510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number: K061413

B. Purpose for Submission: New device clearance

C. Measurand: *Chlamydia trachomatis*

D. Type of Test: Transcription-Mediated Amplification (TMA), and Hybridization Protection Assay (HPA)

E. Applicant: Gen-Probe, Inc

F. Proprietary and Established Names: Gen-Probe APTIMA Assay for *Chlamydia trachomatis (TIGRIS Application)*

G. Regulatory Information:

1. Regulation section: 866.3120
2. Classification: I
3. Product code: MKZ
4. Panel: Microbiology

H. Intended Use: Intended use(s): The APTIMA® Assay for *Chlamydia trachomatis* is a target amplification nucleic acid probe test that utilizes target capture for the *in vitro* qualitative detection of ribosomal RNA (rRNA) from *Chlamydia trachomatis* (CT) to aid in the diagnosis of gonococcal urogenital disease using the TIGRIS® DTS® Automated Analyzer or semi-automated instrumentation as specified. The assay may be used to test the following specimens from symptomatic individuals: clinician-collected endocervical, vaginal and male urethral swab specimens; and patient-collected female and male urine specimens. The assay may be used to test the following specimens from asymptomatic individuals: clinician-collected endocervical and vaginal swab specimens; and patient-collected vaginal swab specimens1 and female and male urine specimens.

[1] Patient-collected vaginal swab specimens are an option for screening women when a pelvic exam is not otherwise indicated. The vaginal swab specimen collection kit is not for home use.

2. Indication(s) for use: NA

3. Special conditions for use statement(s): NA

4. Special instrument requirements: Gen-Probe TIGRIS DTS

I. Device Description: The APTIMA CT Assay combines the technologies of target capture, TMA and HPA. Specimens are collected and transferred into their respective specimen transport tubes. The transport solution in these tubes releases the rRNA target and protects it from degradation during storage. When the APTIMA CT Assay is performed in the laboratory, the target rRNA molecule is isolated from the specimens by the use of a capture oligomer in a method called target capture; magnetic micro particles are another key feature of target capture. The capture oligomer contains a sequence complementary to a specific region of the target molecule as well as a string of deoxyadenosine residues. During the hybridization step, the sequence specific region of the capture oligomer binds to a specific region of the target molecule. The capture oligomer:target complex is then captured out of solution by decreasing the temperature of the reaction to room temperature. This temperature reduction allows hybridization to occur between the deoxyadenosine region on the capture oligomer and the poly-deoxythymidine molecules that are covalently attached to the magnetic particles. The micro particles, including the captured target molecule bound to them, are pulled to the side of the reaction vessel using magnets and the supernatant is aspirated. The particles are washed to remove residual specimen matrix that may contain amplification reaction inhibitors. After the target capture steps are completed, the specimens are ready for amplification. Target amplification assays are based on the ability of complementary oligonucleotide primers to specifically anneal and allow enzymatic amplification of the target nucleic acid strands. The Gen-Probe TMA reaction replicates a specific region of the rRNA from CT via DNA intermediates. A unique set of primers is used for the target molecule. Detection of the rRNA amplification product sequences (amplicon) is achieved using nucleic acid hybridization. A single-stranded chemiluminescent DNA probe, which is complementary to a region of the target amplicon, is labeled with an acridinium ester molecule. The labeled DNA probe combines with amplicon to form stable RNA:DNA hybrids. The Selection Reagent differentiates hybridized from unhybridized probe, eliminating the generation of signal from unhybridized probe. During the detection step, light emitted from the labeled RNA:DNA hybrids is measured as photon signals in a luminometer, and are reported as Relative Light Units (RLU).

The device is similar to the Gen-Probe Aptima Assay for *Chlamydia trachomatis* in that the target organism is *C. trachomatis*. The primary difference between the two assays is the platform on which the assays are performed. The TIGRIS fully automates all steps necessary to perform the ACT Assay from sample processing through amplification, detection, and data reduction.

J. Substantial Equivalence Information:

1. Predicate device name(s): Gen-Probe APTIMA Assay for *Chlamydia trachomatis*

2. Predicate 510(k) number(s): K043072

3. Comparison with predicate: The reagents for the TIGRIS DTS APTIMA CT Assay are unchanged from the initial submission (K043072). No new QC test methods were developed and/or validated for any material or reagents since the QC test methods have been developed and/or validated for the ACT Assay on DTS Systems. There are no new materials that require further QC testing.

   The Magellan system software is used for both the AC2 and ACT Assays, and since the initial clearance of the AC2 Assay on the TIGRIS DTS System, there have been no substantive changes to the hardware or system software components of the TIGRIS DTS System. This application supports modifications to the package insert for the TIGRIS DTS APTIMA CT Assay test system as a result of clinically validating all specimen types on the TIGRIS DTS System for the ACT Assay.

K. Standard/Guidance Document Referenced (if applicable): NA

L. Test Principle: The APTIMA CT Assay combines the technologies of target capture, TMA and HPA. Specimens are collected and transferred into their respective specimen transport tubes. The transport solution in these tubes releases the rRNA target and protects it from degradation during storage. When the APTIMA CT Assay is performed in the laboratory, the target rRNA molecule is isolated from the specimens by the use of a capture oligomer in a method called target capture; magnetic micro particles are another key feature of target capture. The capture oligomer contains a sequence complementary to a specific region of the target molecule as well as a string of deoxyadenosine residues. During the hybridization step, the sequence specific region of the capture oligomer binds to a specific region of the target molecule. The capture oligomer:target complex is then captured out of solution by decreasing the temperature of the reaction to room temperature. This temperature reduction allows hybridization to occur between the deoxyadenosine region on the capture oligomer and the poly-deoxythymidine molecules that are covalently attached to the magnetic particles. The micro particles, including the captured target molecule bound to them, are pulled to the side of the reaction vessel using magnets and the supernatant is aspirated. The particles are washed to remove residual specimen matrix that may contain amplification reaction inhibitors. After the target capture steps are completed, the specimens are ready for amplification. Target amplification assays are based on the ability of complementary oligonucleotide primers to specifically anneal and allow enzymatic amplification of the target nucleic acid strands. The Gen-Probe TMA reaction replicates a specific region of the 16S rRNA from CT via DNA intermediates. A unique set of primers is used for the target molecule. Detection of the rRNA amplification product sequences (amplicon) is

achieved using nucleic acid hybridization. A single-stranded chemiluminescent DNA probe, which is complementary to a region of the target amplicon, is labeled with an acridinium ester molecule. The labeled DNA probe combines with amplicon to form stable RNA:DNA hybrids. The Selection Reagent differentiates hybridized from unhybridized probe, eliminating the generation of signal from unhybridized probe. During the detection step, light emitted from the labeled RNA:DNA hybrids is measured as photon signals in a luminometer, and are reported as Relative Light Units (RLU)

M. Performance Characteristics (if/when applicable): See Attached

DTS Systems Expected Values Prevalence

The prevalence of CT in patient populations depends on risk factors such as age, gender, the presence of symptoms, the type of clinic, and the test method. A summary of the prevalence of CT, by specimen type as determined by the APTIMA CT Assay is shown in Table 1 for two multi-center clinical investigations by clinical site and overall.

