

Detection of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) in Co-infected Patients Using the Gen-Probe APTIMA® Combo 2™ (AC2) Nucleic Acid Amplification Test (NAAT)

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ABSTRACT

Background: This study reports the results of a new NAAT (AC2) that incorporates target capture in a Dual Kinetic Assay chemiluminescent detection system for detection of CT and GC in co-infected patients. **Methods:** Seven clinical centers enrolled 1363 men and 1569 women from high and low risk populations. Four endocervical swabs per patient were used to compare three NAATs and GC culture. Three male urethral swabs per patient were used to compare two NAATs and GC culture. Matched first catch urine (FCU) specimens were compared to three NAATs. Assays were performed according to the manufacturer's recommendations. AC2 was compared to "infected patient status," which defined a patient as infected whenever any two comparator results were positive. **Results:** 146 (5.0%) of all patients were identified as co-infected. 89 cases were male of which 82 were symptomatic and 7 asymptomatic. 56 cases were female of which 42 were symptomatic and 14 asymptomatic. Based on infected patient status, AC2 detected 93.8% of patients having both CT and GC and either CT or GC in 99.3% of co-infected patients. Overall performance for the 2932 patients was as follows: AC2 CT and GC sensitivity respectively for endocervical swabs when compared to infected patient status was 94.2% and 99.2% with a specificity of 97.6% and 98.7%. AC2 CT and GC sensitivity for female FCU when compared to infected patient status was 94.7% and 91.3% with a specificity of 98.9% and 99.3%. AC2 CT and GC sensitivity respectively for male swabs when compared to infected patient status was 95.9% and 99.1% with a specificity of 97.5% and 97.8%. AC2 CT and GC sensitivity for male FCU when compared to infected patient status was 97.9% and 98.5% with a specificity of 98.5% and 99.6%. **Conclusions:** AC2 exhibited excellent overall performance and excellent performance in detection of CT and GC co-infections.

INTRODUCTION

Sexually transmitted disease caused by *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) continues to be a serious threat to infected individuals and their partners. The number of reported cases continues to rise making the need for early, accurate diagnosis and adequate treatment even more critical. Nucleic acid amplification tests (NAATs) have been developed and are now in routine use in many laboratories around the world. The performance of these tests using both swab and first catch urine (FCU) specimens has been documented in numerous clinical studies. Many studies tell us that the health of certain individuals and populations could be improved and that the overall cost to society could be reduced through early detection and treatment of cases identified through the use of NAATs. The newest United States Food and Drug Administration (FDA)-cleared NAAT is the AC2, which was cleared in May 2001 and is available for routine clinical use. AC2 utilizes the TMA technology, which targets rRNA. AC2 incorporates the "target capture" technique, which uses hybridization and magnetic particles to isolate the target sequences and separate them from the specimen matrix, including amplification inhibitors that may be present. Combo 2 is a multiplex second-generation TMA test in which specific regions of the CT and GC rRNA are isolated and amplified using a separate capture oligomer and a unique set of primers for each target. Simultaneous detection and differentiation of the CT and GC amplicons is achieved using the Dual Kinetic Assay (DKA) method. DKA uses DNA probes labeled with two different types of acridinium ester (AE) molecules. Differences in the kinetic profiles of the labeled probes specific for CT and GC differentiate the results for the two pathogens. Studies from the early 1980s showed that nearly half of patients infected with gonorrhea also had chlamydia infection. Other studies have shown lower rates, but the percentages are significant. This study reports the results of AC2 for detection of CT and GC in co-infected patients.

MATERIALS AND METHODS

Endocervical swabs and a matched FCU were collected from 1391 female patients. Urethral swabs and a matched FCU were collected from 1095 male patients. FCU specimens from all patients were tested for CT by Combo 2, LCx, and PCR AmpliCor COBAS assays and for GC by AC2 and LCx assays. Female swab specimens were tested for CT by Combo 2, LCx, and AmpliCor assays; and GC by AC2 and LCx assays, and by GC culture. Male swab specimens were tested for GC by culture if one swab was provided. If two additional swabs were provided, the specimens were tested for CT and GC by Combo 2 and LCx. Each laboratory followed manufacturer's recommendations for NAAT's and standard procedures for GC culture. The clinical performance of AC2 was assessed by comparing assay results to "infected patient status," which defined a patient as infected when two or more positive results were obtained by the comparator assays' swab and urine results in any combination. For specimens where the AC2 result was positive and the comparator results were negative, the AC2 specimen was tested at Gen-Probe by another amplification assay using primers that target for alternate sequences on the rRNA target molecules.

