

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

A. 510(k) Number:

K053446

B. Purpose for Submission:

The Gen-Probe APTIMA[®] Assay for *Chlamydia trachomatis* (ACT) is a nucleic acid amplification test (NAAT) intended for the qualitative detection of ribosomal RNA from *Chlamydia trachomatis* (CT) in endocervical, male urethral and vaginal swab specimens and in female and male urine specimens. The assay originally received FDA clearance in 2004 (K043072). The current application is for the additional indication of testing specimens collected and processed with the Cytoc ThinPrep 2000 System and the SurePath PrepStain System. New SurePath and PreservCyt labeling for the approved ancillary liquid pap Specimen Transfer Kit is included in the current submission.

C. Measurand:

Chlamydia trachomatis (CT) ribosomal RNA

D. Type of Test:

NAAT

E. Applicant:

GEN-PROBE, INC.

F. Proprietary and Established Names:

GEN-PROBE[®] APTIMA[®] Assay for *Chlamydia trachomatis*

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
<u>MKZ</u>	<u>Class I</u>	<u>21 CFR 866.3210</u>	<u>Microbiology (83)</u>

H. Intended Use:

1. 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) in clinician-collected endocervical, vaginal and male urethral swab specimens, patient-collected vaginal swab specimens, and female and male urine specimens. The assay is also intended for use with the testing of gynecological specimens collected in the PreservCyt[®] Solution and processed with the Cytoc ThinPrep[®] 2000 System. 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:

See Intended Use above.

3. Special conditions for use statement(s):

This device is for prescription use only

4. Special instrument requirements:

Gen-Probe DTS[®] System

I. Device Description:

The GEN-PROBE[®] APTIMA[®] Assay for *Chlamydia trachomatis* is a nucleic acid amplification test (NAAT). See Test Principle below for more details.

J. Substantial Equivalence Information:

Addition of a PreservCyt (PC) specimen indication to the previously cleared device. Collection device and media are different, as are specimen handling and storage conditions.

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

Not Applicable

L. Test Principle:

The GEN-PROBE APTIMA Assay for *Chlamydia trachomatis* combines the technologies of target capture, Transcription-Mediated Amplification (TMA), and Hybridization Protection Assay (HPA). During target capture, rRNA molecules are isolated from specimens by capture oligomers on magnetic microparticles. After target capture, the specimens are ready for TMA. The GEN-PROBE APTIMA Assay for *Chlamydia trachomatis* reaction replicates a specific region of the 16S rRNA from *C. trachomatis* via DNA intermediates. Detection of the rRNA amplicons is achieved using single-stranded chemiluminescent DNA probes, which are labeled with different acridinium ester molecules. The labeled DNA probes combine with amplicon to form stable RNA:DNA hybrids and light emitted from the labeled RNA:DNA hybrids is reported as Relative Light Units (RLU). Assay results are determined by a cut-off based on the total RLU.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

PreservCyt specimen within-laboratory precision with the ACT assay was determined by spiking PreservCyt vials with 20 CT IFU per vial (0.1 IFU per reaction) and 100 CT IFU per vial (0.5 IFU per reaction). Vials containing 1,000 CT IFU per vial (5 IFU per reaction) and unspiked PreservCyt vials were tested as positive and negative controls. Ten vials spiked at each IFU level and ten unspiked vials were divided between two operators. The operators vortexed the vials and then transferred 14 aliquots (1.0 mL each) per vial into 14 APTIMA Transfer Tubes as per the APTIMA Specimen Transfer Kit package insert. The operators were blinded to the samples' titers. Each of the resulting Pap-STM samples was tested once in the ACT assay. A total of five runs were performed over a five day period for 140 results at each IFU level. The results are summarized below:

Table 10. APTIMA CT Assay Within-Laboratory Precision Data for PreservCyt using a 4-Member Precision Panel containing 0 to 1000 IFU/20 ml of CT cells