Table 1: Prevalence of *C. trachomatis* by Clinical Site and Overall as Determined by APTIMA CT Assay Results % (#positive / #tested)

Site	M S		M U		F S		F U		P V S		C V S	
1	27.0	(68/252)	25.0	(63/252)	16.5	(38/230)	17.0	(39/229)	19.2	(42/219)	19.1	(44/230)
2	27.7	(98/354)	26.6	(94/354)	35.0	(70/200)	26.5	(53/200)	30.8	(61/198)	33.0	(66/200)
3	25.0	(1/4)	25.0	(1/4)	11.4	(13/114)	8.8	(10/113)	10.8	(12/111)	11.5	(13/113)
4	N/A	N/A	N/A	N/A	11.6	(31/267)	8.1	(22/271)	9.3	(25/268)	12.2	(33/270)
5	8.0	(16/200)	8.0	(16/200)	9.0	(18/199)	7.5	(15/199)	8.0	(16/199)	10.1	(20/199)
6	22.7	(69/304)	20.0	(61/305)	14.3	(42/294)	13.2	(39/295)	15.2	(44/290)	16.2	(48/296)
7	5.8	(12/207)	6.3	(13/207)	7.8	(8/102)	9.8	(10/102)	12.7	(13/102)	8.8	(9/102)
8	N/A	N/A	N/A	N/A	8.2	(4/49)	6.1	(3/49)	12.5	(6/48)	7.8	(4/51)
All	20.0	(264/1321)	18.8	(248/1322)	15.4	(224/1455)	13.1	(191/1458)	15.3	(219/1435)	16.2	(237/1461)

MS = Male Urethral Swab; **MU** = Male Urine; **FS** = Female Endocervical Swab; **FU** = Female Urine; **PVS** = Patient-Collected Vaginal Swab; **CVS** = Clinician-Collected Vaginal Swab.

Positive and Negative Predictive Values for Hypothetical Prevalence Rates in North America

The estimated positive and negative predictive values (PPV and NPV) for different hypothetical prevalence rates using the APTIMA CT Assay are shown in Table 2. These calculations are based on hypothetical prevalence rates and the overall sensitivity and specificity estimated from the patient infected status for three multi-center clinical investigations. The overall sensitivity and specificity for CT were 96.7% and 96.8%, respectively (Table 2). The actual PPV and NPV for clinician-collected endocervical, vaginal and male urethral swab, patient-collected vaginal swab, and male and female urine specimens are shown in Table 5 for each clinical site and overall.

Table 2: Positive and Negative Predictive Values for Hypothetical Prevalence Rates Hypothetical

Prevalence Rate (%) (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV
1	96.7	96.8	23.5	100.0
2	96.7	96.8	38.3	99.9
5	96.7	96.8	61.6	99.8
10	96.7	96.8	77.2	99.6
15	96.7	96.8	84.3	99.4
20	96.7	96.8	88.4	99.2
25	96.7	96.8	91.0	98.9
30	96.7	96.8	92.9	98.6

APTIMA CT Assay RLU Distribution

Figure 2 shows the RLU distribution for the APTIMA CT Assay for all specimen types in the clinical study. Table 3 summarizes the RLU distribution for the total positive and total negative results, as well as the false positive and false negative results for each specimen type relative to infected patient status. Across certain specimen types, there is a trend toward an increasing proportion of true positives as the RLU values increase.

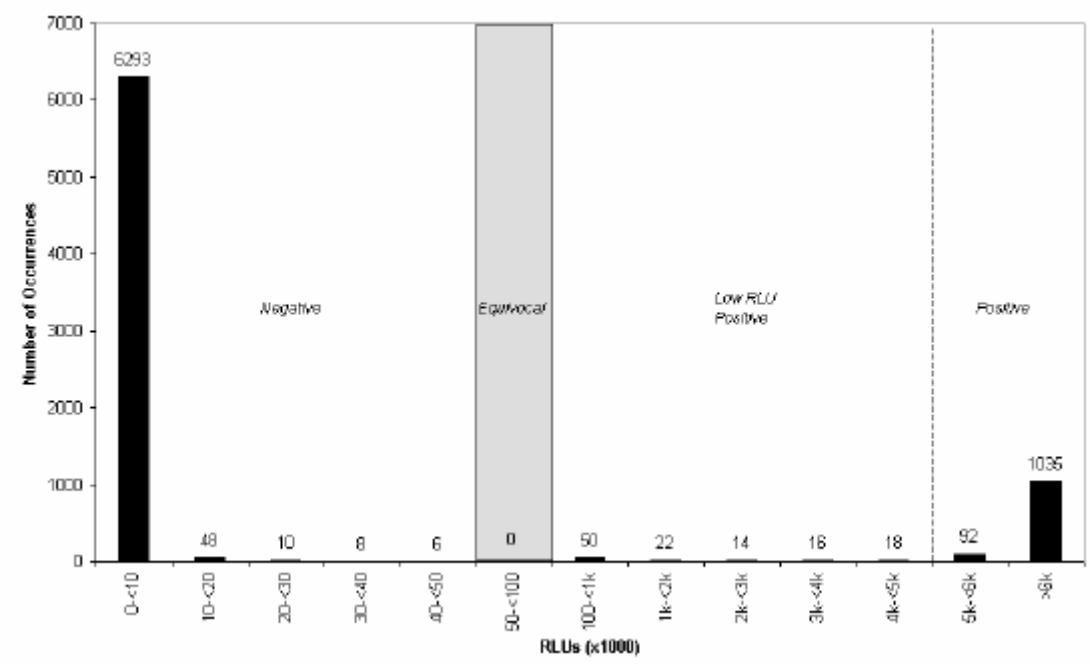

Figure 2. Frequency of RLU Distribution for the APTIMA CT Assay

Table 3: APTIMA CT Assay RLU Distribution

	RLUs (x 1000)											
	0 - <10	**10 - <20**	**20 - <30**	**30 - <40**	**40 - <50**	**50 - <100**	**100 -<1K**	**1K - <2K**	**2K - <3K**	**3K-<4K**	**4K - <5K**	**5K - <6K**
Total Positives						0	**50**	**22**	**14**	**16**	**18**	**92**
Total False Positives						0	**43**	**17**	**7**	**11**	**10**	**25**
CVS						0	18	4	1	4	4	6
PVS						0	7	5	2	1	2	2
FS						0	9	2	3	2	2	5
MS						0	3	4	0	1	0	3
FU						0	5	2	0	1	0	6
MU						0	1	0	1	2	2	3
Total Negatives	**6293**	**48**	**10**	**8**	**6**	0						
Total False Negatives	**31**	**1**	**0**	**1**	**0**	0						
CVS	4	0	0	1	0	0						
PVS	1	0	0	0	0	0						
FS	3	0	0	0	0	0						
MS	4	1	0	0	0	0						
FU	10	0	0	0	0	0						
MU	9	0	0	0	0	0						

CVS = Clinician-Collected Vaginal Swab; **PVS** = Asymptomatic Patient-Collected Vaginal Swab; **FS** = Female Endocervical Swab; **MS** = Male Urethral Swab; **FU** = Female Urine; **MU** = Male Urine.

Shaded column denotes equivocal zone.

DTS Systems Clinical Performance Characteristics

See *TIGRIS DTS System Clinical Specimen Agreement* following the *DTS Systems Analytical Performance Characteristics* section for TIGRIS DTS System-specific clinical performance characteristics.

Clinical Study Results

The performance characteristics of the APTIMA CT Assay were established in three multi-center clinical investigations conducted in North America. In the first clinical investigation, two studies were conducted. One, the clinical specimen study, established the sensitivity, specificity, and predictive values of the APTIMA CT Assay using clinician-collected endocervical, vaginal, and male urethral swab specimens, patient-collected vaginal swab specimens, and male and female urine specimens. The second study evaluated the precision of the APTIMA CT Assay when performed according to NCCLS Guidelines (16).

Clinical Specimen Study: Endocervical Swab, Male Urethral Swab, Vaginal Swab, and Urine Specimens

Clinician-collected endocervical, vaginal and male urethral swab, patient-collected vaginal swab, and male and female urine specimens were collected from 2,787 symptomatic and asymptomatic, male and female subjects attending OB/GYN, sexually transmitted disease (STD), teen, and family planning clinics at eight geographically diverse clinical sites in North America. Subjects were classified as symptomatic if symptoms such as discharge, dysuria, and pelvic pain were reported by the subject. Subjects were classified as asymptomatic if the subject did not report symptoms. Of the 1,392 asymptomatic subjects enrolled in the study, 2 were less than 16 years of age, 237 were between the ages of 16 and 20, 423 were between the ages of 21 and 25, and 730 were greater than 25 years of age. Of the 1,395 symptomatic subjects enrolled in the study, 211 were between the ages of 16 and 20, 494 were between the ages of 21 and 25, and 690 were greater than 25 years of age. Three specimens were collected from each of the 1,322 eligible male subjects. Five specimens were collected from each of the 1,465 eligible female subjects. For male subjects, two randomized urethral swabs were collected followed by one urine specimen. For female subjects, one urine specimen was collected followed by one patient-collected vaginal swab, one clinician-collected vaginal swab, and two randomized endocervical swabs. APTIMA CT Assay and APTIMA Combo 2 Assay CT results were generated from the two vaginal swabs, one endocervical swab, one male urethral swab, and a male and female urine aliquot. The remaining endocervical swab, male urethral swab, and a male and female urine aliquot were tested using another commercially-available NAAT. Endocervical and male urethral swab specimens and male and female urine specimens tested in the APTIMA Combo 2 Assay and the other commercially available NAAT were used as the reference NAATs to determine infected status for each subject. Specimen testing was conducted either at the site of subject enrollment or at an external testing site.

