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

A. 510(k) Number:
K043072

B. Purpose for Submission:
New device clearance

C. Analyte:
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:
APTIMA CT Assay, ACT

G. Regulatory Information:
    1. <u>Regulation section:</u>
    21 CFR Part 866.3120
    2. <u>Classification:</u>
    Chlamydia Serological Reagents
    3. <u>Product Code:</u>
    MKZ
    4. <u>Panel:</u>
    83 Microbiology

H. Intended Use:
    1. <u>Intended use(s):</u>
    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* in clinician-collected endocervical, vaginal and male urethral swab specimens, patient-collected vaginal swab specimens, and female and male urine specimens. The assay may be used to test specimens from symptomatic and asymptomatic individuals to aid in the diagnosis of chlamydial urogenital disease.

    *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 condition for use statement(s):
The device is for prescription use only
4. Special instrument Requirements:
NA

I. Device Description:

The GEN-PROBE APTIMA CT Assay combines the technologies of target capture, Transcription-Mediated Amplification (TMA), and Hybridization Protection Assay (HPA).

Swab or urine 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 urine and swab samples by the use of a capture oligomer in a method called target capture; magnetic microparticles 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 microparticles, 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 *C. trachomatis* 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)."

J. Substantial Equivalence Information:
1. <u>Predicate device name(s):</u>
Gen-Probe™ Aptima Combo 2 Assay (*Chlamydia trachomatis* and *Neisseria Gonorrhoeae*)
2. <u>Predicate K number(s):</u>
K003395
3. <u>Comparison with predicate:</u>

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

L. Test Principle:
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M. Performance Characteristics (if/when applicable):

    1. <u>Analytical performance:</u>

        *a. **Precision/Reproducibility:***

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. APTIMA CT Assay Precision Data 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-operator, inter-lot, inter-run, and intra-run variability.

Table 8. APTIMA CT Assay Precision Data

Concentration	N	Mean RLU (x1000)	% Agrmt.	Inter-Site SD(RLU x1000)	CV (%)	Inter-Lot SD (RLU x1000)	CV (%)	Inter-Operator SD(RLU x1000)	CV (%)	Inter-Run SD(RLU x1000)	CV (%)	Intra-Run SD (RLU x1000)	CV (%)
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	N/A
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	2.8
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	2.3
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	2.2
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	3.2

"SD" = Standard Deviation; "CV(%)" = Percent Coefficient of Variation; "% Agrmt." = Percent Agreement
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).

b. *Linearity/assay reportable range:*

NA

c. *Traceability, Stability, Expected values (controls, calibrators, or method):*

NA

d. *Detection limit:*

C. trachomatis analytical sensitivity (limit of detection) was determined by directly comparing dilutions of *C. trachomatis* organisms in cell culture in the APTIMA CT assay. The analytical sensitivity claim for the assay is one Inclusion-Forming Unit (IFU) per assay (7.25 IFU/swab, 5 IFU/mL urine) for all 15 *C. trachomatis* serovars. However, dilutions of less than one IFU/assay of all serovars tested positive.

e.   Assay cut-off:

	0 - <10	10 - <20	20 - <30	30 - <40	40 - <50	50 - <100	100 - <1K	1 - <2K	2 - <3K	3-<4K	4 - <5K	5 - <6K	>6K
RLUs (x 1000)													
Total True Positives						**0**	50	27	15	16	18	101	1035
Total False Positives						**0**	43	18	7	11	10	29	126
CVS						*0*	*18*	*4*	*1*	*4*	*4*	*6*	*28*
PVS						*0*	*7*	*6*	*2*	*1*	*2*	*6*	*6*
FS						*0*	*9*	*2*	*3*	*2*	*2*	*5*	*26*
MS						*0*	*3*	*4*	*0*	*1*	*0*	*3*	*32*
FU						*0*	*5*	*2*	*0*	*1*	*0*	*6*	*12*
MU						*0*	*1*	*0*	*1*	*2*	*2*	*3*	*22*
Total True 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.

