



CANADIAN ASSOCIATION FOR CLINICAL MICROBIOLOGY  
AND INFECTIOUS DISEASES

66th Conjoint Meeting on Infectious Diseases  
In Conjunction With The CIDS Annual Meeting

Holiday Inn on King  
Toronto, Ontario

November 8th - 12th, 1998

**EVALUATION OF FOUR TRANSPORT SYSTEMS  
FOR THE SURVIVAL OF N. GONORRHOEAE (NG).**

JC Arbique, KR Forward.  
Department of Pathology and Laboratory Medicine,  
Queen Elizabeth II HSC, Halifax, Nova Scotia, Canada.

# EVALUATION OF FOUR TRANSPORT SYSTEMS FOR THE SURVIVAL OF *N. GONORRHOEAE* (NG).

JC Arbique, KR Forward. Department of Pathology and Laboratory Medicine, Queen Elizabeth II HSC, Halifax, Nova Scotia, Canada.

## ABSTRACT

**Objective:** To challenge a variety of commercial transport systems with a standardized inoculum of clinical isolates of *N. gonorrhoeae* (NG), and assess survival after storage at room temperatures (RT) and refrigeration temperatures for periods of 6, 24 and 48 h, in an attempt to simulate conditions of specimen submission.

**Methods:** Suspensions of clinical isolates of NG were standardized, aliquoted and adsorbed onto four swab types: Culturette EZ (Becton Dickinson (BD), Cockeysville, MD); Cultureswab (Difco Laboratories, Detroit, MI); & Venturi Transystem (Copan Italia, Bovezzo, Italy); and a recently modified Starswab (Starplex Scientific, Etobicoke, Ontario). Swabs were plated to chocolate agar at 0, 6, 24 and 48 h and colonies counted after incubation at 35°C for 72 h in 5% carbon dioxide. Tests were run in quadruplicate.

**Results:** There was a dramatic reduction in NG CFUs after only 6 h incubation with each of the swabs tested. Survival was best using Copan and Difco transport (mean reduction at 6 h: 84.1% and 83.5% respectively); superior to BD and Starplex transport (mean reduction at 6 h: 97.9% and 94.8% respectively). All isolates were recovered after 24 h from Copan transport under all conditions and from the Difco transport at refrigeration. None of the strains were consistently recovered from the Starplex transport after 24 h in ambient air.

**Conclusion:** None of the systems tested had less than a 10-fold decrease in recovered colonies after 6 h. Further studies are required to determine how poor transport influences the number of positive cultures and what are the public health implications. Of the swabs tested both Difco and Copan were most acceptable.

## OBJECTIVES

To compare the survival of *N. gonorrhoeae* in four commercially available transport systems.

To assess the effect of storage at ambient and refrigeration temperatures for periods of 6, 24 and 48 hours to simulate conditions of specimen transport.

## INTRODUCTION

Maintaining organism viability while specimens are in transport is an extremely important part of microbiology and is ignored when choosing a transport system and setting guidelines for physicians submitting specimens.

The recovery rate for NG at the Queen Elizabeth II Health Sciences Centre is approximately three per thousand cultures (0.3%). Although a number of factors may be involved with the relatively low recovery rate, false negative cultures may make a significant contribution.

Transfer rates of organism from swabs to solid culture media may vary depending on handling and various other factors including:

- Type of swab fibre and entrapment variations
- Type of media used in transport (Modified Stuart's, Amies with and without charcoal)
- Effectiveness of media in maintaining viability and inhibiting overgrowth of competing organisms present in the sample<sup>1</sup>.

Specimen transport is often delayed by 24 - 48 hours from time of collection. The role of transport media is to inhibit the effects of toxic by-products, oxidation, dehydration, and bacterial overgrowth during this time<sup>2,3</sup>.

Despite general agreement in the literature that the presence of charcoal is imperative for the survival of NG<sup>2,4</sup>, a number of non-charcoal products are available from manufacturers who maintain that NG will survive on their products.

## METHODS

Each isolate of NG was used in one of four test runs. Isolates were suspended in phosphate buffered saline (pH 7.2)  $2.13 \times 10^5$  cfu/ml.