All performance calculations were based on the total number of APTIMA CT Assay results for endocervical, vaginal and male urethral swab, and male and female urine specimens compared to a patient infected status algorithm for each gender. In the algorithm, the designation of a subject as being
infected or not infected with CT was based on endocervical swab and urine specimen results from the commercially-available APTIMA Combo 2 Assay and the other commercially-available NAAT. Subjects were considered infected with CT if two of the four endocervical swab and urine specimens tested positive in the APTIMA Combo 2 Assay and the other reference NAAT (one specimen testing positive in each NAAT). Subjects were considered non-infected if less than two reference NAAT results were positive. A total of 8,406 APTIMA CT Assay results were used to calculate sensitivity and specificity. Sensitivity and specificity for CT by gender, specimen type and symptom status are presented in Table 4. Table 5 shows the APTIMA CT Assay sensitivity, specificity, and predictive values compared to patient infected status for each clinical site and overall. Tables 6a-6d summarize the number of results from symptomatic and asymptomatic subjects designated as infected or non-infected with CT according to the patient infected status algorithm.

Of the 2,787 subjects enrolled, there were 13 subjects with unknown CT patient infected status. Subjects were designated with an unknown patient infected status if results were missing that prevented conclusive determination of infected status. These subjects' results were not included in any performance calculations. Of the 8,452 APTIMA CT Assay results from the multi-center clinical study, there was a small percentage (8, 0.09%) of specimens that initially tested invalid for CT. Upon repeat testing, there were no equivocal or invalid results.

Table 4: Sensitivity and Specificity of the APTIMA CT Assay Relative to Patient Infected Status by Symptom Status and Overall

Specimen		Symptom Status	N	TP	FP	TN	FN		Sensitivity (95% C.I.)		Specificity (95% C.I.)
		Symptomatic	576	131	23[a]	418	4	97.0	(92.6 - 99.2)	94.8	(92.3 - 96.7
	Swab	Asymptomatic	745	90	20[b]	634	1	98.9	(94.0 - 100)	96.9	(95.3 - 98.1
		All	1321	221	43[c]	1052	5	97.8	(94.9 - 99.3)	96.1	(94.7 - 97.1
Male											
		Symptomatic	576	127	14[d]	427	8	94.1	(88.7 - 97.4)	96.8	(94.7 - 98.3
	Urine	Asymptomatic	746	90	17[e]	638	1	98.9	(94.0 - 100)	97.4	(95.9 - 98.5
		All	1322	217	31[f]	1065	9	96.0	(92.6 - 98.2)	97.2	(96.0 - 98.1
		Symptomatic	807	114	28[g]	664	1	99.1	(95.3 - 100)	96.0	(94.2 - 97.3
	Swab	Asymptomatic	636	59	22[h]	553	2	96.7	(88.7 - 99.6)	96.2	(94.3 - 97.6
		All	1443	173	50[i]	1217	3	98.3	(95.1 - 99.6)	96.1	(94.8 - 97.1
Female											
		Symptomatic	809	107	13[j]	682	7	93.9	(87.8 - 97.5)	98.1	(96.8 - 99.0
	Urine	Asymptomatic	639	58	13[k]	565	3	95.1	(86.3 - 99.0)	97.8	(96.2 - 98.8
		All	1448	165	26[l]	1247	10	94.3	(89.7 - 97.2)	98.0	(97.0 - 98.7
Patient-	**Vaginal**										

Collected	**Swab**	Asymptomatic	629	60	25[m]	543	1	98.4	(91.2 - 100)	95.6	(93.6 - 97.1
Clinician-	**Vaginal**	Symptomatic	811	111	33[n]	663	4	96.5	(91.3 - 99.0)	95.3	(93.4 - 96.7
Collected	**Swab**	Asymptomatic	638	60	32[o]	545	1	98.4	(91.2 - 99.0)	94.5	(92.3 - 96.2
		All	1449	171	65[p]	1208	5	97.2	(93.5 - 99.1)	94.9	(93.5 - 96.0

N = Negative; TP = True Positive; **FP** = False Positive; **TN** = True Negative; **FN** = False Negative.
APTIMA Combo 2 Assay CT results: # positive results / # specimens tested a: 9/23; b: 14/20; c: 23/43; d: 6/14; e: 6/17; f: 12/31; g: 14/28; h:11/22; i: 25/50; j: 7/13; k: 5/13; l: 12/26; m: 15/25; n: 17/33; o: 15/32; p: 32/65.

Table 5: Sensitivity, Specificity and Predictive Values of the APTIMA CT Assay Relative to Patient Infected Status by Clinical Site and Overall

Specimen	**Site**	**N**	**TP**	**FP**	**TN**	**FN**	**Prev. (%)**	**Sensitivity (95% C.I.)**		**Specificity (95% C.I.)**		**PPV (%)**	**NPV**
	1	252	54	14	183	1	21.8	98.2	(90.3 - 100)	92.9	(88.4 - 96.1)	79.4	99.5
	2	354	83	15	252	4	24.6	95.4	(88.6 - 98.7)	94.4	(90.9 - 96.8)	84.7	98.4
	3	4	1	0	3	0	25.0	100	(2.5 - 100)	100	(29.2 - 100)	100	100
	4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Swab	5	200	12	4	184	0	6.0	100	(73.5 - 100)	97.9	(94.6 - 99.4)	75.0	100
	6	304	59	10	235	0	19.4	100	(93.9 - 100)	95.9	(92.6 - 98.0)	85.5	100
	7	207	12	0	195	0	5.8	100	(73.5 - 100)	100	(98.1 - 100)	100	100
	8	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	All	1321	221	43	1052	5	17.1	97.8	(94.9 - 99.3)	96.1	(94.7 - 97.1)	83.7	99.4
Male													
	1	252	54	9	188	1	21.8	98.2	(90.3 - 100)	95.4	(91.5 - 97.9)	85.7	99.5
	2	354	85	9	258	2	24.6	97.7	(91.9 - 99.7)	96.6	(93.7 - 98.4)	90.4	99.2
	3	4	1	0	3	0	25.0	100	(2.5 - 100)	100	(29.2 - 100)	100	100
	4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Urine	5	200	12	4	184	0	6.0	100	(73.5 - 100)	97.9	(94.6 - 99.4)	75.0	100
	6	305	53	8	238	6	19.3	89.8	(79.2 - 96.2)	96.7	(93.7 - 98.6)	86.9	97.5
	7	207	12	1	194	0	5.8	100	(73.5 - 100)	99.5	(97.2 - 100)	92.3	100
	8	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	All	1322	217	31	1065	9	17.1	96.0	(92.6 - 98.2)	97.2	(96.0 - 98.1)	87.5	99.2
	1	228	36	2	190	0	15.8	100	(90.3 - 100)	99.0	(96.3 - 99.9)	94.7	100
	2	198	52	18	128	0	26.3	100	(93.2 - 100)	87.7	(81.2 - 92.5)	74.3	100
	3	114	9	4	101	0	7.9	100	(66.4 - 100)	96.2	(90.5 - 99.0)	69.2	100
	4	260	19	11	229	1	7.7	95.0	(75.1 - 99.9)	95.4	(91.9 - 97.7)	63.3	99.6
Swab	5	199	13	5	181	0	6.5	100	(75.3 - 100)	97.3	(93.8 - 99.1)	72.2	100
	6	294	33	9	252	0	11.2	100	(89.4 - 100)	96.6	(93.6 - 98.4)	78.6	100
	7	102	8	0	92	2	9.8	80.0	(44.4 - 97.5)	100	(96.1 - 100)	100	97.9
	8	48	3	1	44	0	6.3	100	(29.2 - 100)	97.8	(88.2 - 99.9)	75.0	100
	All	1443	173	50	1217	3	12.2	98.3	(95.1 - 99.6)	96.1	(94.8 - 97.1)	77.6	99.8
Female													
	1	227	34	5	187	1	15.4	97.1	(85.1 - 99.9)	97.4	(94.0 - 99.1)	87.2	99.5
	2	198	51	2	144	1	26.3	98.1	(89.7 - 100)	98.6	(95.1 - 99.8)	96.2	99.3
	3	113	9	1	103	0	8.0	100	(66.4 - 100)	99.0	(94.8 - 100)	90.0	100
	4	265	18	4	241	2	7.5	90.0	(68.3 - 98.8)	98.4	(95.9 - 99.6)	81.8	99.2
Urine	5	199	11	4	182	2	6.5	84.6	(54.6 - 98.1)	97.8	(94.6 - 99.4)	73.3	98.9
	6	295	29	10	252	4	11.2	87.9	(71.8 - 96.6)	96.2	(93.1 - 98.2)	74.4	98.4
	7	102	10	0	92	0	9.8	100	(69.2 - 100)	100	(96.1 - 100)	100	100
	8	49	3	0	46	0	6.1	100	(29.2 - 100)	100	(92.3 - 100)	100	100
	All	1448	165	26	1247	10	12.1	94.3	(89.7 - 97.2)	98.0	(97.0 - 98.7)	86.4	99.2
Specimen	**Site**	**N**	**TP**	**FP**	**TN**	**FN**	**Prev. (%)**	**Sensitivity (95% C.I.)**		**Specificity (95% C.I.)**		**PPV (%)**	**NPV**
	1	70	14	4	52	0	20.0	100	(76.8 - 100)	92.9	(82.7 - 98.0)	77.8	100
	2	46	13	4	29	0	28.3	100	(75.3 - 100)	87.9	(71.8 - 96.6)	76.5	100
	3	45	4	2	39	0	8.9	100	(39.8 - 100)	95.1	(83.5 - 99.4)	66.7	100