The patient infected status was designated inconclusive if the comparator assay results included: one single positive result along with three negative results or with negative results together with missing results or three negative results with one missing result for female subjects or two negative results with one missing result for male subjects. Samples positive in only the AC2 assay were tested in a TMA system targeting alternate sequences.

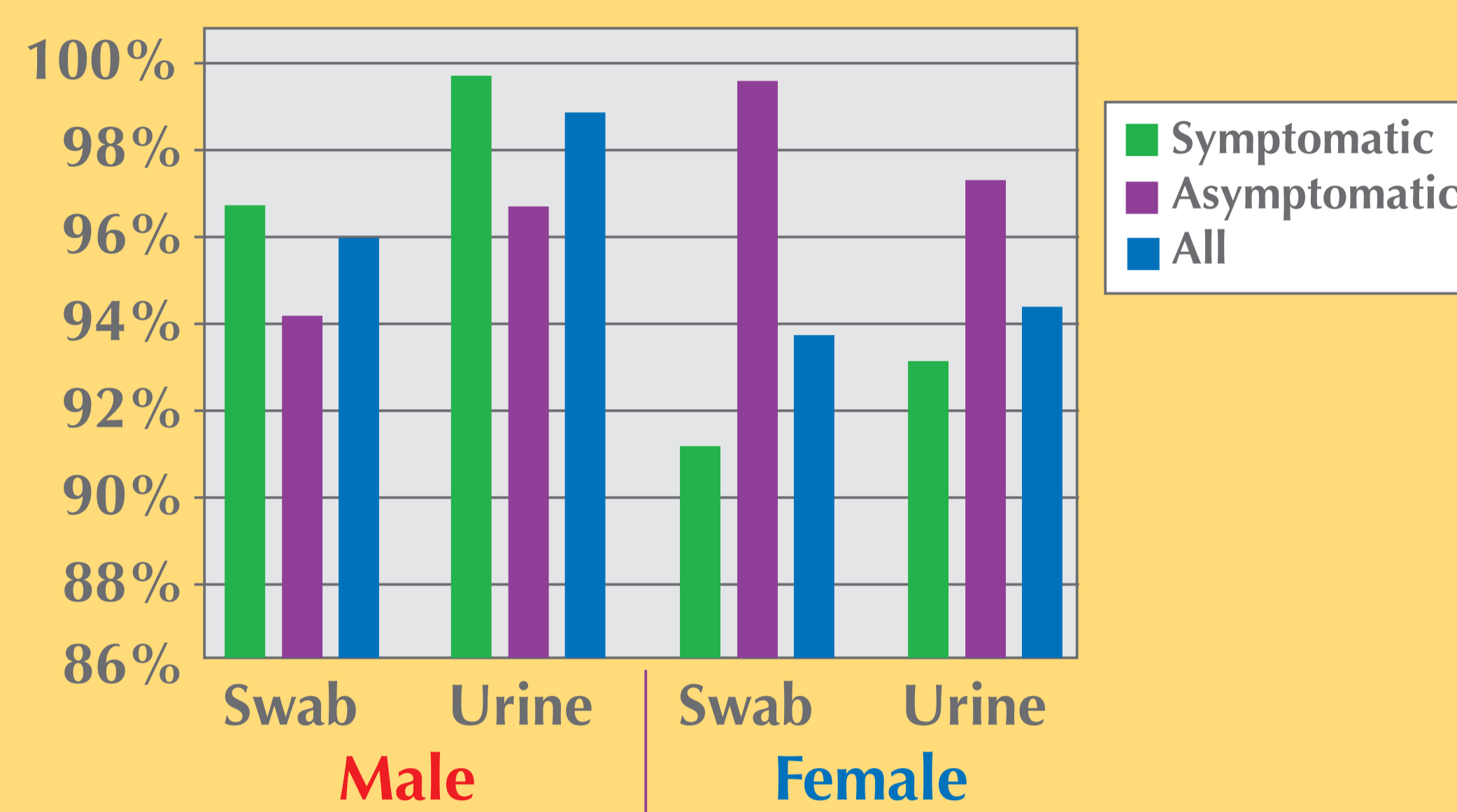
Subject Demographics and Clinical Characteristics