    2. <u>Comparison studies:</u>
NA

    3. <u>Clinical studies:</u>

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 *C. trachomatis* 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 *C. trachomatis* 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. Table 4 shows the APTIMA CT Assay sensitivity, specificity, and predictive values compared to patient infected status for each clinical site and overall. Tables 5a-5c summarize the number of results from symptomatic and asymptomatic subjects designated as infected or non-infected with *C. trachomatis* 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 or equivocal 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**

Gender	Specimen	Symptom Status	N	TP	FP	TN	FN	Sensitivity (95% C.I.)		Specificity (95% C.I.)	
Male	Urethral Swab	Symptomatic	576	131	23[a]	418	4	97.0	(92.6 - 99.2)	94.8	(92.3 - 96.7)
		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)
	Urine	Symptomatic	576	127	14[d]	427	8	94.1	(88.7 - 97.4)	96.8	(94.7 - 98.3)
		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)
Female	Endocervical Swab	Symptomatic	807	114	28[g]	664	1	99.1	(95.3 - 100)	96.0	(94.2 - 97.3)
		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)
	Urine	Symptomatic	809	107	13[j]	682	7	93.9	(87.8 - 97.5)	98.1	(96.8 - 99.0)
		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-Collected Vaginal Swab	Asymptomatic	629	60	25[m]	543	1	98.4	(91.2 - 100)	95.6	(93.6 - 97.1)

		N	TP	FP	TN	FN	Sensitivity %	95% CI	Specificity %	95% CI
Clinician-Collected Vaginal Swab	Symptomatic	811	111	33[n]	663	4	96.5	(91.3 - 99.0)	95.3	(93.4 - 96.7)
	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)

"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 4 continued
Sensitivity (57/75) x 100 = 76% (95% CI, 65.2% to 84.3%)
Specificity: (184/188) x 100 = 97.9% (95% CI, 94.7% - 99.2%)
Agreement (241/263) x 100 = 91.6% (95%CI, 87.7% to 94.4%)

  a. *Method comparison with predicate device:*
  b. *Matrix comparison:*
     NA
4. Clinical studies:

  a. *Clinical sensitivity:*
     See above
  b. *Clinical specificity:*
     See above
  c. *Other clinical supportive data (when a and b are not applicable):*

5. Clinical cut-off:
   NA

6. Expected Values

Prevalence

The prevalence of *C. trachomatis* 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 *C. trachomatis*, by specimen type as determined by the APTIMA CT Assay is shown in Prevalence by clinical site and overall.

Positive and Negative Predictive Values for Hypothetical Prevalence Rates

The estimated positive and negative predictive values (PPV and NPV) for different hypothetical prevalence rates using the APTIMA CT Assay are shown in **Error! Reference source not found.**. These calculations are based on hypothetical prevalence rates and the overall sensitivity and specificity calculated from the patient infected status. The overall sensitivity and specificity for *C. trachomatis* was 97.0% and 96.4%, respectively (**Error! Reference source not found.**). The actual PPV and NPV for clinician-collected endocervical, vaginal and male urethral swab, patient-collect vaginal swab, and male and female urine specimens are shown in **Error! Reference source not found.**d for each clinical site and overall.

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

Hypothetical Prevalence Rate (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
2	97.0	96.4	35.4	99.9
5	97.0	96.4	58.6	99.8
10	97.0	96.4	74.9	99.7
15	97.0	96.4	82.6	99.4
20	97.0	96.4	87.0	99.2
25	97.0	96.4	90.0	99.0
30	97.0	96.4	92.0	98.7

Table 1. Prevalence of *C. trachomatis* by Clinical Site and Overall as Determined by APTIMA CT Assay Results:

	% (#positive / #tested)					
Site	Male Urethral Swab	Male Urine	Endocervical Swab	Female Urine	Patient-Collected Vaginal Swab	Clinician-Collected Vaginal Swab
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)

N. Conclusion:

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