Panel Member	IFU/20mL PreservCyt	IFU/ rxn	n	Agreed	% Agmt.	Mean RLU (x1000)	Within-Operator		Between-Day		Between-Operator		Total	
							SD (x1000)	CV (%)	SD (x1000)	CV (%)	SD (x1000)	CV (%)	SD (x1000)	CV (%)
A	20	0.1	140	140	100	6501.7	734.8	11.3	0	0.0	546.9	8.4	916	14.1
B	100	0.5	140	138*	98.6	6337.7	1054.7	16.6	0	0.0	947.2	14.9	1417.6	22.4
C	1000	5	140	140	100	6521.9	909	13.9	247.1	3.8	393.9	6	1021	15.7
D	0	0	140	140	100	1.2	0.6	N/A	0	N/A	0.4	N/A	0.9	N/A

* discordant results were one negative result and 1 equivocal result

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 panel members. Operator = Run. Samples with discordant results were included in the signal variability analysis.

b. Linearity/assay reportable range:

Not Applicable

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

Not Applicable

d. Detection limit:

A study was performed that showed the ACT Assay detected CT cells at the analytical sensitivity claim (1 IFU/assay) for 3 replicates of each of 15 CT serovars tested in PC media. See results below:

Table 5.5-10: PreservCyt Analytical Sensitivity for Detection of CT

Serovar	IFU/ Assay	Replicate 1		Replicate 2		Replicate 3	
		RLU	Result	RLU	Result	RLU	Result
A	10	5,651,000	CT+	5,855,000	CT+	5,812,000	CT+
	1.0	5,882,000	CT+	5,923,000	CT+	5,777,000	CT+
	0.1	5,597,000	CT+	5,820,000	CT+	5,637,000	CT+
B	10	5,687,000	CT+	5,579,000	CT+	5,575,000	CT+
	1.0	5,657,000	CT+	5,856,000	CT+	5,756,000	CT+
	0.1	5,866,000	CT+	5,540,000	CT+	5,510,000	CT+
Ba	10	5,513,000	CT+	5,599,000	CT+	5,758,000	CT+
	1.0	5,647,000	CT+	5,661,000	CT+	5,588,000	CT+
	0.1	5,613,000	CT+	5,474,000	CT+	5,662,000	CT+
C	10	5,506,000	CT+	5,456,000	CT+	5,521,000	CT+
	1.0	5,539,000	CT+	5,576,000	CT+	5,326,000	CT+
	0.1	5,527,000	CT+	5,623,000	CT+	5,421,000	CT+
D	10	5,443,000	CT+	5,672,000	CT+	5,627,000	CT+
	1.0	5,406,000	CT+	5,436,000	CT+	5,422,000	CT+
	0.1	5,508,000	CT+	5,565,000	CT+	5,573,000	CT+
E	10	5,445,000	CT+	5,529,000	CT+	5,412,000	CT+
	1.0	5,501,000	CT+	5,269,000	CT+	5,480,000	CT+
	0.1	5,653,000	CT+	5,575,000	CT+	5,614,000	CT+
F	10	5,451,000	CT+	5,424,000	CT+	5,351,000	CT+
	1.0	5,609,000	CT+	5,589,000	CT+	5,556,000	CT+
	0.1	5,511,000	CT+	5,423,000	CT+	5,386,000	CT+
G	10	5,558,000	CT+	5,589,000	CT+	5,485,000	CT+
	1.0	5,360,000	CT+	5,389,000	CT+	5,543,000	CT+
	0.1	5,588,000	CT+	5,601,000	CT+	5,519,000	CT+