	Total (n=2932)
Age (mean years, ±SD)	28
≤ 20 years, n(%)	751 (25.6)
21 - 25 years, n(%)	770 (26.3)
26 - 30 years, n(%)	489 (16.7)
31 - 35 years, n(%)	371 (12.7)
36 - 40 years, n(%)	231 (7.9)
> 40 years, n(%)	320 (10.9)
Symptomatic, n(%)	1824 (62.2)
Asymptomatic, n(%)	1104 (37.6)
Undetermined, n(%)	4 (0.1)

RESULTS

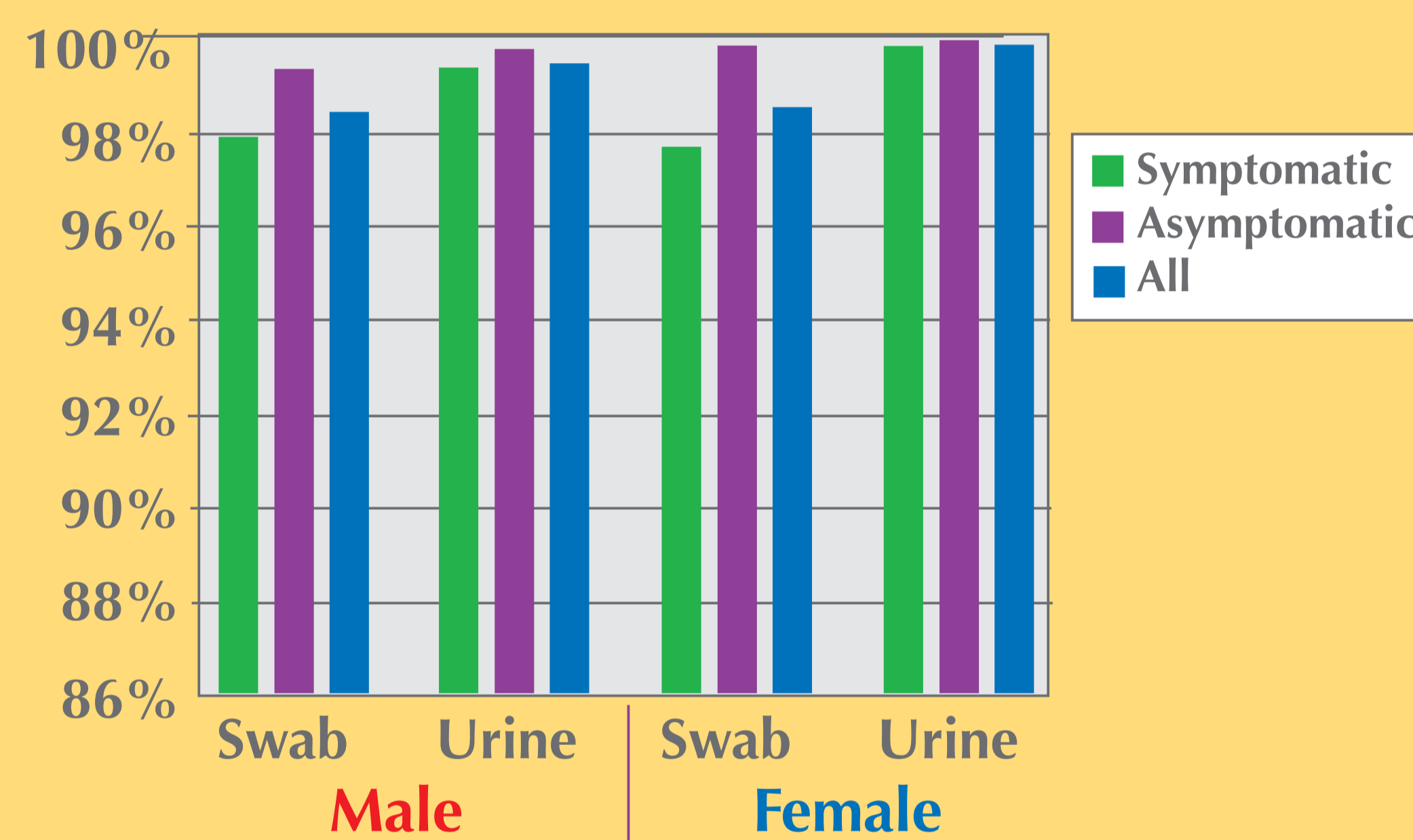
CT Sensitivity

AC2 CT Sensitivity: Female swab by symptom (symp, asymp, total), female urine, male swab and male urine



