Comparison of Second Generation Molecular Assays for *Chlamydia trachomatis* in Female Urines and Self-Collected Vaginal Swabs

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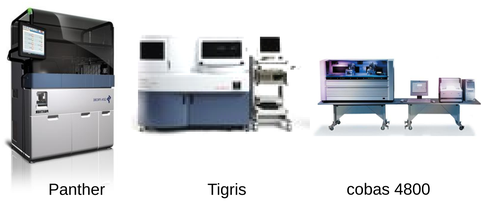
Abstract

Background
Less invasive screening samples such as self-collected vaginal swabs (SCVS) and first catch urine (FCU) are suitable for *C. trachomatis* testing in second generation molecular assays on automated instruments. The objectives were to test FCU and four SCVS from women.
Methods
From July 2012 to August 2013, 575 women self-collected FCU and four SCVS using collection kits from Abbott Molecular RealTi*m*e CT/NG, Becton Dickinson BD ProbeTec ET CT/GC Q^X, Roche Diagnostics cobas 4800 CT/NG and Hologic/GenProbe Aptima Combo 2 (AC2). A proportion of each sample was spiked with known concentrations of *C. trachomatis* to detect inhibitors. A patient infected status of two positive tests for a sample type or two different samples positive in a single test were used for comparisons.
Results
The analytical sensitivity of AC2 assay was 10-100 fold more sensitive than the other tests. There was no inhibition in either specimen type for all assays. The sensitivities for SCVS were 96.3% for AC2 on both TIGRIS and PANTHER, 96.2% for m2000, 88.9% for ProbeTec ET Q^X on a Viper instrument and 83.0% for cobas 4800. For FVU testing, sensitivity of AC2 was 95.9% on TIGRIS and 95.8% on PANTHER, 79.6% for m2000, 80.0% for ProbeTec Q^X on Viper and 86.0% for cobas 4800. All assays demonstrated specificities above 99%.
Conclusion
Inhibitors of amplification did not impact on the sensitivity of the second generation assays in SCVS and FCU. AC2 and RealTi*m*e assays were more sensitive than ProbeTec ET Q^X and cobas 4800 tests on SCVS whereas the AC2 assay which detects *C. trachomatis* rRNA was considerably more sensitive than the three DNA tests on FCU. The clinical sensitivities observed in this head to head study are likely determined by analytical sensitivity and the concentration of target analytes in clinical samples.

Background

Chlamydia trachomatis infections of the female genital tract present a diagnostic challenge because many patients are asymptomatic.[1] Nucleic acid amplification tests (NAATs) for *Chlamydia trachomatis* have been commercially available for over 15 years.[2] The package inserts for second-generation assays show that FDA approval is granted by comparing an investigational assay to a patient infected status (PIS), with infection based on obtaining positive results from at least 2 cleared assays when testing 2 different specimen types.[3] Comparisons that use the Gen-Probe Aptima Combo 2 (AC2) assay may garner misleading performance estimates for the investigational assay, due to the extra positive results detected by the more-sensitive AC2 test being classified as false-positives. Head to head comparisons of several assays on multiple sample types show more accurate findings of sensitivity and specificity.[4]



Panther Tigris cobas 4800

m2000 RealTime Viper XTR

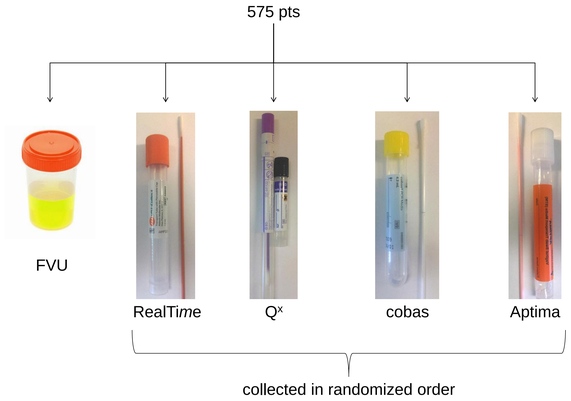
Figure 1: Five automated platforms used to compare performance of four second-generation assays.

