Molecular Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* is Enhanced by Flocked Swabs and Universal Transport Media

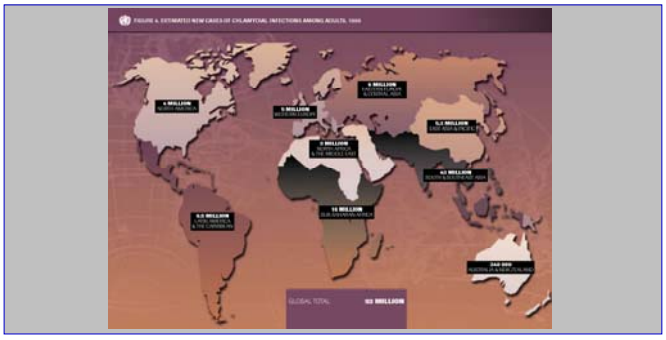
M. Chernesky, S. Castriciano, D. Jang, M. Smieja
McMaster University, St. Joseph's Healthcare, Hamilton, ON, CANADA

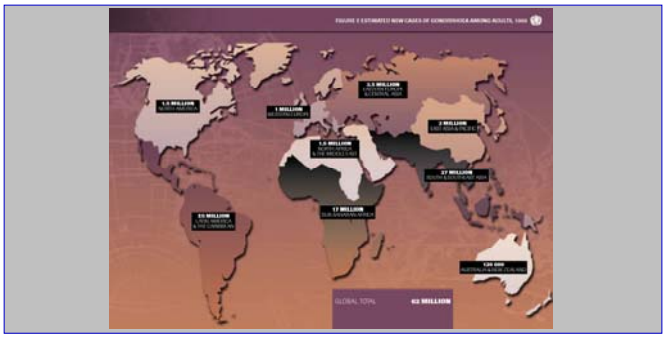
REVISED ABSTRACT

Background: Nucleic acid amplification tests (NAATs) are used to diagnose *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. A universal transport system would enable confirmatory testing. The objective was to compare a room temperature universal transport media (UTM-RT) and flocked swabs (FS, Copan, Diagnostics Inc.) to swabs and transport systems of commercial NAATs in mocked specimens.
Methods: Laboratory strains of CT and GC were diluted in UTM-RT from 10^{-1} to 10^{-10} and aliquoted into tubes for each dilution using FS, ProbeTec swabs (PT, Becton Dickinson), Amplicor swabs (AMP, Roche Diagnostics), and APTIMA Combo 2 swabs (AC2, Gen-Probe Inc.). Each was processed into kit-dilution buffers as a mocked sample. Dilutions of CT in UTM-RT were inoculated into McCoy cells and scored for fluorescent inclusions. GC dilutions in UTM-RT were compared to dilutions in TSB with colony counts.
Results: For CT the 100% endpoints for FS and kit swabs (KS) were equal in AC2 (10^{-7}) and PT (10^{-6}). In AMP, the FS 100% endpoint was 10^{-7} compared to 10^{-6} for the Roche swab. Small numbers of replicates were positive to higher dilutions for all swab types (for AC2 the FS yielded 50% and the KS 10% positive between 10^{-8} and 10^{-10}). The CT culture endpoint was 10^{-6}. For GC the 100% endpoints for FS and KS were equal for AC2 (10^{-6}), PT and AMP (10^{-5}); but at 10^{-7}, 90% of FS and 70% of KS were positive in AC2 and higher proportions of replicates were positive with FS at 10^{-6} in the other 2 assays. GC infectivity titrated to 10^{-6}.
Conclusions: The UTM/FS system enhanced the ability of commercial NAATs to detect low levels of CT or GC. The use of this universal system might yield more positives from clinical specimens and enable confirmatory testing from one assay to another.

BACKGROUND

Chlamydia trachomatis are the most prevalent sexually transmitted bacteria with high prevalence in all parts of the world. Similarly, but with less prevalence, *Neisseria gonorrhoeae* are often infecting the same patients as Chlamydia. In most populations these are young, sexually active men and women. When symptoms are present in the lower genital tract, the patient may have cervicitis in women or urethritis in men. Upper tract infections in women can cause complications such as pelvic inflammatory disease (PID), ectopic pregnancy or infertility. The main reason for high prevalence is high rate of asymptomatic infections. For CT, more than 75% of women and 50% of men with infections are asymptomatic. These rates are slightly lower for gonorrhoeae infections.