Table 5.5-10: (con't) PreservCyt Analytical Sensitivity for Detection of CT

Serovar	IFU/ Assay	Replicate 1		Replicate 2		Replicate 3	
		RLU	Result	RLU	Result	RLU	Result
H	10	5,593,000	CT+	5,746,000	CT+	5,661,000	CT+
	1.0	5,591,000	CT+	5,668,000	CT+	5,685,000	CT+
	0.1	5,675,000	CT+	5,594,000	CT+	5,681,000	CT+
I	10	5,764,000	CT+	5,897,000	CT+	5,952,000	CT+
	1.0	6,122,000	CT+	5,924,000	CT+	5,873,000	CT+
	0.1	5,742,000	CT+	5,594,000	CT+	5,621,000	CT+
J	10	5,457,000	CT+	5,017,000	CT+	5,334,000	CT+
	1.0	5,137,000	CT+	5,290,000	CT+	5,373,000	CT+
	0.1	5,348,000	CT+	5,477,000	CT+	5,501,000	CT+
K	10	5,317,000	CT+	5,065,000	CT+	5,155,000	CT+
	1.0	5,357,000	CT+	5,450,000	CT+	5,495,000	CT+
	0.1	5,532,000	CT+	5,619,000	CT+	5,346,000	CT+
L1	10	5,300,000	CT+	5,332,000	CT+	5,391,000	CT+
	1.0	5,452,000	CT+	5,431,000	CT+	5,517,000	CT+
	0.1	5,277,000	CT+	4,955,000	CT+	5,203,000	CT+
L2	10	5,317,000	CT+	5,401,000	CT+	5,394,000	CT+
	1.0	5,419,000	CT+	5,320,000	CT+	5,117,000	CT+
	0.1	4,979,000	CT+	5,281,000	CT+	5,317,000	CT+
L3	10	5,534,000	CT+	5,416,000	CT+	5,394,000	CT+
	1.0	5,277,000	CT+	5,369,000	CT+	5,198,000	CT+
	0.1	5,329,000	CT+	5,109,000	CT+	5,337,000	CT+

Table 5.5-11: SurePath Analytical Sensitivity for Detection of CT

Serovar	IFU/ Assay	Replicate 1		Replicate 2		Replicate 3	
		RLU	Result	RLU	Result	RLU	Result
A	10	5,707,000	CT+	5,224,000	CT+	5,371,000	CT+
	1.0	5,316,000	CT+	5,835,000	CT+	5,809,000	CT+
	0.1	5,592,000	CT+	5,364,000	CT+	5,495,000	CT+
B	10	5,772,000	CT+	5,572,000	CT+	5,763,000	CT+
	1.0	5,747,000	CT+	5,467,000	CT+	5,216,000	CT+
	0.1	5,544,000	CT+	5,638,000	CT+	5,876,000	CT+
Ba	10	5,432,000	CT+	5,495,000	CT+	5,125,000	CT+
	1.0	5,158,000	CT+	5,328,000	CT+	5,143,000	CT+
	0.1	5,453,000	CT+	5,619,000	CT+	5,761,000	CT+
C	10	5,917,000	CT+	5,874,000	CT+	5,712,000	CT+
	1.0	5,825,000	CT+	5,767,000	CT+	5,661,000	CT+
	0.1	5,624,000	CT+	5,748,000	CT+	5,644,000	CT+
D	10	5,903,000	CT+	5,933,000	CT+	5,967,000	CT+
	1.0	5,648,000	CT+	5,950,000	CT+	5,815,000	CT+
	0.1	5,883,000	CT+	5,704,000	CT+	5,905,000	CT+
E	10	5,818,000	CT+	5,771,000	CT+	5,756,000	CT+
	1.0	5,743,000	CT+	5,611,000	CT+	5,910,000	CT+
	0.1	5,835,000	CT+	5,715,000	CT+	5,610,000	CT+
F	10	5,491,000	CT+	5,573,000	CT+	5,345,000	CT+
	1.0	5,538,000	CT+	5,854,000	CT+	5,396,000	CT+
	0.1	4,394,000	CT+	5,446,000	CT+	5,057,000	CT+
G	10	5,423,000	CT+	5,523,000	CT+	5,561,000	CT+
	1.0	5,762,000	CT+	5,744,000	CT+	5,646,000	CT+
	0.1	5,725,000	CT+	5,526,000	CT+	5,497,000	CT+

