FVU culture can increase GC sample size for monitoring gonococcal antibiotic resistance

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Introduction

Nucleic Acid Amplification Testing [NAAT] is displacing traditional culture methods for diagnosing gonorrhoea. Consequently gonococcal isolates are no longer available for antimicrobial susceptibility determination.

However gonococci continue to develop resistance to antibiotic treatments currently in use and molecular methods are currently unable to determine this resistance. This means that gonococcal isolates are still required for monitoring antimicrobial resistance in *Neisseria gonorrhoea*. The value of this monitoring is enhanced when a large and representative gonococcal sample is examined.

It is well established that gonococci may be cultured at a variable rate from first-void urine [FVU] samples.

This investigation examined the utility and practical applications of using FVU samples as a cost-effective source of gonococcal isolates for surveillance of antimicrobial resistance in *N. gonorrhoeae*.

Aim

We investigated the use of targeted culture of FVU from males as a source of gonococcal isolates.

Specifically we examined:

(i) The capacity of FVU collected for NAAT to maintain gonococcal viability over time;

(ii) The physical characteristics of urine that affected gonococcal viability;

(iii) The survival of different gonococcal subtypes in FVU

Methods

Male patients attending the Sydney Sexual Health Clinic complaining of a urethral discharge had the discharge Gram stained and cultured on Modified NYC medium (time zero) and a FVU collected for examination for *Chlamydia trachomatis* by NAAT.

The FVU was separated into 5ml aliquots that were stored at 4ºC. At 24h hr intervals until the sample was extinguished, the aliquots were centrifuged and the resulting deposit inoculated onto Modified NYC medium. Gonococcal growth obtained at sequential time intervals was compared with that derived at time zero.

The pH of the FVU was determined using a standard pH meter, osmolarity by osmometry and antibacterial activity by spot inoculation of urine onto paper discs on nutrient agar seeded with a fully sensitive *E. coli*.

The extended phenotype of gonococccal isolates was determined by antibiogram, auxotyping and serotyping (their reaction with standard set of 14 monoclonal antibodies, Boule Diagnostics, Huddinge, Sweden).

Results

Time to culture	< 24h	24 – 48 h	48 – 72 h	> 72 hr
No cultured	53	42	25	6
No (%) culture positive	48	31	21	2
	90%	74%	84%	33%

Table. Proportion of FVU that provided a positive gonococcal culture – by time

Overall 102/126 samples (80%) were culture positive. A high proportion of FVU continued to sustain gonococcal viability up to 72 hours after collection.

However after storage for 24 h or more, culture of the FVU often yielded only low numbers of gonococci. This decreased yield correlated with extremes of urinary pH and urine with a high solute content (high osmolarity). Seven samples contained antibacterial substances, but five of these yielded gonococcal growth.

The phenotype of cultures at time zero was in all instances the same as that subsequently cultured from aliquots of the same FVU.

A total of 14 auxotype/serovar (A/S) classes was identified.

Survival of one A/S class – Nr/B1- may have been more affected by physical characteristics of urine.

Conclusions

Targeted culture for *N. gonorrhoeae* using spun sediment of FVU positive for gonococci on NAAT is a cost-effective means of obtaining gonococcal isolates for antimicrobial susceptibility testing.

Although yields remain high for up to 72 hours after collection, positive cultures are more readily obtained when culture is initiated early. Turn-around times for NAAT may thus influence yields of gonococcal isolates by this stratagem.

Physical characteristics of the urine, especially extremes of pH and high solute content, have a greater influence on growth yields than the particular gonococcal subtype present. Some A/S classes may survive less well than others.

The data presented here were derived from analysis of FVU from culture positive and symptomatic males. Lower yields would be expected from endocervical gonorrhoea in females where gonococcal inocula are known to be significantly lower than in male urethritis.

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