

EFFECTS OF TEMPERATURE ON FASTIDIOUS ORGANISM VIABILITY DURING SWAB TRANSPORT

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INTRODUCTION

Mergers, affiliations, and development of core laboratory testing sites have impacted many microbiology laboratories in the past several years. A shift from in-patient medicine to an out-patient based practice and same-day surgery and clinic visits have produced problems in maintaining specimen integrity during prolonged specimen transport from outlying areas. Chemistry and hematology specimens can often be kept cold or frozen without appreciable analyte deterioration. Maintaining the viability and appropriate ratios of microbes, many of which are fastidious and have been touted as being sensitive to extremes in temperature, presents a larger problem.

Guidelines are inconsistent and dated in suggesting temperatures at which various specimens should be held during transport and storage for the most efficacious recovery of fastidious bacteria. This study was designed to help our laboratory network determine the best temperature for specimen transport. Initial investigation using room temperature and 4°C to test the survival of clinical isolates of fastidious organisms commonly isolated from patient specimens provided results that were surprising, deviated from previous dogma, and were significant enough to prompt a more detailed evaluation of temperature effect.

MATERIALS & METHODS

Saline Broth Study:

1. Inocula of three clinical isolates each of *Haemophilus influenzae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Streptococcus pneumoniae*, and group A streptococcus were prepared by using a 1:10 dilution of suspensions prepared in a BBL Prompt (approximately 1.5×10^8 CFU/ml; Becton Dickinson, Cockeysville, MD.)
2. Tubes containing 2.5 ml of sterile saline and tryptic soy broth (TSB; Remel, Lenexa, KS) were each inoculated with bacterial suspensions of the clinical isolates and placed at 24°C (room temperature; RT) and 4°C for 72h. Periodic colony counts were determined by preparing duplicate plates for each organism/tube/temperature configuration using a 0.001-ml calibrated loop. Colony counts were averaged and expressed as a percentage of the baseline quantitation (0 time).

Swab Study:

1. The protective effects of various swab media types and temperature of storage were studied by inoculating swabs in a previously described manner.¹ Swab devices consisted of (1) a 1 dry swab prepared by removing the stick from a Culturette device (DRY; Becton-Dickinson, Cockeysville, MD), cutting 2.5 cm off the stick and replacing it into the cap. The swab did not contact the pledget and the medium ampule was not activated, (2) a Culturette device used appropriately (CUL); (3) a

Copan Amies gel device (GEL; Copan Diagnostics, Inc., Corona, CA); and (4) a Copan liquid Stuarts swab (COS).

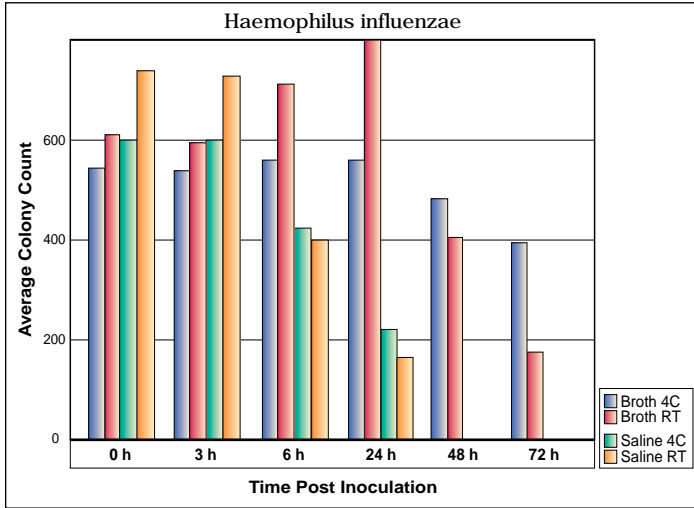
2. Multiple swabs of each of these four configurations were inoculated to allow duplicate plate counts at various time intervals from both RT and 4°C. One hundred sixteen (116) clinical strains consisting of *Streptococcus pneumoniae* (61), *Neisseria gonorrhoeae* (11), *Neisseria meningitidis* (5), and *Haemophilus influenzae* (39) were evaluated and an average percent of the starting inoculum recovered calculated for each species.