Objectives

1. To compare performance of four second-generation assays on first void urine and self-collected vaginal swabs
2. To detect inhibitors by spiking samples with known concentrations of *C. trachomatis*

Materials & Methods

Study Design:
- From July 2012 to August 2013, 575 women attending sexual health centres in Hamilton and Toronto signed consent to participate (Hamilton Integrated REB approved).
- First, self-collected FVU (initial 20mL of urine) were obtained. Four SCVS were collected with kits from Abbott Molecular, Becton Dickinson, Roche Diagnostics, and Hologic Gen-Probe in a randomized order.



Determination of Analytical Sensitivity:
- Serial 10-fold dilutions of *C.* trachomatis strain L2 434 were made with uninfected SCVS and FVU, using 10 replicates per dilution. *C. trachomatis* elementary bodies (EBs) were counted by direct fluorescent antibody staining with monoclonal antibodies specific for major outer membrane proteins. To determine whether inhibitors were present, clinical specimens were spiked with a dilution 100-fold above the endpoint of detection (10/10 replicates positive by an assay for each specimen type).

Laboratory Testing:
- All clinical specimens, consisting of spiked and unspiked FVU and SCVS, were tested blindly within 2 weeks of collection by AC2 on Tigris and Panther, RealTi*m*e CT/NG on m2000, ProbeTec CT/GC Q^x on Viper, and cobas CT/NG on cobas 4800.

Data Analysis:
- Results from each assay were compared with PIS findings based on results from all four assays with both specimen types.
- AC2 results from Tigris testing were used in the PIS analysis. A patient was considered infected if at least two of the four assays yielded positive results for any specimen type.
- Clinical performance was determined by calculating sensitivity, specificity and predictive values.
- McNemar's test was used to compare sensitivity estimates for matched samples.

Results

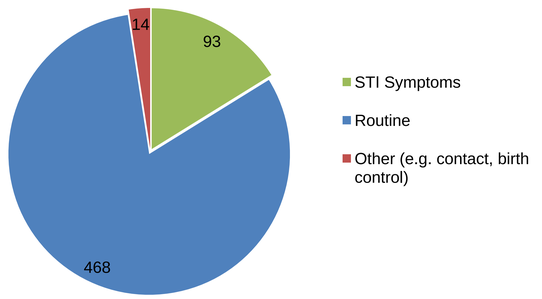


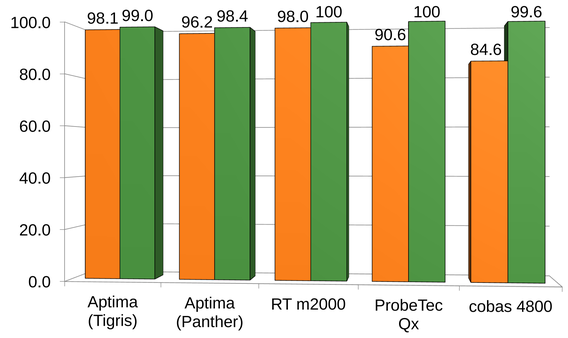
Figure 2: Reason for patient visit. The majority of study recruits were routine visits and were asymptomatic.

Table 1: Endpoints of Detection of C. trachomatis Dilutions in FVU and SCVS

	Assay/Sample Type							
	AC2 (Tigris)		CT/NG RT m2000		CT/GC ProbeTec Q^x		CT/NG cobas 4800	
CT Dilution	SCVS	FVU	SCVS	FVU	SCVS	FVU	SCVS	FVU
10^{-5}	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
10^{-6}	10/10	10/10	4/10	6/10	10/10	4/10	4/10	10/10
10^{-7}	10/10	10/10	0/10	0/10	2/10	0/10	0/10	6/10
10^{-8}	6/10	4/10	0/10	0/10	0/10	0/10	0/10	0/10
10^{-9}	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Probit LOD_{50}	-8.1 (-9.1, -7.1)	-7.9 (-8.9,-6.9)	-5.9 (-6.2,-5.4)	-6.1 (-7.1,-5.1)	-6.7 (-7.7,-5.7)	-5.9 (-6.9,-4.9)	-5.9 (-6.9,-4.9)	-7.1 (-8.1,-6.1)
% rate of inhibition*	0.3	0.5	0.0	0.0	4.5	2.3	0.0	0.0