		4	152	6	3	142	1	4.6	85.7	(42.1 - 99.6)	97.9	(94.1 - 99.6	66.7	99.3
Patient-	**Vaginal**													
		5	130	7	3	120	0	5.4	100	(59.0 - 100)	97.6	(93.0 - 99.5)	70.0	100
Collected	**Swab**													
		6	75	8	5	62	0	10.7	100	(63.1 - 100)	92.5	(83.4 - 97.5)	61.5	100
		7	68	5	2	61	0	7.4	100	(47.8 - 100)	96.8	(89.0 - 99.6)	71.4	100
		8	43	3	2	38	0	7.0	100	(29.2 - 100)	95.0	(83.1 - 99.4)	60.0	100
		All	629	60	25	543	1	9.7	98.4	(91.2 - 100)	95.6	(93.6 - 97.1)	70.6	99.8
		1	228	36	8	184	0	15.8	100	(90.3 - 100)	95.8	(92.0 - 98.2)	81.8	100
		2	198	50	16	130	2	26.3	96.2	(86.8 - 99.5)	89.0	(82.8 - 93.6)	75.8	98.5
		3	113	9	4	100	0	8.0	100	(66.4 - 100)	96.2	(90.4 - 98.9)	69.2	100
		4	263	18	14	229	2	7.6	90.0	(68.3 - 98.8)	94.2	(90.5 - 96.8)	56.3	99.1
Clinician-	**Vaginal**													
		5	199	13	7	179	0	6.5	100	(75.3 - 100)	96.2	(92.4 - 98.5)	65.0	100
Collected	**Swab**													
		6	296	33	15	248	0	11.1	100	(89.4 - 100)	94.3	(90.8 - 96.8)	68.8	100
		7	102	9	0	92	1	9.8	90.0	(55.5 - 99.7)	100	(96.1 - 100)	100	98.9
		8	50	3	1	46	0	6.0	100	(29.2 - 100)	97.9	(88.7 - 99.9)	75.0	100
		All	1449	171	65	1208	5	12.1	97.2	(93.5 - 99.1)	94.9	(93.5 - 96.0)	72.5	99.6

N = Negative; TP = True Positive; **FP** = False Positive; **TN** = True Negative; **FN** = False Negative.

Table 6a: Male Urethral Swab and Urine Results from Subjects Infected or Non-Infected with *C. trachomatis* According to Patient Infected Status

	NAAT 1 (APTIMA Combo 2 Assay)		**NAAT 2**		**APTIMA CT Assay**		**Symptom Status**	
Patient Infected Status	**MS**	**MU**	**MS**	**MU**	**MS**	**MU**	**Sympt.**	**Asympt.**
Infected	+	+	+	+	+	+	96	68
Infected	+	+	+	+	+	-	5	1
Infected	+	+	+	-	+	+	11	7
Infected	+	+	-	+	+	+	13	11
Infected	+	+	-	+	+	-	1	0
Infected	+	+	-	+	-	+	1	0
Infected	+	-	+	+	+	+	2	0
Infected	+	-	+	+	+	-	1	0
Infected	+	-	+	-	+	-	1	0
Infected	-	+	+	+	+	+	1	0
Infected	-	+	-	+	+	+	0	2
Infected	-	+	-	+	-	+	3	1
Infected	-	+	=	+	+	+	0	1
Non-infected	+	+	-	-	+	+	4	4
Non-infected	+	+	-	-	-	+	1	0
Non-infected	+	-	-	-	+	+	1	4
Non-infected	+	-	-	-	+	-	4	6
Non-infected	+	-	-	-	-	+	1	0
Non-infected	+	-	-	-	-	-	3	0
Non-infected	-	+	-	-	+	+	1	0
Non-infected	-	+	-	-	-	+	0	2
Non-infected	-	+	-	-	-	-	1	0
Non-infected	-	-	+	+	+	+	1	0
Non-infected	-	-	-	+	-	-	2	2
Non-infected	-	-	-	-	+	+	1	1
Non-infected	-	-	-	-	+	-	11	5
Non-infected	-	-	-	-	-	+	4	4
Non-infected	-	-	-	-	-	-	403	618
Non-infected	-	-	-	N/A	-	+	0	2
Non-infected	-	-	-	N/A	-	-	1	2
Non-infected	-	-	-	=	-	-	0	4
Non-infected	-	-	=	-	-	-	2	0
Non-infected	N/A	-	-	-	N/A	-	0	1
Total							576	746

N/A = Specimen not obtained or available for testing. The equal symbol (**=**) represents equivocal or indeterminate on repeat testing.
MS = Male Urethral Swab; **MU** = Male Urine.