Inoculum charge was chosen to represent numbers of gonococcal organisms expected from cervical secretions - gonococcal colony counts range from  $5 \times 10^3$  -  $8 \times 10^6$  with 47% of women showing counts  $>10^5$  and 53% with counts  $<10^4$ . Counts are significantly lower in vaginal secretions<sup>5</sup>.

A 0.1ml aliquot was absorbed onto each of the four swab types examined.

- Starswab™ (Starplex Scientific, Ontario, Canada)\*
- Cultureswab™ (Difco Laboratories, Detroit, Michigan)\*
- Venturi Transystem™ (Copan Italia, Bovezzo, Italy)\*
- Culturette EZ™ (Becton Dickinson, Maryland, USA)\*\*

\*Amies medium with charcoal

\*\*Polyurethane foam swab without transport medium.

Swabs were held in the following manner:

- Refrigerate for 48 hours
- Ambient for 48 hours
- Ambient air for 4 hours then refrigerate 44 hours

Tests were run in quadruplicate and the order of swabs was rotated in each subsequent trial to counter balance loss of organism viability during the test period. Results were then averaged for each manufacturer.

Swabs were plated to the entire surface of chocolate agar at 0, 6, 24 and 48 hours and colonies counted after incubation at 35°C for 72 hours in 5% CO<sub>2</sub>.

## RESULTS

There was a dramatic reduction in the number of colonies recovered after only 6 hours incubation with each of the transport systems tested - the mean count dropped from 1997 colonies at zero time to 182 after 6 hours.

Survival was best using Copan and Difco transport (mean reduction at 6 hours 84.1% and 83.5% respectively). Both were superior to BD and Starplex transport (mean reduction at 6 hours: 97.9% and 94.8% respectively).

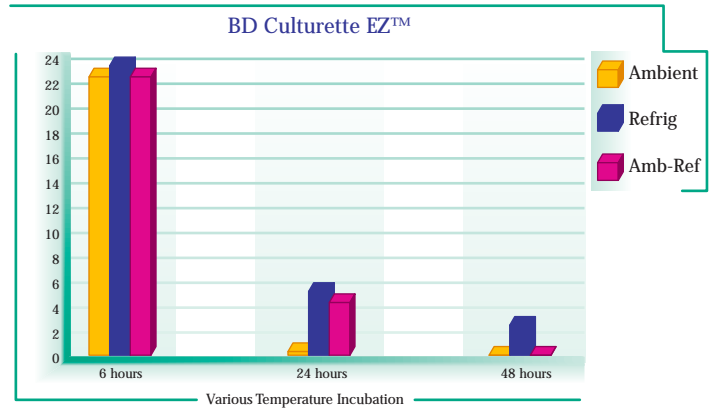
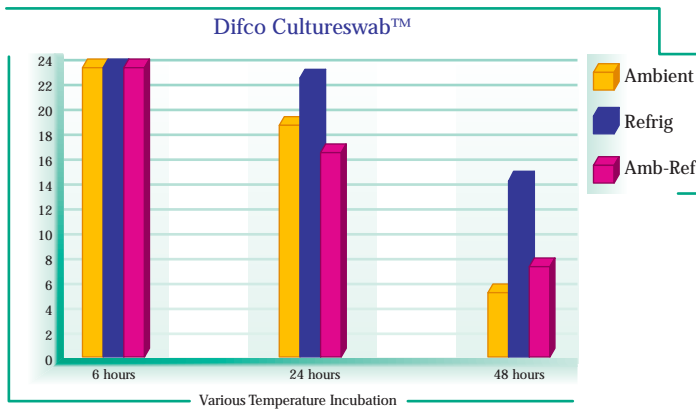
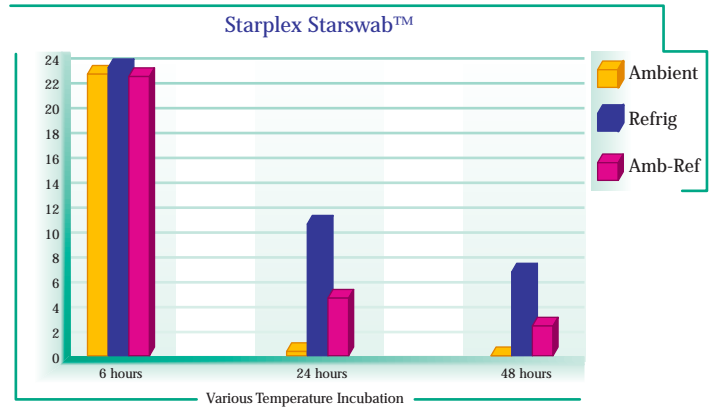
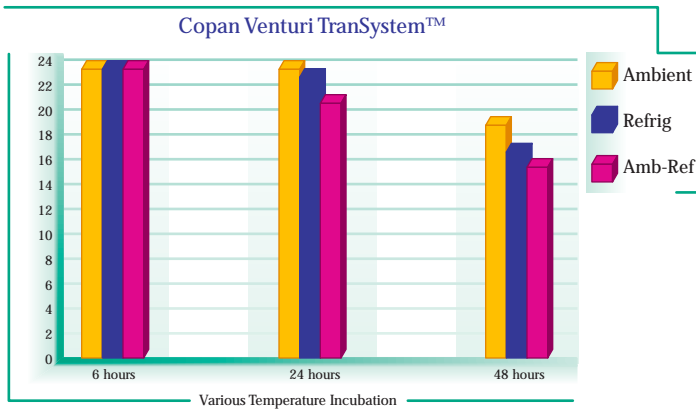
**Copan swabs:** NG isolated from 24/24 cultures after 24 hours at ambient temp and 23/24 cultures after 24 hours refrigeration. Recovery dropped to 19/24 cultures after 48 hours at ambient air and 17/24 after 48 hours refrigeration.

**Difco swabs:** NG isolated from 19/24 cultures after 24 hours at ambient temp and 23/24 cultures after 24 hours refrigeration. Recovery dropped to 6/24 cultures after 48 hours in ambient air and 15/24 cultures after 48 hours refrigeration.

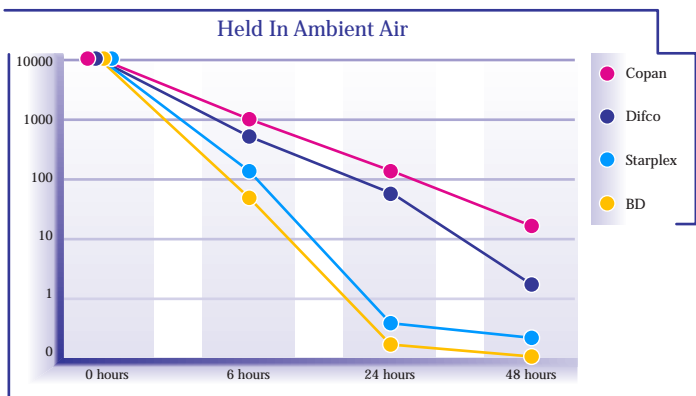
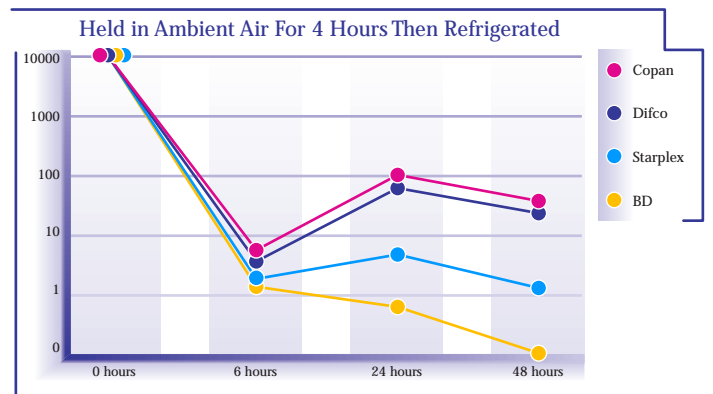
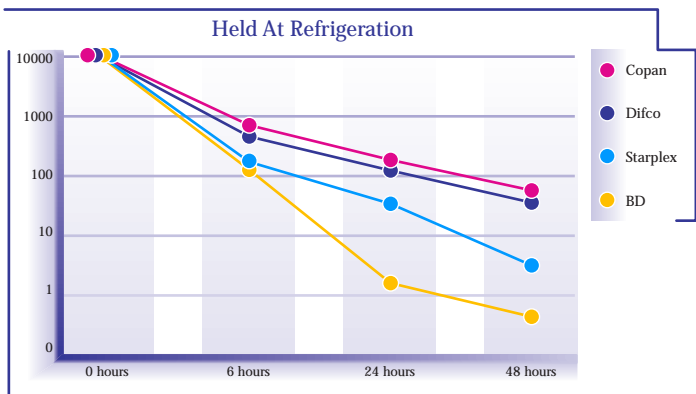
**Starplex swabs:** NG isolated from 1/23 after 24 hours at ambient temp and 11/24 cultures after 24 hours refrigeration. Recovery dropped to 0/24 after 48 hours at ambient temp and 7/23 cultures after 48 hours refrigeration.