Table 5.5-11: (con't) SurePath Analytical Sensitivity for Detection of CT

Serovar	IFU/ Assay	Replicate 1		Replicate 2		Replicate 3	
		RLU	Result	RLU	Result	RLU	Result
H	10	5,224,000	CT+	5,437,000	CT+	5,356,000	CT+
	1.0	5,428,000	CT+	5,233,000	CT+	5,176,000	CT+
	0.1	5,322,000	CT+	5,457,000	CT+	5,202,000	CT+
I	10	5,873,000	CT+	6,011,000	CT+	6,046,000	CT+
	1.0	6,078,000	CT+	6,041,000	CT+	5,917,000	CT+
	0.1	5,786,000	CT+	5,873,000	CT+	5,795,000	CT+
J	10	5,846,000	CT+	5,700,000	CT+	5,695,000	CT+
	1.0	5,454,000	CT+	5,252,000	CT+	5,334,000	CT+
	0.1	5,331,000	CT+	5,271,000	CT+	5,437,000	CT+
K	10	5,179,000	CT+	5,123,000	CT+	5,155,000	CT+
	1.0	4,430,000	CT+	5,034,000	CT+	5,495,000	CT+
	0.1	4,954,000	CT+	5,205,000	CT+	5,346,000	CT+
L1	10	4,914,000	CT+	4,853,000	CT+	4,999,000	CT+
	1.0	4,957,000	CT+	4,987,000	CT+	5,228,000	CT+
	0.1	5,221,000	CT+	5,334,000	CT+	5,116,000	CT+
L2	10	5,206,000	CT+	5,141,000	CT+	5,213,000	CT+
	1.0	5,246,000	CT+	5,462,000	CT+	5,055,000	CT+
	0.1	5,119,000	CT+	5,129,000	CT+	4,862,000	CT+
L3	10	5,183,000	CT+	5,265,000	CT+	5,354,000	CT+
	1.0	5,165,000	CT+	4,996,000	CT+	5,076,000	CT+
	0.1	4,761,000	CT+	4,892,000	CT+	4,903,000	CT+

e. *Analytical specificity:*

The *Chlamydia* species were used to evaluate the analytical specificity of the ACT Assay. A total of 3 culture isolates were tested in the liquid Pap media. None of the 3 culture isolates produced a positive result in the ACT Assay. See results below:

Table 5.5.7.1-01: Specificity of the ACT Assay with PreservCyt Samples

PHYLOGENETIC CROSS-SECTION	GP No.	ATCC No.	Concentration Tested/Assay	Rep #	Results (RLU)
<i>Chlamydia psittaci</i>	1557	VR601	7.9 x 10 ⁴ cells	1	0
				2	0
<i>Chlamydia psittaci</i>	768	VR629	1 x 10 ⁴ CELD ₅₀ /0.2 ml	1	1,000
				2	1,000
<i>Chlamydia pneumoniae</i>	1404	VR1360	4.0 x 10 ³ cells	1	0
				2	0

f. Assay cut-off:

Not Applicable

2. Comparison studies:

a. Method comparison with predicate device:

Not Applicable

3. Clinical studies:

A prospective multi-center clinical study was conducted to evaluate the use of the PreservCyt Solution (a component of the ThinPrep 2000 System) as an alternative medium for gynecological specimens for the detection of CT by the APTIMA CT Assay. One thousand six hundred forty-seven (1,647) symptomatic and asymptomatic female subjects attending OB/GYN, family planning, public health, women's, and STD clinics were evaluated in the clinical study. Of the 1,647 evaluable subjects, 1,288 were asymptomatic subjects and 359 were symptomatic subjects. Subjects were enrolled from sites with CT prevalence that ranged from 2.8% to 14.0%.