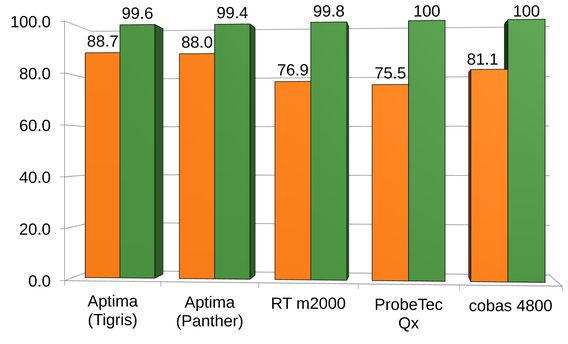
* Numbers of spiked specimens negative/total number of specimens spiked with CT x 100

Figure 3: Sensitivity and Specificity for *C. trachomatis* SCVS (%)



- The C. trachomatis prevalence was 9% (53/575) and 60.4% of infected patients (32/523 patients) tested positive with both specimen types in all tests on all instruments.

Figure 4: Sensitivity and Specificity for *C. trachomatis* FVU (%)



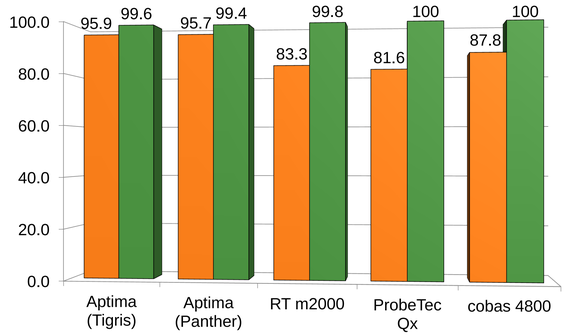
- The sensitivities of all assays in this study were lower than in the manufacturer's package inserts. This may be due to the exclusion of having vaginal swabs as part of the reference test for comparison, i.e. the PIS used was different and the use of first-generation assays for comparison.

Table 2: *C. trachomatis* FVU Testing Profiles of 15 women with Discordant Results

	First Void Urine				
Vaginal	AC2	RealTi*m*e m2000	PTETQ^X	Cobas 4800	Number of Patients
Pos	Neg	Neg	Neg	Neg	4
Pos	Pos	Neg	Neg	Neg	5
Pos	Pos	Neg	Neg	Pos	3
Pos	Pos	Pos	Pos	Neg	1
Pos	Neg	Neg	Neg	Pos	1
Neg	Pos	Pos	Pos	Pos	1

- In this study, 4 women were clearly infected with CT by SCVS testing but their FVU samples were negative in all assays.

Figure 5: Sensitivity and Specificity for *C. trachomatis* FVU Excluding 4 False-Negative Women (%)



- Calculations of sensitivity and specificity based on positive urines as the PIS.

Conclusions

- **Based on the LOD_{50} calculations, AC2 demonstrated the best analytical sensitivity for both SCVS and FVU.**
- **SCVS identified more *C. trachomatis* positives than FVU in all assays.**
- **For SCVS, the AC2 identified significantly more *C. trachomatis* positives (98.1% to 96.2%) than cobas CT/NG test (84.6%; p=0.016).**
- **For FVU, the sensitivities of the assays ranged from 88.0% (Panther) to 75.5% (ProbeTec ET CT/GC Q^X) using a 2 sample PIS and 95.9% to 81.6% using an FVU PIS.**
- **The specificity of all assays was greater than 98.4%.**
- **Inhibition rates were negligible.**

References

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