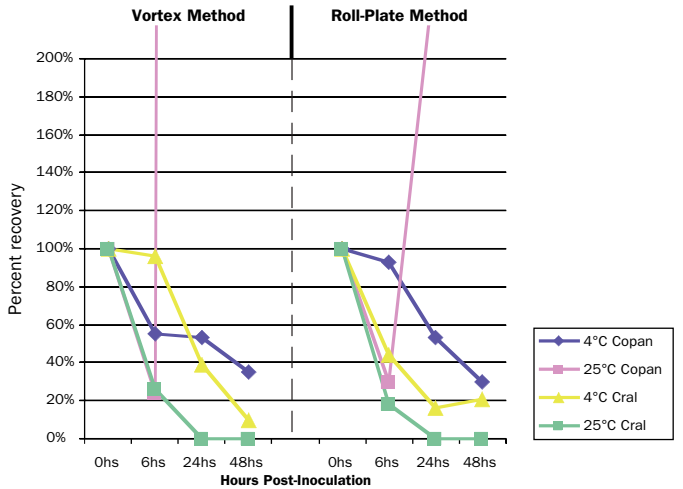
Haemophilus influenzae:



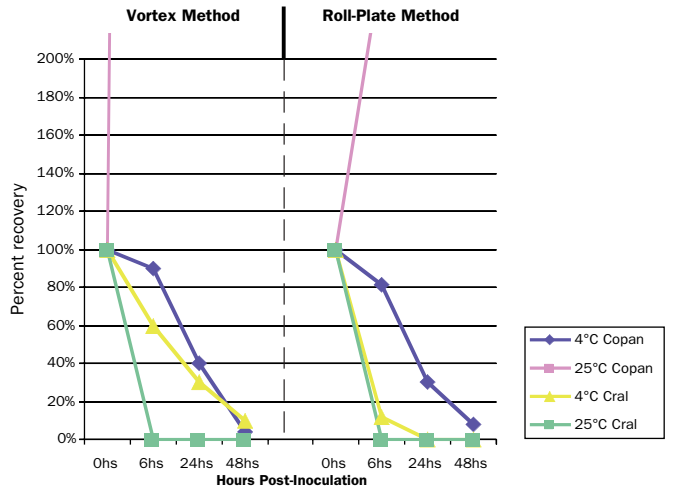
Streptococcus pyogenes:



Streptococcus pneumoniae:



Neisseria meningitidis:



Neisseria gonorrhoeae: