Recovery after 24 Hours and 48 Hours of Gonococci from Eswab and from Charcoal Impregnated Swabs in Stuart's Transport Medium

Hoffmann S & Berthelsen L

Department of Bacteriology, Mycology, and Parasitology [hof@ssi.dk; lbe@ssi.dk]
Statens Serum Institut, Artillerivej 5, Copenhagen, DK-2300, Denmark

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Objectives

The aim of this in-vitro study was to evaluate the recovery by culture of gonococci kept for 24 h and 48 h at room temperature in two transport systems: Eswab, and charcoal swabs in Stuart's transport medium.

Methods

Transport systems

- **Eswab**: Flocked swabs in 1 ml of liquid Amies medium (AM), both from COPAN (Brescia, Italy).
- **CSTM**: Charcoal impregnated cotton tipped swabs in modified Stuart's transport medium (Statens Serum Institut, Copenhagen, Denmark).

Gonococcal strains

One type strain (ATCC 43069) and 13 fresh clinical isolates were used.

Gonococcal suspensions

Suspensions from 18-20 h chocolate agar plate cultures were prepared in 0.9% NaCl followed by 10-fold serial dilution in 0.9% NaCl, plating, incubation, and counting of colony forming units (CFUs).

Inoculation

All swabs were allowed to absorb 75 microliters from appropriate tubes.

- Swabs for retrieval after 48 h were charged from the undiluted suspensions and from tubes 10^{-1} and 10^{-2}.
- Swabs to be stored for 24 h were charged from tubes 10^{-2}, 10^{-3}, and 10^{-4}.
- Swabs for retrieval after 0 h were charged from tubes 10^{-4}, 10^{-5}, and 10^{-6}.
- Each combination of transport system, bacterial inoculum, and storage time was done in duplicate.

Retrieval procedure

After 0 h, 24 h, and 48 h at room temperature swabs from CSTM and 100 microliters of AM from the Eswab were seeded onto chocolate agar plates. CFUs were counted after incubation.

Calculation of recovery

The average number of CFUs counted on the two retrieval plates was divided by the calculated initial number of CFUs on the corresponding swab.

Background

- For culture of gonococci (GC) from clinical specimens a sufficient number of GC must be viable after transportation.
- Therefore, transportation time should be < 24 h. Relatively often, however, transportation time is 48 h or even longer.
- Even with transportation time < 24 h, the number of GC may be below the threshold of detection by culture.
- Diagnosis by PCR or other nucleic acid amplification tests (NAATs) is much less sensitive to long transportation time and low initial inoculum.
- A liquid transport medium could facilitate the conduct of several assays on the same specimen, e.g. initial diagnostic NAAT followed by culture of NAAT positive specimens to enable antimicrobial susceptibility testing and surveillance.

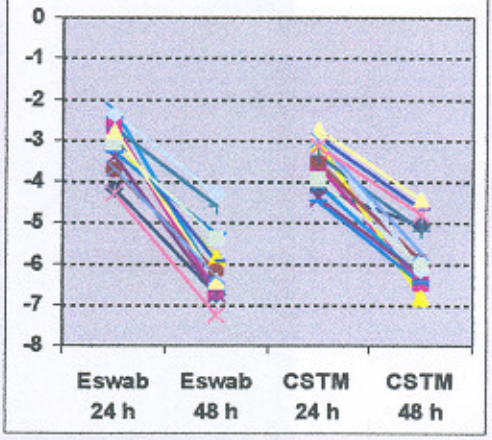
Conclusion

- There was no substantial difference between the recovery of gonococci from the two transport systems, neither after 24 h or after 48 h.
- The decrease in viability count was considerable in both transport systems, even at 0 h.

Results

- The undiluted suspensions contained 2.2×10^8 to 1.4×10^9 CFUs/ml.
- The recovery at 0 h with Eswab as well as CSTM was 0.04 to 0.10 of the calculated initial inoculum.
- The recovery at 24 h was 5×10^{-5} to 5×10^{-3} from Eswab, and 3×10^{-5} to 2×10^{-3} from CSTM (Figure 1).
- The recovery at 48 h was 5×10^{-8} to 5×10^{-5} from Eswab, and 2×10^{-7} to 4×10^{-5} from CSTM (Figure 1).
- The ratio between the recovery in Eswab and the recovery in CSTM was 0.08 to 64 at 24 h, and 0.003 to 67 at 48 h (Figure 2).

Figure 1. Recovery compared to calculated inoculum (log scale)



Discussion

- In both transport systems the decrease in viable count after 24 h and 48 h was substantial.
- The recovery in the two transport systems varied greatly (Figure 1). Neither of the two systems appeared to be clearly superior to the other, as also evidenced by the ratio of recoveries (Figure 2).
- Preferably, a comparative study of the two transport systems should be performed with clinical specimens, including detection by NAAT.

Figure 2. Ratio of recovery from Eswab to recovery from Stuart's transport medium