**BD swabs:** NG isolated from 1/24 cultures after 24 hours at ambient temp and 6/24 cultures after 24 hours refrigeration. Recovery dropped to 0/24 cultures after 48 hours at ambient temp and 3/24 cultures after 48 hours refrigeration.

# Number of Cultures Positive From Swabs Held at Various Temperatures



# Colony Counts From Swabs



## DISCUSSION

It is difficult to simulate clinical conditions in an in vitro survival study. Limitations of study include:

Absence of protein, mucus and exudate which might affect organism viability and overgrowth by competing organisms.

Use of pure rather than mixed cultures so that effects of bacterial competition are not seen

Rapid loss of NG viability in suspension. Even though inoculation onto transport systems was completed within 30 minutes (mean 25.5 min) loss of viability ranged from 11.9 - 64% (mean 40.1%) during the testing period.

It is difficult to maintain a constant ambient temperature or to provide for continuous refrigeration of specimens being submitted for culture.

Although the effects of refrigeration and ambient temperatures were evaluated the effects of temperature extremes encountered in the summer and winter months were not evaluated.

There was considerable strain to strain variability in the survival of NG on transport swabs. Further studies with a larger number of strains are necessary to definitively determine the effects of strain variability on survival.

Recovery was better when transport swabs were held at refrigeration with the exception of the Copan swabs, which preferred ambient temperatures.

The results obtained reflect those which can be expected when using extremely fresh transport media, presumably activity will reduce as transport systems reach their expiry dates and are affected by evaporation of the agar component and permeation by oxygen from the atmosphere.

The difference in results obtained with Copan and Difco swabs may in part be due to shelf life of products - the Difco product was manufactured nine months earlier than the product that was obtained directly from Copan.

### CONCLUSIONS

Of the four transport media evaluated, Copan products, which are also available from Difco, Que-Lab and Pro-Lab, were most acceptable.

NG survival on both Starplex and BD swabs was significantly reduced when compared to the other two swabs.

There was variability between products from the same manufacturer showing the need for careful QC on products and the importance of notification when changes in formulations are made so that laboratory acceptance criteria can be modified to reflect these changes.

Despite the many changes in transport media since Stuart's original formulation in 1946, laboratories are still struggling with the limitations of transport media for fastidious organisms. However, ease of use, cost and the availability of organisms for susceptibility testing still make it popular.

Further studies are required to determine how conditions of transport influence the number of positive cultures and what the public health implications are.

Further studies are required to compare the difference between results obtained using a phosphate buffered saline suspension medium versus a protein supplemented medium more closely resembling cervical secretions.

### REFERENCES

1. Perry JL, Ballou DR, Salyer JL, 1997  
Inhibitory properties of a swab transport device.  
J. Clin. Microbiol. 35: 3367 - 68.
2. Human RP and Jones GA, 1986  
Survival of bacteria in swab transport packs.  
Med. Lab. Sci. 43: 14-18.
3. Stuart RD, Toshach SR, 1954  
The problem of transport of specimens for culture of gonococci.  
Can. J. Public Health. 45: 73 - 83
4. Amies CR, 1967  
A modified formula for the preparation  
of Stuart's transport medium.  
Can. J. Public Health. 58: 296 - 300
5. Young H, Safafian SK, Harris AB and McMillan A, 1983  
Non-cultural detection of Neisseria gonorrhoeae in cervical  
and vaginal washings.  
Med Microbiol. 16: 183 - 191