Table 6b: Female Endocervical Swab and Urine Results from Subjects Infected or Non-Infected with *C. trachomatis* According to Patient Infected Status

	NAAT 1 (APTIMA Combo 2 Assay)		NAAT 2		APTIMA CT Assay		Symptom Status		
Patient Infected Status	**FS**	**FU**	**FS**	**FU**	**FS**	**FU**	**Sympt.**	**Asympt.**	**Total**
Infected	+	+	+	+	+	+	80	43	123
Infected	+	+	+	+	+	-	1	1	2
Infected	+	+	+	-	+	+	10	5	15
Infected	+	+	+	=	+	+	1	0	1
Infected	+	+	-	+	+	+	9	3	12
Infected	+	-	+	+	+	+	3	1	4
Infected	+	-	+	+	+	-	2	2	4
Infected	+	-	+	-	+	+	2	0	2
Infected	+	-	+	-	+	-	4	0	4
Infected	+	-	+	-	+	N/A	1	0	1
Infected	-	+	+	+	+	+	0	1	1
Infected	-	+	-	+	+	+	1	3	4
Infected	-	+	-	+	-	+	1	2	3
Non-infected	+	+	-	-	+	+	1	2	3
Non-infected	+	+	-	N/A	+	+	1	0	1
Non-infected	+	-	-	-	+	+	0	2	2
Non-infected	+	-	-	-	+	-	12	7	19
Non-infected	+	-	-	-	-	-	0	1	1
Non-infected	-	+	-	-	+	+	1	0	1
Non-infected	-	+	-	-	-	+	4	3	7
Non-infected	-	+	-	-	-	-	0	1	1
Non-infected	-	-	+	-	-	-	1	1	2
Non-infected	-	-	-	+	-	-	1	2	3
Non-infected	-	-	-	-	+	+	0	2	2
Non-infected	-	-	-	-	+	-	11	9	20
Non-infected	-	-	-	-	-	+	5	4	9
Non-infected	-	-	-	-	-	-	636	526	1162
Non-infected	-	-	-	-	-	N/A	1	0	1
Non-infected	-	-	-	N/A	-	-	2	3	5
Non-infected	-	-	-	=	-	-	12	10	22
Non-infected	-	-	=	-	-	-	1	1	2
Non-infected	-	N/A	-	-	-	N/A	1	1	2
Non-infected	N/A	-	-	-	N/A	-	5	4	9
Non-infected	=	-	-	-	+	+	1	0	1
Non-infected	=	-	-	-	+	-	1	0	1
Total							812	640	1452

N/A = Specimen not obtained or available for testing. The equal symbol (**=**) represents equivocal or indeterminate on repeat testing.
FS = Female Endocervical Swab; **FU** = Female Urine. **Sympt**. = Symptomatic; **Asympt** . = Asymptomatic.

Table 6c: Asymptomatic Patient-Collected Vaginal Swab Results from Subjects Infected or Non-Infected with *C. trachomatis* According to Patient Infected Status

	NAAT 1 (APTIMA Combo 2 Assay)		NAAT 2		APTIMA CT Assay	
Patient Infected Status	**FS**	**FU**	**FS**	**FU**	**PVS**	**Total**
Infected	+	+	+	+	+	44
Infected	+	+	+	-	+	5
Infected	+	+	-	+	+	3
Infected	+	-	+	+	+	3
Infected	-	+	+	+	+	1
Infected	-	+	-	+	+	4
Infected	-	+	-	+	-	1
Non-infected	+	+	-	-	+	2
Non-infected	+	-	-	-	+	4
Non-infected	+	-	-	-	+	1
Non-infected	+	-	-	-	-	2
Non-infected	+	-	-	-	-	3
Non-infected	-	+	-	-	+	2
Non-infected	-	+	-	-	-	2
Non-infected	-	-	+	-	-	1
Non-infected	-	-	-	+	-	2
Non-infected	-	-	-	-	+	5
Non-infected	-	-	-	-	+	10
Non-infected	-	-	-	-	-	15
Non-infected	-	-	-	-	-	500
Non-infected	-	-	-	-	-	1
Non-infected	-	-	-	-	N/A	1
Non-infected	-	-	-	-	N/A	9
Non-infected	-	-	-	N/A	-	2
Non-infected	-	-	-	N/A	N/A	1
Non-infected	-	-	-	=	-	1
Non-infected	-	-	-	=	-	8
Non-infected	-	-	-	=	-	1
Non-infected	-	-	=	-	-	1
Non-infected	-	N/A	-	-	-	1
Non-infected	N/A	-	-	-	+	1
Non-infected	N/A	-	-	-	-	3
Total						640

N/A = Specimen not obtained or available for testing. The equal symbol (**=**) represents equivocal or indeterminate on repeat testing.
FS = Female Endocervical Swab; **FU** = Female Urine; **CVS** = Clinician-Collected Vaginal Swab;
PVS = Asymptomatic Patient-Collected Vaginal Swab.

Table 6d: Clinician-Collected Vaginal Swab Results from Subjects Infected or Non-Infected with *C. trachomatis* According to Patient Infected Status

	NAAT 1 (APTIMA Combo 2 Assay)		NAAT 2		APTIMA CT Assay	Symptom Status		
Patient Infected Status	**FS**	**FU**	**FS**	**FU**	**CVS**	**Sympt.**	**Asympt.**	**Total**
Infected	+	+	+	+	+	76	44	120
Infected	+	+	+	+	-	2	0	2
Infected	+	+	+	+	+	2	0	2
Infected	+	+	+	+	+	1	0	1
Infected	+	+	+	-	+	8	5	13
Infected	+	+	+	-	-	1	0	1
Infected	+	+	+	-	+	1	0	1
Infected	+	+	+	=	+	1	0	1
Infected	+	+	-	+	+	9	3	12
Infected	+	-	+	+	+	5	3	8
Infected	+	-	+	-	+	7	0	7
Infected	-	+	+	+	+	0	1	1
Infected	-	+	-	+	+	1	4	5
Infected	-	+	-	+	-	1	0	1
Infected	-	+	-	+	-	0	1	1
Non-infected	+	+	-	-	+	1	2	3
Non-infected	+	+	-	N/A	+	1	0	1
Non-infected	+	-	-	-	+	3	4	7
Non-infected	+	-	-	-	-	0	1	1
Non-infected	+	-	-	-	+	2	2	4
Non-infected	+	-	-	-	-	5	3	8
Non-infected	+	-	-	-	+	1	0	1
Non-infected	+	-	-	-	-	1	0	1
Non-infected	-	+	-	-	+	5	2	7
Non-infected	-	+	-	-	-	0	2	2
Non-infected	-	-	+	-	-	1	1	2
Non-infected	-	-	-	+	-	1	2	3
Non-infected	-	-	-	-	+	4	5	9
Non-infected	-	-	-	-	-	6	10	16
Non-infected	-	-	-	-	+	16	15	31
Non-infected	-	-	-	-	-	614	500	1114
Non-infected	-	-	-	-	N/A	0	1	1
Non-infected	-	-	-	-	+	0	1	1
Non-infected	-	-	-	-	-	13	9	22
Non-infected	-	-	-	N/A	-	2	2	4
Non-infected	-	-	-	N/A	-	0	1	1
Non-infected	-	-	-	=	+	0	1	1
Non-infected	-	-	-	=	-	12	8	20
Non-infected	-	-	-	=	N/A	0	1	1

Non-infected	-	-	=	-	-	1	1	2
Non-infected	-	N/A	-	-	-	0	1	1
Non-infected	-	N/A	-	-	N/A	1	0	1
Non-infected	N/A	-	-	-	-	0	1	1
Non-infected	N/A	-	-	-	-	5	3	8
Non-infected	=	-	-	-	-	2	0	2
Total						812	640	1452

N/A = Specimen not obtained or available for testing. The equal symbol (**=**) represents equivocal or indeterminate on repeat testing.
FS = Female Endocervical Swab; **FU** = Female Urine; **CVS** = Clinician-Collected Vaginal Swab. **Sympt**. = Symptomatic;

Asympt. = Asymptomatic.

Precision Study

APTIMA CT Assay precision (i.e., reproducibility) was evaluated at two external clinical sites and at Gen-Probe. APTIMA CT Assay precision was evaluated across three APTIMA CT Assay kit lots, three study sites, six operators and 108 APTIMA CT Assay runs. Two operators at each of the three testing sites performed a total of six APTIMA CT Assay runs per kit lot for a total of 36 runs per kit lot. Each run was composed of a 12-member precision panel containing 0 to 2,000 fg/assay of CT rRNA. Reproducibility was established using spiked swab transport medium with rRNA. Reproducibility when testing swab and urine specimens containing target organism has not been determined. Table 7 presents the precision RLU data in terms of Mean, Standard Deviation, Coefficient of Variation (CV), and percent agreement with expected results for calculations of inter-site, inter-lot, inter-operator, inter-run, and intra-run variability.