Specimens
Men - urethral, meatal, anal swabs and FCU
Women – cervical, urethral, anal, vaginal, vulvar or introital swabs and FCU

A variety of specimen types have been examined for the diagnosis of CT and GC. Since so many CT and GC infections are asymptomatic; less invasive samples are required for screening such as FCU, self-collected vaginal swabs and samples already taken for Pap smears. Laboratory testing technologies show that the nucleic acid amplification tests performed on less invasive samples are the best approach.

Molecular Amplification Technologies

Target	Polymerase chain reaction (PCR)
	Self-sustaining sequence replication (3SR)
	Strand displacement amplification (SDA)
	Transcription-mediated amplification (TMA)
	Nucleic acid sequence-based amplification (NASBA)
Probe	Ligase chain reaction (LCR)
	Q-Beta replicase (QBR)
Signal	Branch probe technology (BPT)

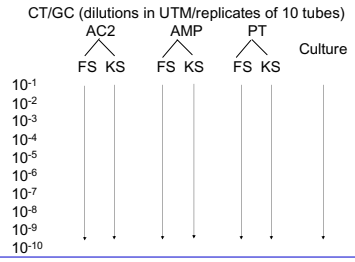
NAAT Clinical Sensitivity Determinants

- Analytical sensitivity on mocked specimens
- Amount of target in specimen
- Efficiency of nucleic extraction
- Inhibitors of amplification present in the sample
- Collection of target
- Preservation of target

OBJECTIVE

To compare flocked swabs to kit swabs saturated with serially diluted mocked samples of CT and GC by three commercial assays.

METHODS



The study algorithm used CT/GC organisms diluted and tested in the APTIMA Combo 2 (Gen-Probe Inc., Amplicor (Roche Diagnostics) and ProbeTec (Becton Dickinson) using Flocked (Copan) or kit swabs.

Swabs
AC2 AMP PT FS

Collection Tubes
AC2 AMP PT FS

RESULTS

Table 1: Detection of CT diluted in UTM from FS and KS processed in AC2, AMP and PT Assays

Dilution of UTM	Commercial NAAT AC2 KS	AC2 FS	AMP KS	AMP FS	PT KS	PT FS	Culture (inclusions)
10^{-5}	10	10	10	10	10	10	41
10^{-6}	10	10	10	10	10	10	8
10^{-7}	10	10	7	10	1	3	0
10^{-8}	2	8	0	1	0	0	0
10^{-9}	1	4	0	0	0	0	0
10^{-10}	0	3	0	0	0	0	0
(%) BELOW ENDPOINT (10^{-6})	13/40 (32.5)	25/40 (62.5) **(p< 0.001)**	7/40 (17.5)	11/40 (27.5) (p=0.13)	1/40 (2.5)	3/40 (7.5) (p= 1.0)	

In the Chlamydia testing component, AC2 endpointed at 10^{-7} for both FS and kit swabs. AMP and PT assays were tenfold lower at 10^{-6}. The FS enhanced the results in each tests but more dramatic enhancement was in the AC2 assay where the increase in number of positives below 10^{-6} dilution was 62.5% for FS compared to 32.5% for kit swabs (p<0.001). As expected, all tests detected well beyond the culture endpoint. The data for GC assays were very similar.

Table 2: Detection of GC diluted in UTM from FS and KS processed in AC2, AMP and PT Assays

Dilution of UTM	Commercial NAAT AC2 KS	AC2 FS	AMP KS	AMP FS	PT KS	PT FS	Culture (colonies/10ul)
10^{-4}	10	10	10	10	10	10	>1000
10^{-5}	10	10	10	10	10	10	48
10^{-6}	10	10	4	6	6	8	7
10^{-7}	7	9	0	2	1	3	0
10^{-8}	0	3	0	0	0	0	0
(%) BELOW ENDPOINT (10^{-5})	17/30 (56.6)	22/30 (73.3) **(p< 0.06)**	4/30 (13.3)	8/30 (26.6) (p=0.13)	7/30 (23.3)	11/30 (36.6) (p= 0.013)	

CONCLUSIONS

1. Flocked swabs enhanced the analytical sensitivity of each test performed on mocked specimens.
2. UTM and FS could allow confirmatory testing from one test to another.
3. A clinical trial is warranted on patient specimens to determine effects of FS on clinical sensitivity