Two specimens were collected from each eligible subject: one PreservCyt Solution liquid Pap specimen and one endocervical swab specimen. PreservCyt Solution liquid Pap specimens were collected with the spatula/cyto-brush or a broom-like brush cervical sampling device. The distribution of cervical sampling devices is summarized in Table 4 by specimen collection site and overall.

PreservCyt Solution liquid Pap specimens were processed in accordance with the ThinPrep 2000 Processor Operator's Manual and APTIMA Specimen Transfer Kit Package Insert. After processing the PreservCyt Solution liquid Pap specimen with the ThinPrep 2000 Processor, the specimen was transferred into the APTIMA Specimen Transfer Kit for testing with the APTIMA CT Assay.

Sensitivity and specificity of the APTIMA CT Assay in PreservCyt Solution liquid Pap specimens were calculated by comparing results to a patient infected status algorithm. The algorithm included APTIMA Combo 2 Assay and APTIMA CT Assay results in endocervical swab specimens. Both reference NAATs were required to be positive to establish an infected patient status. At least one reference NAAT was required to be negative to establish a non-infected patient status. Table 7e summarizes the frequency of test outcomes for the two reference NAATs.

Table 5b shows the sensitivities and specificities of the APTIMA CT Assay by symptom status and overall. Overall sensitivity was 95.6% (86/90). In symptomatic and asymptomatic subjects, sensitivities were 96.7% (29/30) and 95.0% (57/60), respectively. Overall specificity was 98.8% (1539/1557). In symptomatic and asymptomatic subjects, specificities were 98.8% (325/329) and 98.9% (1214/1228), respectively.

Table 6b shows the sensitivities and specificities of the APTIMA CT Assay by specimen collection site and overall. Sensitivities ranged from 92.9% to 100%. Specificities ranged from 96.5% to 100%.

Table 4. Distribution of Cervical Sampling Device Used for PreservCyt Solution Liquid Pap Specimens

Cervical Sampling Device Used	Clinical Collection Site						Total
	1	2	3	4	5	6	
Spatula/Cytobrush	0	124	475	287	57	364	1307
Broom-Type Device	100	0	0	0	240	0	340

Table 7e. PreservCyt Solution Liquid Pap Specimen Analysis for Patient Infected Status

Patient Infected Status	Endocervical Swab		Symptom Status	
	APTIMA COMBO 2 Assay	APTIMA CT Assay	Symptomatic	Asymptomatic
Infected	Positive	Positive	30	60
Non-Infected	Negative	Negative	322	1214
Non-Infected	Negative	Positive	4	12
Non-Infected	Positive	Negative	3	2
Total			359	1288

Table 5b. Sensitivity and Specificity of the APTIMA CT Assay Relative to Patient Infected Status by Symptom Status and Overall for PreservCyt Solution Liquid Pap Specimens

	APTIMA CT PreservCyt Solution result	+/+	+/-	-/+	-/-	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)
Symptomatic	Positive	29	0	1	3	96.7 (29/30) (82.8 – 99.9)	98.8 (325/329) (96.9 – 99.7)
	Negative	1	3	3	319		
	Total	30	3	4	322		
Asymptomatic	Positive	57	0	1	13	95.0 (57/60) (86.1 – 99.0)	98.9 (1214/1228) (98.1 – 99.4)
	Negative	3	2	11	1201		
	Total	60	2	12	1214		
All	Positive	86	0	2	16	95.6 (86/90) (89.0 – 98.8)	98.8 (1539/1557) (98.2 – 99.3)
	Negative	4	5	14	1520		
	Total	90	5	16	1536		

+/+ = Positive endocervical swab specimen result in the APTIMA COMBO 2 Assay/Positive endocervical swab specimen result in the APTIMA CT Assay