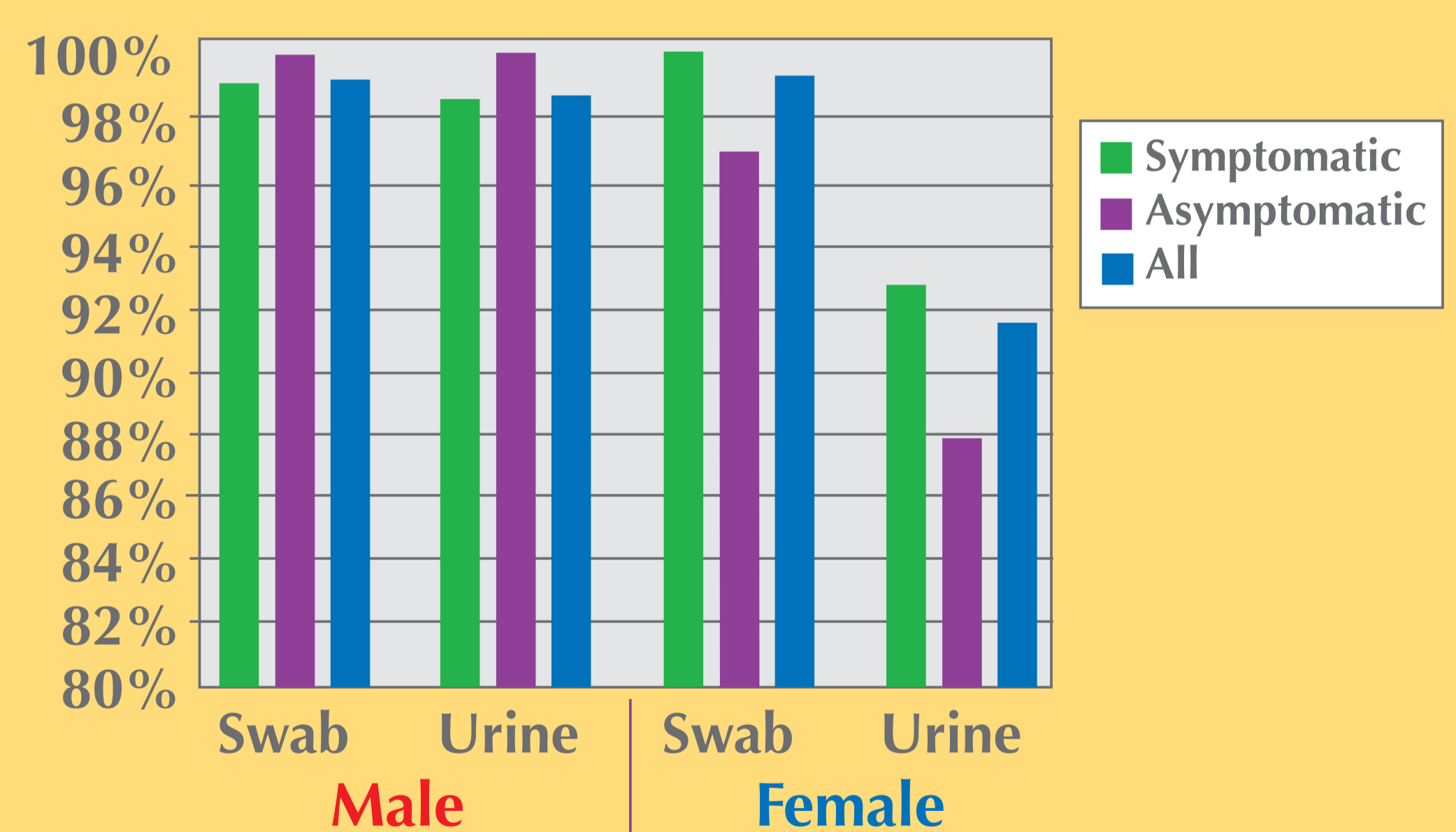
CT Specificity

AC2 CT Specificity: Female swab by symptom (symp, asymp, total), female urine, male swab and male urine