Table 7: APTIMA CT Assay Precision Data

				Inter-Site		Inter-Lot		Inter-Operator		Inter-Run		Intra-Run	
Concentration	**N**	**Mean (RLUx1000)**	**% Agrmt.**	**SD (RLUx1000)**	**CV (%)**	**SD (RLUx1000)**	**CV (%)**	**SD (RLUx1000)**	**CV (%)**	**SD (RLUx1000)**	**CV (%)**	**SD (RLUx1000)**	
Neg (0 fg/mL)	540	0.7	100	0.5	N/A	0.3	N/A	0.4	N/A	0	N/A	0.7	
Low (12 fg/mL)	216	7143.4	100	335.6	4.7	207.7	2.9	537.3	7.5	558.8	7.8	200.3	
Mid (250 fg/mL)	108	7084.9	100	275.1	3.9	159.5	2.3	546.3	7.7	578.2	8.2	162.2	
Mid (2,500 fg/mL)	108	6991.1	100	279.4	4.0	117.8	1.7	532.3	7.6	534.9	7.7	150.7	
High (5,000 -5,135 fg/mL)	324	7133.4	100	301.0	4.2	129.0	1.8	531.7	7.5	618.3	8.7	229.2	

SD = Standard Deviation; **CV(%)** = Percent Coefficient of Variation; **% Agrmt.** = Percent Agreement. **N/A** = not applicable for negative analyte.
Note: Variability from some factors may be numerically negative, which can occur if the variability due to those factors is very small. When this occurs, the variability as measured with SD and %CV is set to zero (16). **N/A** = not applicable for negative analyte.

DTS Systems Analytical Performance Characteristics

See *TIGRIS DTS System Analytical Performance Characteristics* following the Figure *TIGRIS DTS System Clinical Specimen Agreement* section for TIGRIS DTS System-specific analytical performance characteristics. **Analytical Sensitivity** *C. trachomatis* analytical sensitivity (limit of detection) was determined by directly comparing dilutions of CT organisms in cell culture and in the APTIMA CT assay. The analytical sensitivity claim for the assay is one Inclusion-Forming Unit (IFU) per assay (7.25 IFU/ swab and 5 IFU/mL urine for all 15 CT serovars. However, dilutions of less than one IFU/assay of all serovars tested positive. **Analytical Specificity** A total of 154 culture isolates were evaluated using the APTIMA CT Assay. These isolates included 86 organisms that may be isolated from the urogenital tract and 68 additional organisms that represent a phylogenetic cross-section of organisms. The tested organisms included bacteria, fungi, yeast, parasites and viruses. All organisms except *C. psittaci, C. pneumoniae, U. urealyticum* and the viruses were

tested at $1.0x10^6$ cells/assay in Kova-trol/Urine Transport Media and 60 organisms were tested in Swab Transport Media. *C. psittaci* VR601 was tested at $8x10^4$ cells/assay and *C. psittaci* VR125 was tested at $1x10^5$ cells/assay. *C. pneumoniae* was tested at $4x10^3$ cells/assay and U. urealyticum was tested at $6.7x10^6$ cells/assay. The viruses were tested as follows: (a) herpes simplex virus I: $2.5x10^4$ $TCID_{50}$/assay, (b) herpes simplex virus II: $6.0x10^4$ $TCID_{50}$/assay, (c) human papillomavirus 16: $2.9x10^6$ DNA copies/assay and (d) cytomegalovirus: $4.8x10^5$ cells/assay. The list of organisms tested is shown in Table 8.

Table 8: Analytical Specificity

Organism	Organism	Organism
Achromobacter xerosis	*Escherichia coli*	*Neisseria mucosa (3)*
Acinetobacter calcoaceticus	*Flavobacterium meningosepticum*	*Neisseria sicca (3)*
Acinetobacter Iwoffi	*Fusobacterium nucleatum*	*Neisseria subflava (14)*
Actinomyces israelii	*Gardnerella vaginalis*	*Neisseria perflava*
Actinomyces pyogenes	*Gemella haemolysans*	*Neisseria polysaccharea*
Aerococcus viridans	*Haemophilus ducreyi*	*Paracoccus denitrificans*
Aeromonas hydrophila	*Haemophilus influenzae*	*Peptostreptococcus anaerobius*
Agrobacterium radiobacter	*Herpes simplex virus I*	*Peptostreptococcus productus*
Alcaligenes faecalis	*Herpes simplex virus II*	*Plesiomonas shigelloides*
Bacillus subtilis	*Human papilloma virus 16*	*Propionibacterium acnes*
Bacteriodes fragilis	*Kingella dentrificans*	*Proteus mirabilis*
Bacteriodes ureolyticus	*Kingella kingae*	*Proteus vulgaris*
Bifidobacterium adolescentis	*Klebsiella oxytoca*	*Providencia stuartii*
Bifidobacterium brevi	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*
Branhamella catarrhalis	*Lactobacillus acidophilus*	*Pseudomonas fluorescens*
Brevibacterium linens	*Lactobacillus brevis*	*Pseudomonas putida*
Campylobacter jejuni	*Lactobacillus jensonii*	*Rahnella aquatilis*
Candida albicans	*Lactobacillus lactis*	*Rhodospirillum rubrum*
Candida glabrata	*Legionella pneumophila (2)*	*Saccharomyces cerevisiae*
Candida parapsilosis	*Leuconostoc paramensenteroides*	*Salmonella minnesota*
Candida tropicalis	*Listeria monocytogenes*	*Salmonella typhimurium*
Chlamydia pneumoniae	*Micrococcus luteus*	*Serratia marcescens*
Chlamydia psittaci (2)	*Moraxella lacunata*	*Staphylococcus saprophyticus*
Chromobacterium violaceum	*Moraxella osloensis*	*Staphylococcus aureus*
Citrobacter freundii	*Morganella morganii*	*Staphylococcus epidermidis*
Clostridium perfringens	*Mycobacterium smegmatis*	*Streptococcus agalactiae*
Corynebacterium genitalium	*Mycoplasma genitalium*	*Streptococcus bovis*
Corynebacterium xerosis	*Mycoplasma hominis*	*Streptococcus mitis*
Cryptococcus neoformans	*N. meningitidis Serogroup A*	*Streptococcus mutans*
Cytomegalovirus	*N. meningitidis Serogroup B*	*Streptococcus pneumoniae*
Deinococcus radiodurans	*N. meningitidis Serogroup C (4)*	*Streptococcus pyogenes*
Derxia gummosa	*N. meningitidis Serogroup D*	*Streptococcus salivarius*
Eikenella corrodens	*N. meningitidis Serogroup Y*	*Streptococcus sanguis*
Enterobacter aerogenes	*N. meningitidis Serogroup W135*	*Streptomyces griseinus*
Enterobacter cloacae	*Neisseria cinerea (4)*	*Trichomonas vaginalis*
Entercoccus avium	*Neisseria dentrificans*	*Ureaplasma urealyticum*
Entercoccus faecalis	*Neisseria elongata (3)*	*Vibrio parahaemolyticus*
Entercoccus faecium	*Neisseria flava*	*Yersinia enterocolitica*
Erwinia herbicola	*Neisseria flavescens (2)*	
Erysipelothrix rhusiopathiae	*Neisseria lactamica (9)*	

(n) = number of strains tested. All organisms tested produced a negative result in the APTIMA CT Assay.

Interfering Substances

The following interfering substances were individually spiked into swab and/or urine specimens: 10% blood, contraceptive jelly, spermicide, moisturizer, hemorrhoidal anesthetic, body oil, powder, anti-fungal cream, vaginal lubricants, feminine spray and leukocytes ($1x10^6$ cells/mL). The following interfering substances were individually spiked into urine specimens: 30% blood, urine analytes, protein, glucose, ketones, bilirubin, nitrate, urobilinogen, pH 4 (acidic), pH 9 (alkaline), leukocytes ($1x10^6$ cells/mL), cellular debris, vitamins, minerals, acetaminophen, aspirin and ibuprofen. All were tested for potential assay interference in the absence and presence of CT at the estimated rRNA equivalent of 1cell/assay (5 fg/assay). The rRNA equivalents were calculated based on the genome size and estimated DNA:RNA ratio/cell of each organism. No interference was observed with any of the tested substances. No inhibitors of amplification were observed in the APTIMA CT Assay.