RESULTS

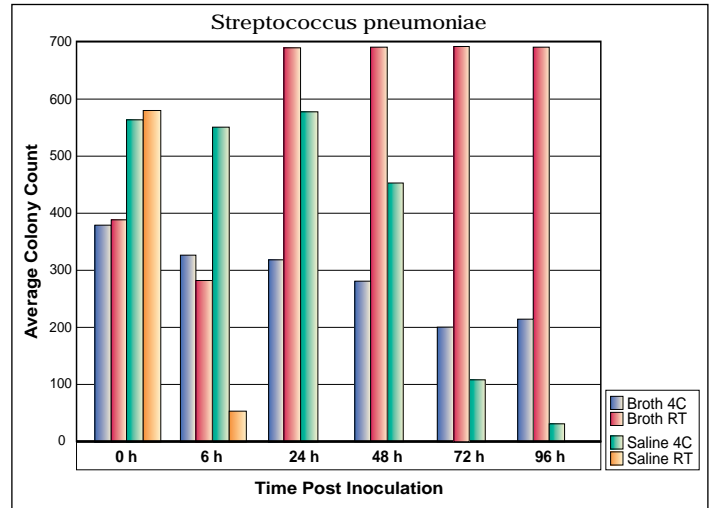
- Graphs 1 – 5 detail the effects of storage temperature on microorganisms maintained in sterile saline and TSB. The most dramatic effect occurred with *Neisseria gonorrhoeae* stored at RT in saline. Overall, 4°C provided better recovery than RT whether in saline or TSB. TSB suspensions survived longer than saline suspensions.
- Graphs 6 – 9 illustrate the effects of swab type and media when subjected to temperature extremes. Dry swabs, as expected, did not maintain organism viability for any duration. Devices incorporating a transport medium and held at 4°C provided the best fastidious organism survival in this study.
- Amies gel and liquid Stuarts configurations provided recoverable numbers of all organisms at 24h post inoculation but significant decreases were observed with specimens held at RT. Viable organisms were detected at 48h and 72h only from swabs held at 4°C.

CHARTED RESULTS

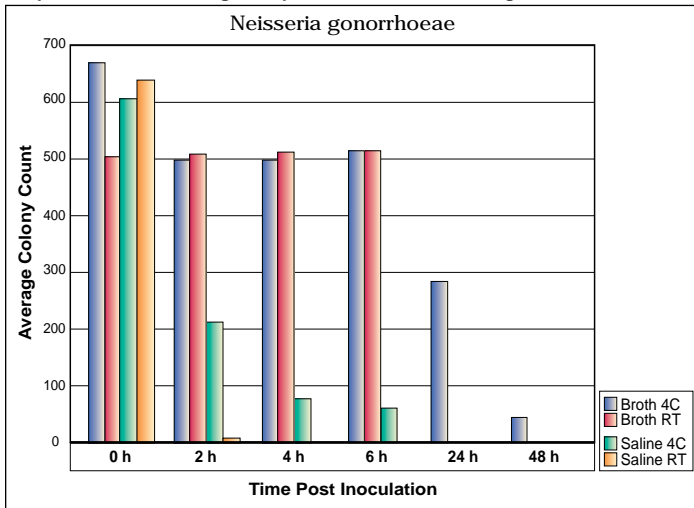
Graph 1: Effects of Storage Temperature on Fastidious Organisms