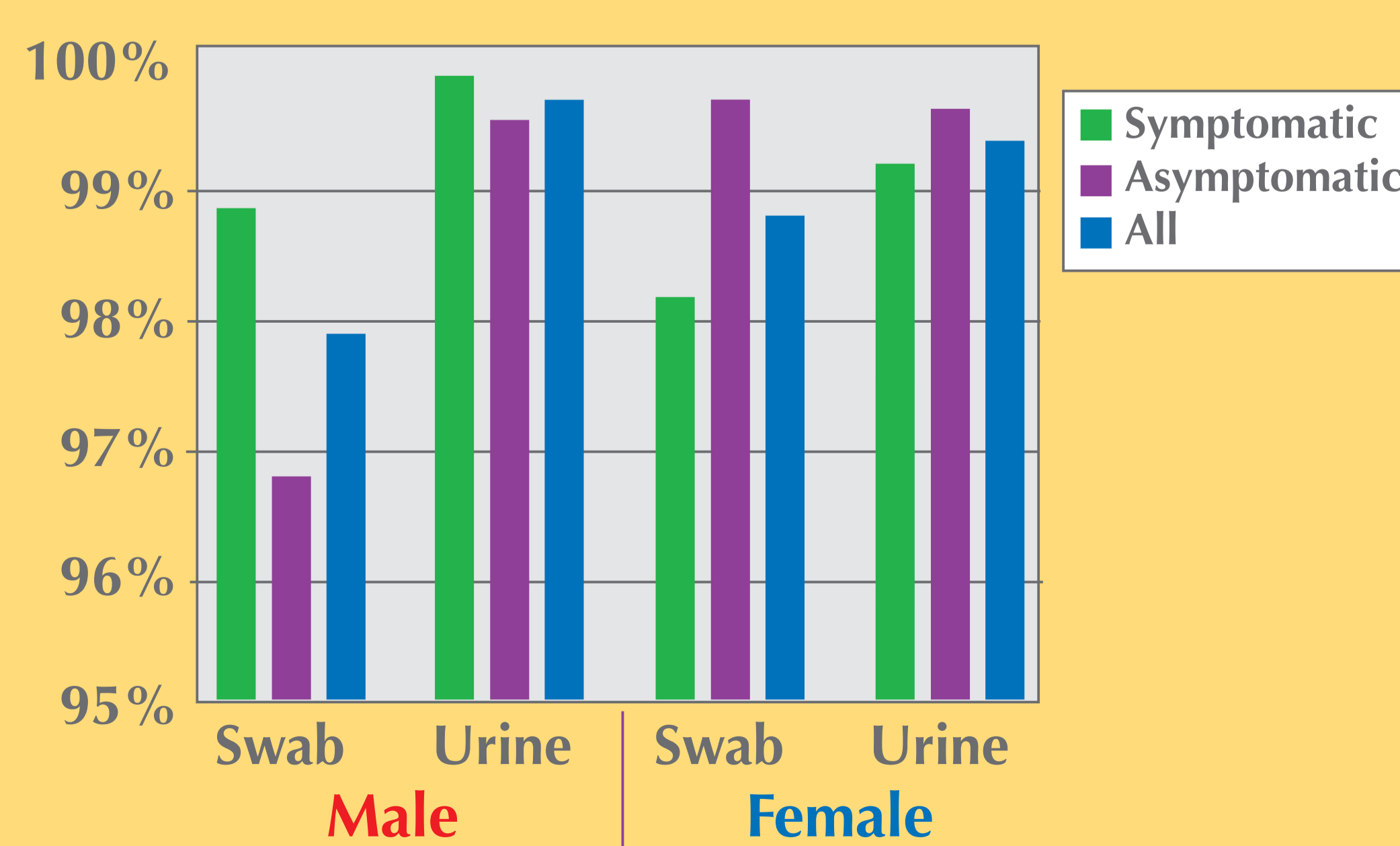
GC Sensitivity

AC2 GC Sensitivity: Female swab by symptom (symp, asymp, total), female urine, male swab and male urine



GC Specificity

AC2 GC Specificity: Female swab by symptom (symp, asymp, total), female urine, male swab and male urine



Overall AC2 Assay Performance

Gender	Specimen	Symptoms	CT		GC	
			Sensitivity	Specificity	Sensitivity	Specificity
Male	Swab	Symptomatic	96.40%	96.90%	99.00%	98.80%
		Asymptomatic	94.60%	98.40%	100.00%	96.70%
		All	95.90%	97.50%	99.10%	97.80%
Female	Swab	Symptomatic	98.50%	98.40%	98.40%	99.80%
		Asymptomatic	96.30%	98.80%	100.00%	99.50%
		All	97.90%	98.50%	98.50%	99.60%
Female	Urine	Symptomatic	92.40%	96.70%	100.00%	98.10%
		Asymptomatic	98.40%	98.80%	96.90%	99.60%
		All	94.20%	97.60%	99.20%	98.70%
Female	Urine	Symptomatic	93.80%	98.80%	92.60%	99.10%
		Asymptomatic	96.80%	99.00%	87.50%	99.50%
		All	94.70%	98.90%	91.30%	99.30%

