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A Universal Transport Medium and Flocked Swabs Enhanced the Detection of Chlamydia trachomatis and Neisseria gonorrhoeae in Nucleic Acid Amplification Assays

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REVISED ABSTRACT

Background: *Chlamydia trachomatis* [CT] and *Neisseria gonorrhoeae* [GC] infections are common and nucleic acid amplification tests [NAAT] are used extensively for their diagnosis. The objective was to compare a room temperature universal transport media [UTM-RT] and flocked swabs [FS] (Copan, Diagnostics Inc.) to the swabs and transport systems of Gen-Probe Aptima Combo 2 [AC2] Amplicor [AMP] swabs [Roche] and Becton Dickinson ProbeTec ET [PT] for the detection of CT and GC in mocked specimens.

Methods: Laboratory strains of CT and GC were serially diluted ten fold in UTM-RT from 10^{-1} to 10^{-10} and 0.3 ml of each dilution was aliquoted into tubes for each dilution using FS, and commercial swabs. Each swab was processed into appropriate kit-dilution buffers as a mocked sample. Dilutions of CT in UTM-RT were also inoculated into shell vials containing McCoy cells and scored after 48 hours for fluorescent inclusions. GC dilutions in UTM-RT were compared to dilutions in TSB [both with FS] 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 combination from Copan enhanced the ability of each NAAT to detect samples containing low levels of CT or GC. The use of this system might yield more positives from clinical specimens and enable confirmatory testing of low level positives 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

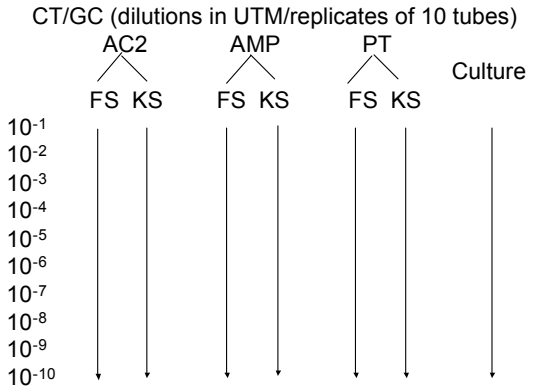
- Analytical sensitivity on mocked specimens
- Amount of target in specimen
- Efficiency of nucleic extraction
- Inhibitors of amplification present in the sample
- Collection of target
- Preservation of target

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.



RESULTS

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

	Commercial NAAT						
Dilution of UTM	AC2		AMP		PT		Culture
	KS	FS	KS	FS	KS	FS	(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)	

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

	Commercial NAAT						
Dilution of UTM	AC2		AMP		PT		Culture
	KS	FS	KS	FS	KS	FS	(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)	

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.

CONCLUSION

1. Flocked swabs enhanced the analytical sensitivity of each test per formed 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