Recovery

Escherichia coli, Gardnerella vaginalis, *Lactobacillus acidophilus*, *Bacteroides ureolyticus,* and *Staphylococcus epidermidis* (1×10^8 cells/assay) were added to samples containing the rRNA equivalent of approximately one CT IFU (5 fg). These additions did not interfere with the amplification and detection of CT rRNA using the APTIMA CT Assay.

Swab and Urine Specimen Stability Studies

Data to support the recommended shipping and storage conditions for endocervical, urethral and vaginal swab samples were generated with pooled negative swab samples. Pooled samples were spiked with CT at a final concentration of 1 IFU per reaction. The spiked samples were held at -70°C, -20°C, 4°C, and 30°C. Samples were tested in duplicate at days 0, 20, 77, and 117. All test conditions were positive for CT at all times and temperatures.

Data to support the recommended shipping and storage conditions for urine samples were generated with female and male negative urine samples. The urine samples were spiked with CT at a final concentration of 10 IFU per reaction. Two sets of the spiked urine samples were held at 30°C for 24 hours prior to being added to the Urine Transport Media (UTM). The two sets of UTM samples then were held at 4°C and 30°C, and tested in triplicate at days 0, 1, 5, 20, and 35. All samples were positive for CT at all timepoints. The two sets of UTM samples were also tested after 116 days of storage at -20°C and -70°C. All samples were positive for CT under both storage conditions.

TIGRIS DTS System Clinical Specimen Agreement

TIGRIS DTS System Agreement

Agreement between APTIMA CT Assay results generated on the fully automated TIGRIS DTS System and semi-automated DTS Systems was evaluated by testing endocervical swab, male urethral swab, male and female urine, and vaginal swab specimens. Each of the clinical specimens was tested individually with the APTIMA CT Assay on both the TIGRIS DTS System and DTS Systems at Gen-Probe. The order of testing was not randomized. Specimens identified for inclusion were tested on the TIGRIS DTS System followed by testing on DTS Systems.

Clinical Specimen Agreement Study - Endocervical Swab, Male Urethral Swab, Female and Male Urine, and Vaginal Swab Specimens

Female and male subjects attending STD, family planning, and OB/GYN clinics from eight geographically diverse sites with low to high prevalence for CT contributed endocervical swab, male urethral swab, female and male urine, and vaginal swab specimens. The specimens were transferred directly to Gen-Probe for testing. At Gen-Probe, endocervical swab, male urethral swab, female and male urine specimens were first screened with APTIMA Combo 2 Assay on the TIGRIS DTS System and the vaginal swab specimens were screened with APTIMA Combo 2 Assay on the DTS Systems. Specimens with final invalid or equivocal results were not selected in the APTIMA CT Clinical Specimen Agreement Study.Two hundred and five female swabs (87 endocervical and 118 vaginal), 120 male urethral swab, 98 female urine, and 115 male urine specimens with APTIMA Combo 2 Assay CT positive and negative results were selected for comparison testing between the TIGRIS DTS System and the DTS Systems for the APTIMA CT Assay. Specimens with initial invalid or equivocal results were retested using the same system on which the result was generated. One female urine specimen had an initial equivocal result on the DTS Systems; when retested, the final result was valid. One male urine specimen had an initial invalid result on the TIGRIS DTS System; when retested, the final result was valid. One female urine specimen had an initial equivocal result on the TIGRIS DTS System; this specimen was retested, however, the specimen had expired, so the final result was equivocal. Table 9 shows the positive, negative, and overall agreements for all paired results for each specimen type by symptomatic status. Specimens are relatively imbalanced by symptomatic and asymptomatic status but overall agreements for symptomatic subjects were 98.5% (131/133) for female swabs (combined endocervical and vaginal swabs), 98.2% (55/56) for female urine specimens, 100% (60/60) for male urethral swab, and 100% (60/60) for male urine specimens. For asymptomatic subjects, overall agreements were 100% for 72 female swabs, 60 male urethral swabs, 42 female urine, and 55 male urine specimens, respectively. For 'All' (symptomatic and asymptomatic combined) subjects, overall agreement was 99.0% (203/205) for female swab (combined endocervical and vaginal swabs), 100% (120/120) for male urethral swab, 99.0% (97/98) for female urine, and 100% (115/115) for male urine specimens. Due to relatively smaller specimen number from asymptomatic subjects, these findings may not be generalizable to ACT-TIGRIS testing with specimens from asymptomatic subjects. Refer to Tables 4 and 5 for APTIMA CT Assay sensitivity and specificity estimates from testing on the DTS Systems. Sensitivity and specificity of the APTIMA CT Assay when using the TIGRIS DTS System would be expected to be similar given the agreement findings.

Table 9: Clinical Specimen Agreement Study: Positive, Negative, and Overall Agreements by Symptom Status

Symptom	Specimen	Gender	n	DTS+	DTS+	DTS-	DTS-	Positive % Agreement	Negative % Agreement	Overal Agreer

				TIGRIS+	TIGRIS-	TIGRIS+	TIGRIS-			
								(95% CI)	(95% CI)	(95% C
		Female*	133	63	1	1	68	98.4 (91.6-100)	98.6 (92.2-100)	98.5 (9
	Swab									
		Male	60	42	0	0	18	100 (91.6-100)	100 (81.5-100)	100 (94
Sympt.										
		Female	56	33	0	1	22	100 (89.4-100)	95.7 (78.1-99.9)	98.2 (9
	Urine									
		Male	60	41	0	0	19	100 (91.4-100)	100 (82.4-100)	100 (94
		Female*	72	41	0	0	31	100 (91.4-100)	100 (88.8-100)	100 (95
	Swab									
		Male	60	23	0	0	37	100 (85.2-100)	100 (90.5-100)	100 (94
Asympt.										
		Female	42	23	0	0	19	100 (85.2-100)	100 (82.4-100)	100 (91
	Urine									
		Male	55	20	0	0	35	100 (83.2-100)	100 (90.0-100)	100 (93
		Female*	205	104	1	1	99	99.0 (94.8-100)	99.0 (94.6-100)	99.0 (9
	Swab									
		Male	120	65	0	0	55	100 (94.5-100)	100 (93.5-100)	100 (97
All										
		Female	98	56	0	1	41	100 (93.6-100)	97.6 (87.4-99.9)	99.0 (9
	Urine									
		Male	115	61	0	0	54	100 (94.1-100)	100 (93.4-100)	100 (96

"+" denotes a positive result, "-" a negative result, CI = confidence interval
*Endocervical and Vaginal Swab samples combined
[1]Specimen had a final equivocal result on the TIGRIS DTS System

Precision Study

The effect of several factors on the variability of APTIMA CT Assay performance on the TIGRIS DTS System was evaluated using 12-member STD reproducibility panels. Panel members contained 0 to 5,000 fg CT rRNA/assay. The panel included panel members with CT concentrations at the analytical sensitivity claim of 5 fg CT rRNA/assay.The panels were tested at one external testing site and at Gen-Probe using two APTIMA CT Assay reagent lots. At Gen-Probe, two operators each performed three valid worklists per reagent lot on each of two TIGRIS DTS System instruments. At the external testing site, two operators each performed three valid worklists per reagent lot on one TIGRIS DTS System instrument. One worklist consisted of run controls and six 12-member panels. Reproducibility was determined by calculating the agreement between the final assay results and the expected outcome for each panel member. Reproducibility was also assessed by calculating the SD and coefficient of variation (CV) of signal with respect to sites, operators, lots, and worklists. CVs were not calculated for CT-negative panel members due to low signal values that could theoretically equal zero. Table 10 shows the reproducibility results. All APTIMA CT Assay results on the TIGRIS DTS System agreed with the expected results. CV values were less than or equal to 3.4%. These data indicate excellent reproducibility of the APTIMA CT Assay using the TIGRIS DTS System.