+/- = Positive endocervical swab specimen result in the APTIMA COMBO 2 Assay/Negative endocervical swab specimen result in the APTIMA CT Assay

-/+ = Negative endocervical swab specimen result in the APTIMA COMBO 2 Assay/Positive endocervical swab specimen result in the APTIMA CT Assay

-/- = Positive endocervical swab specimen result in the APTIMA COMBO 2 Assay/Positive endocervical swab specimen result in the APTIMA CT Assay

Table 6b. Sensitivity, Specificity and Predictive Values of the APTIMA CT Assay Relative to Patient Infected Status by Clinical Site and Overall for PreservCyt Solution Liquid Pap Specimens

Site	APTIMA CT PreservCyt Solution Result	+/+	+/-	-/+	-/-	Prev (%)	Sensitivity (%) (95% C.I.)	Specificity (%) (95% C.I.)	PPV (%)	NPV (%)
1	Positive	14	0	1	2	14.0	100 (14/14) (78.8 – 100)	98.5 (83/86) (90.1 – 99.3)	82.4	100
	Negative	0	0	0	83					
	Total	14	0	1	85					
2	Positive	4	0	0	0	3.2	100 (4/4) (39.8 – 100)	100 (120/120) (97.0 – 100)	100	100
	Negative	0	0	2	118					
	Total	4	0	2	118					
3	Positive	29	0	0	6	6.5	93.5 (29/31) (78.6 – 99.2)	98.6 (438/444) (97.1 – 99.5)	82.9	99.5
	Negative	2	0	2	436					
	Total	31	0	2	442					
4	Positive	8	0	0	4	2.8	100 (8/8) (83.1 – 100)	98.6 (275/279) (96.4 – 99.6)	66.7	100
	Negative	0	3	1	271					
	Total	8	3	1	275					
5	Positive	13	0	0	3	4.7	92.9 (13/14) (86.1 – 99.8)	98.9 (280/283) (96.9 – 99.8)	81.3	99.6
	Negative	1	1	4	275					
	Total	14	1	4	278					
6	Positive	18	0	1	1	5.2	94.7 (18/19) (74.0 – 99.9)	99.4 (343/345) (97.9 – 99.9)	90.0	99.7
	Negative	1	1	5	337					
	Total	19	1	6	338					
All	Positive	88	0	2	16	5.5	95.8 (86/90) (89.0 – 98.8)	98.8 (1539/1557) (98.2 – 99.3)	82.7	99.7
	Negative	4	5	14	1520					
	Total	90	5	16	1536					

+/+ = Positive endocervical swab specimen result in the APTIMA COMBO 2 Assay/Positive endocervical swab specimen result in the APTIMA CT Assay

+/- = Positive endocervical swab specimen result in the APTIMA COMBO 2 Assay/Negative endocervical swab specimen result in the APTIMA CT Assay

-/+ = Negative endocervical swab specimen result in the APTIMA COMBO 2 Assay/Positive endocervical swab specimen result in the APTIMA CT Assay

-/- = Positive endocervical swab specimen result in the APTIMA COMBO 2 Assay/Positive endocervical swab specimen result in the APTIMA CT Assay

4. Clinical cut-off:

Not Applicable

5. Expected values/Reference range:

The prevalence of *C. trachomatis* disease 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* as determined by the APTIMA[®] Assay for *Chlamydia trachomatis* (ACT) results on PreservCyt liquid Pap specimens is shown below by clinical site and overall.

Table 1b. Prevalence of *C. trachomatis* by Clinical Site and Overall as Determined by APTIMA CT Assay Results Using PreservCyt Solution Liquid Pap Specimens

Site	% (#positive / #tested)	
1	17.0	(17/100)
2	3.2	(4/124)
3	7.4	(35/475)
4	4.2	(12/287)
5	5.4	(16/297)
6	5.5	(20/364)
All	6.3	(104/1647)

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.