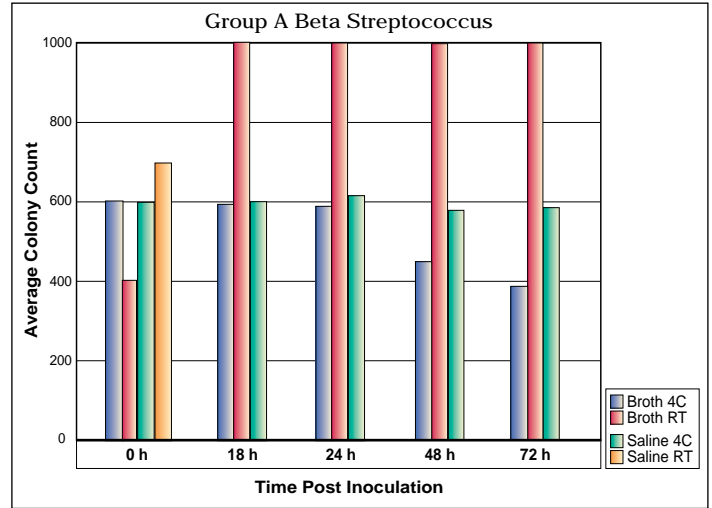
Graph 4: Effects of Storage Temperature on Fastidious Organisms



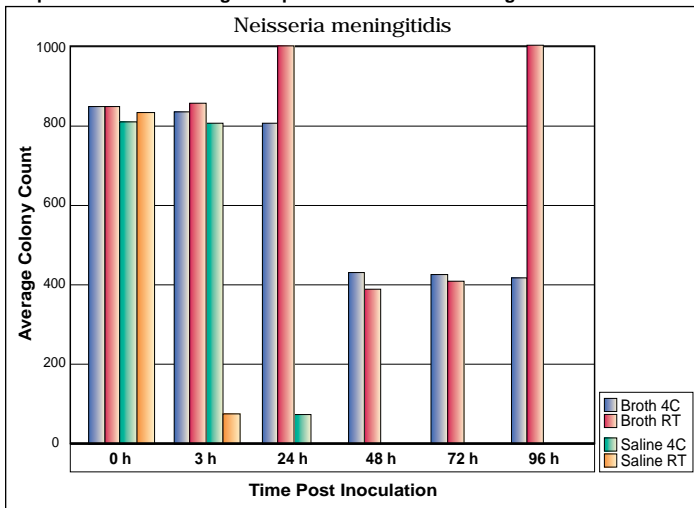
Graph 2: Effects of Storage Temperature on Fastidious Organisms



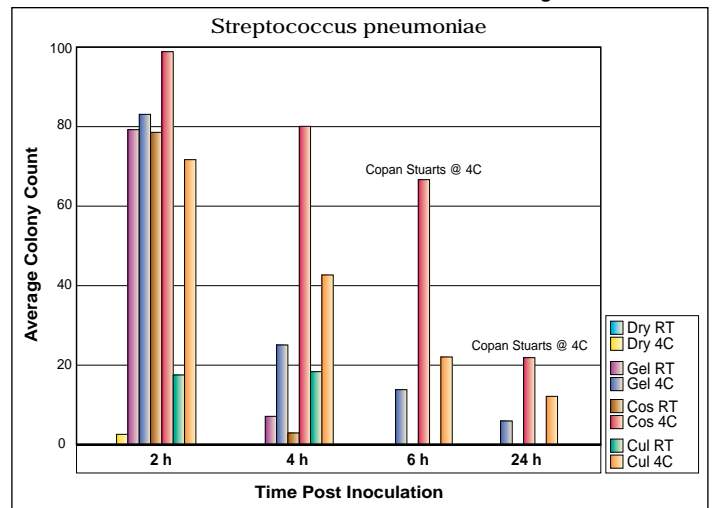
Graph 5: Effects of Storage Temperature on Fastidious Organisms



Graph 3: Effects of Storage Temperature on Fastidious Organisms



Graph 6: ORGANISM SURVIVAL-Effects of Transport/Storage Temperature on Various Swab Configurations



DISCUSSION

The purpose of this study was to determine the best temperature for shipping a variety of clinical specimens from distant outpatient clinics to a core laboratory for processing. Control of organism type and numbers coupled with the vagaries of specimen volume rendered a study of actual patient specimens impractical. Extrapolation of these data, utilizing clinical isolates and transport devices, to raw clinical specimens would be quite a leap. However, some sense of temperature effects and a greater awareness of the protective effects of standard transport media can be appreciated.