Table 10: TIGRIS DTS System Precision Data

Conc (fg rRNA per assay)	n	Mean RLU (x1000)	% Agrmt	Inter-Site SD[1] (x1000)	CV[1] (%)	Inter-Operator SD (x1000)	CV (%)	Inter-Lot SD[1] (x1000)	CV (%)	Inter-Worklist SD (x1000)	CV (%)	Intra-W SD (x1000
0	863	2.9	100	1.4	N/A	0.3	N/A	0.0	N/A	0.2	N/A	2.2
5	432	7041	100	32.0	0.5	217	3.1	63.7	0.9	174	2.5	206
50	433[2]	7090	100	0.0	0.0	224	3.2	93.1	1.3	168	2.4	189
500	431[3]	7130	100	0.0	0.0	240	3.4	96.9	1.4	164	2.3	217
5,000	432	7152	100	0.0	0.0	208	2.9	85.7	1.2	179	2.5	211

Agrmt = Agreement, Conc = Concentration, CV = Coefficient of Variation, N/A = Not Applicable for negative samples, RLU = Relative Light Units, SD = Standard Deviation
[1] SD and CV values are set to 0 and 0.0%, respectively, according to the random effects model, if the variability due to this source relative to random errors and/or variati other sources is numerically negative.
[2] One worklist included 1 additional replicate of a panel member with 50 fg rRNA/assay.
[3] One worklist was missing 1 replicate of a panel member with 500 fg rRNA/assay.

TIGRIS DTS System Analytical Performance Characteristics

Analytical Sensitivity Equivalence Study

Sensitivity panels in endocervical swab pool, vaginal specimen pool, and urine specimen pool were prepared at CT rRNA equivalent of 1 IFU per assay (7.25 IFU/swab and 5 IFU/mL urine) and tested 60 replicates on the TIGRIS DTS System. Percent positivity (95% C.I.) on the TIGRIS DTS System for endocervical swab specimen was 100% (95.1 - 100), for urine specimen was 100% (95.1 - 100), and for vaginal swab specimen was 100% (95.1 - 100).

CT rRNA Spiked Clinical Panel Study

The CT rRNA spiked clinical panel study evaluated agreement between the two systems using five Gen-Probe prepared CT clinical panels spiked with 0 to 5,000 fg rRNA/assay of CT. The CT clinical panels were created from endocervical swab, vaginal swab, urethral swab, male urine, and female urine specimens that had negative APTIMA CT results on the DTS Systems when tested at Gen-Probe. The negative specimens were pooled by specimen type, spiked or not spiked with CT rRNA and aliquotted as replicates of each panel member. Replicates of each of 5-panel members with different spiked rRNA levels were combined to create one clinical panel for each specimen type. Each panel contained a total of 132 replicates.Table 11 shows the percent agreement for each level of rRNA in the endocervical swab, vaginal swab, urethral swab, male urine, and female urine panels, respectively, with expected CT results for the TIGRIS DTS System and for the DTS Systems. The concentration ranged from 1 log below to 3 logs above the 5 fg rRNA/assay for CT. Also shown in Table 11 are the overall percent agreements of the clinical panel study between the TIGRIS DTS System and DTS Systems.

Table 11: CT rRNA Spiked Clinical Panel Agreement Study

Specimen		Panel Member	Concentration (fg rRNA/Assay)	Replicates	TIGRIS % Agreement	DTS % Agreement	Overall % Agreement between TIGRIS and DTS (95% CI)
Swab	Endocervical	No Target	0	12	100	100	100 (97.2-100)
		Very Low	0.5	30	100	100	
		Low	5	30	100	100	
		Medium	50	30	100	100	
		High	5,000	30	100	100	
	Vaginal	No Target	0	12	100	100	100 (97.2-100)
		Very Low	0.5	30	100	100	
		Low	5	30	100	100	
		Medium	50	30	100	100	
		High	5,000	30	100	100	
	Urethral	No Target	0	12	100	100	100 (97.2-100)
		Very Low	0.5	30	100	100	
		Low	5	30	100	100	
		Medium	50	30	100	100	
		High	5,000	30	100	100	
Urine	Male	No Target	0	12	91.7 (11/12)	100	99.2 (95.9-100)
		Very Low	0.5	30	100	100	
		Low	5	30	100	100	
		Medium	50	30	100	100	
		High	5,000	30	100	100	

	No Target	0	12	100	100	
	Very Low	0.5	30	100	100	
Female	Low	5	30	100	100	100 (97.2-100)
	Medium	50	30	100	100	
	High	5,000	30	100	100	

Analytical Specificity Equivalence Study

For a nucleic acid amplification assay, analytical specificity with respect to individual organisms is largely determined by the chemistry of the assay (e.g. oligonucleotide sequences) rather than by the platform. Because the reagents for the APTIMA CT Assay are identical between the TIGRIS DTS System and the DTS Systems, analytical specificity experiments on the TIGRIS DTS System were designed to focus on the most challenging culture isolates. These organisms included those known to cross-react in other amplification assays. Twenty-four (24) culture isolates were selected from the panel of organisms in Table 8, including 3 organisms that are most closely related to CT. All of the organisms tested produced negative results on the TIGRIS DTS System.

Interfering Substances Equivalence Study Whole blood, a substance commonly found in urogenital specimens and known to interfere in some amplification assays, was used to establish that the TIGRIS DTS System tolerates similar levels of potentially interfering substances as does the DTS Systems. Fresh blood was added to clinical swab, vaginal swab, and urine specimen pools, then tested for potential assay interference in the absence and presence of CT target at the estimated rRNA equivalent of one CT IFU/assay (5 fg/assay). The rRNA equivalents were calculated based on the genome size and estimated DNA:RNA ratio/cell of each organism. Specimens were tested on two TIGRIS DTS Systems. All samples containing target nucleic acid were positive when tested at a level of 10% blood in swab specimens and vaginal swab specimens, and 30% blood in urine specimens. All samples that did not contain target were negative for CT. These results indicate that at the levels tested, whole blood is unlikely to affect the CT result on the TIGRIS DTS System.

Carryover Studies

To establish that the TIGRIS DTS System minimizes the risk of false positive results arising from carryover contamination, a study was conducted using spiked panels on three TIGRIS DTS Systems. The study used 20% high-target samples containing 1 x 10_6 fg CT rRNA/mL, which were randomly spaced amongst 80% negative samples containing swab transport media. In the study, 576 high-target samples and 2,376 negative samples were tested across the three TIGRIS DTS Systems. Table 12 shows the overall carryover rate was averaged at 0.21% (5/2364). A total of 12 negative samples were reported as invalid and were excluded from the calculation. A separate analysis was conducted on a subset of the study population comprised of the negative samples that immediately followed a high-target positive. The carryover rate for this subset of the population was averaged at 0.47% (2/424). For false positives in this subset, the carryover rate ranged from 0% to 1.43% across the three TIGRIS DTS Systems. These results demonstrate that carryover contamination is minimized on the TIGRIS DTS System.

Table 12: Summary of Overall TIGRIS DTS System Carryover

Instrument	**# Valid Negative Tests**	**Total # CT False Positive Results**	**% CT False Positive Results**	**Confidence Intervals (95% CI)**
TIGRIS 1	789	2[a]	0.25	0.03 - 0.91
TIGRIS 2	783	3[b]	0.38	0.08 - 1.12
TIGRIS 3	792	0[c]	0.00	0.00 - 0.38
All Instruments	2364	5	0.21	0.07 - 0.49

a. TIGRIS 1 had no false CT positive result directly following a high-target positive
b. TIGRIS 2 had two false CT positive results directly following a high-target positive
c. TIGRIS 3 had no false CT positive result directly following a high-target positive

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

1. The submitted information in this premarket notification is complete and supports a substantial equivalence decision.

2. The petition for Evaluation of Automatic Class III Designation for this device is accepted. The device is classified as Class II under regulation 21 CFR 866.3390.

P. Other Supportive Device and Instrument Information: NA