AC2 Assay Performance in Detecting Both CT and GC in Co-Infected Subjects

Gender	Specimen	Symptoms	Infected Status ¹ CT Pos/GC Pos N	Combo 2 Assay			
				CT pos/GC pos n (% of N)	CT pos/GC neg n (% of N)	CT neg/GC pos n (% of N)	CT neg/GC neg n (% of N)
Male	Swab	Symptomatic	82 ²	77 (93.9)	1 (1.2)	2 (2.4)	1 (1.2)
		Asymptomatic	7	4 (57.1)	0 (0.0)	3 (42.9)	0 (0.0)
		Total	89	81 (91.0)	1 (1.1)	5 (5.6)	1 (1.1)
Female	Swab	Symptomatic	82	79 (96.3)	1 (1.2)	1 (1.2)	1 (1.2)
		Asymptomatic	7	7 (100)	0 (0.0)	0 (0.0)	0 (0.0)
		Total	89	86 (96.6)	1 (1.1)	1 (1.1)	1 (1.1)
Female	Urine	Symptomatic	42	40 (95.2)	0 (0.0)	2 (4.8)	0 (0.0)
		Asymptomatic	14	14 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
		Total ³	56	55 (96.5)	0 (0.0)	2 (3.5)	0 (0.0)
All ¹	Swab	Symptomatic	124	117 (94.4)	1 (0.8)	4 (3.2)	1 (0.8)
		Asymptomatic	21	18 (85.7)	0 (0.0)	3 (14.3)	0 (0.0)
		Total	146	136 (93.2)	1 (0.7)	7 (4.8)	0 (0.7)
All ¹	Urine	Symptomatic	124	116 (93.6)	4 (3.2)	3 (2.4)	1 (0.8)
		Asymptomatic	21	20 (95.2)	1 (4.8)	0 (0.0)	0 (0.0)
		Total	146	137 (93.8)	5 (3.4)	3 (2.1)	0 (0.7)

¹ As determined by the criteria for patient infected status.
² Includes 2 female subjects for whom symptoms were not reported.
³ Includes one subject who is missing a Combo 2 Assay result.

Summary of AC2 Co-Infected Patient Data

Gender	Specimen	CT and GC infected patients	AC2 CT+ and GC+	% (95% C.I.)
Female	Swab	56	54	96.4 (87.7 - 99.6)
	Urine	56	50	89.3 (78.1 - 96.0)
Male	Swab	89	81	91.0 (83.1 - 96.0)
	Urine	89	86	96.6 (90.5 - 99.3)

CONCLUSIONS

- The AC2 assay showed comparable performance to comparator NAAT's for the detection of CT and GC co-infections in female and male patients for both swab and FCU specimens.
- AC2 Assay sensitivity for FCU in this study is higher than is generally reported in similar studies of other NAAT's. Target capture may account for the enhanced sensitivity of the AC2 Assay for detection of CT female urine specimens by eliminating potential inhibitors that may be present in the specimen.
- A very high female CT specimen sensitivity (96.8%) and specificity (99.0%) for the AC2 assay was found in asymptomatic female FCU specimens. This is particularly important to FCU screening programs where asymptomatic patients are most vulnerable to silent infection advancing to more serious disease. Individuals with asymptomatic infection continue to serve as a reservoir of infection and the argument can be made that asymptomatic patients are the most important to identify in order to decrease the overall prevalence of chlamydia.
- Based upon infected patient status, the percentage of total GC infected patients who were co-infected with CT was 32.4% (146/450).
- Based upon infected patient status, the percentage of total GC infected male patients who were co-infected with CT was 27.5.4% (89/324)
- Based upon infected patient status, the percentage of total GC infected female patients who were co-infected with CT was 44.4% (56/126).
- Based on infected patient status, AC2 detected 93.8% of patients having both CT and GC and either CT or GC in 99.3% of co-infected patients.
- Due to the high percentage of infected patients who were co-infected, and the ease of testing using urine based screening, it may be a cost effective approach to routinely screen for both CT and GC.