It is quite evident why dry swabs were supplemented with protective transport media reducing the deleterious effects of oxidation, with Stuart's medium appearing around 1946 and Amies gel medium in 1967.² Less evident is documentation that various specimen sources should not be placed at refrigerator temperatures due to the antibacterial effect of the cold. Specimens such as penile/urethral, cervical/endocervical and cerebral spinal fluid have historically been attached to a caveat that pathogens found in these specimens, e.g. *Neisseria gonorrhoeae*, *N. meningitidis*, and *Haemophilus influenzae* cannot tolerate refrigerator temperatures.^{3,4,5}

Although this study evaluated only 116 clinical isolates and used seeded transport devices, it suggests some provocative outcomes not expected by the author and worthy of further investigation.

CONCLUSIONS

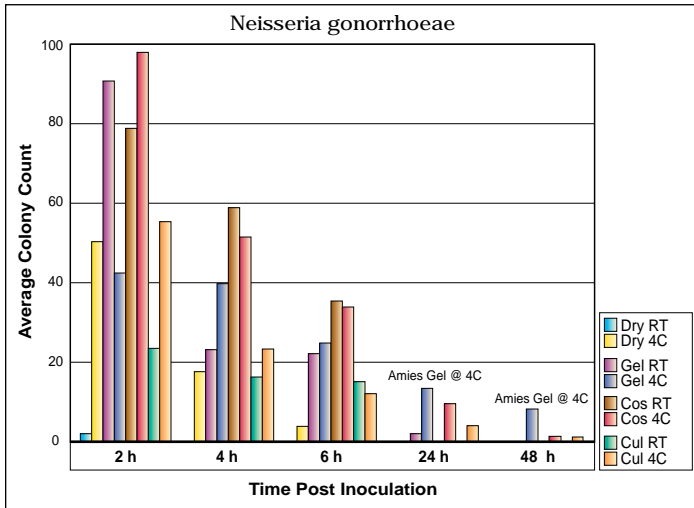
Although handbooks, manuals and guidelines warn of the deleterious effects of cold temperatures on the viability of some fastidious pathogenic bacteria, this study suggests that prolonged survival of these organisms depends upon their storage at 4°C. Adherence to historical findings for specimen transport/storage policies may not accurately reflect the capabilities of modern collection and transport devices. Extrapolation of these data to non-swab specimens requires further study.

LITERATURE CITED

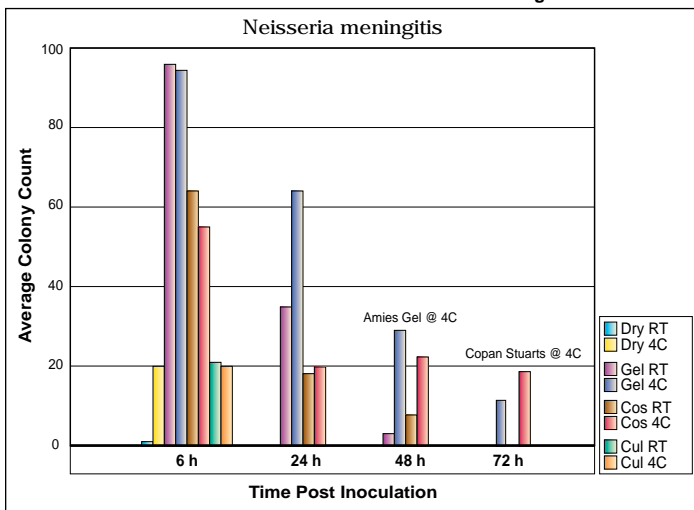
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Graph 7: ORGANISM SURVIVAL-Effects of Transport/Storage Temperature on Various Swab Configurations



Graph 8: ORGANISM SURVIVAL-Effects of Transport/Storage Temperature on Various Swab Configurations



Graph 9: ORGANISM SURVIVAL-Effects of Transport/Storage Temperature on Various Swab Configurations

