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GEN-PROBE K962217

510(k) SUMMARY

SUMMARY OF SAFETY AND EFFECTIVENESS

GEN-PROBE AMPLIFIED Chlamydia Trachomatis Assay Kit

General Information

Submitter's Name: Company Name/Contact

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Device Name

Trade Name:	GEN-PROBE® AMPLIFIED Chlamydia Trachomatis Assay Kit
Common or Usual Name:	rRNA Target-amplified nucleic acid probe test for the *in vitro* diagnostic detection of *Chlamydia trachomatis*
Classification Names:	*Chlamydia*, DNA reagents Serological Reagents (Microbiology Classification Device List)

000042

Classification Codes:

Class I

Panel: Microbiology
Number: CFR 866.3120
Name: Chlamydia Serological Reagents

Reagents used to identify *Chlamydia* directly from clinical specimens and/or cultured isolates derived from clinical specimens.

Substantially Equivalent Device

Substantially Equivalent Device: Tissue culture media and components, synthetic cells and tissue culture.

Device Description

Background on the Disease and Principle of the Test

The Disease

C. trachomatis infections are a major cause of sexually transmitted diseases (STD) in the United States. More than four million new cases of chlamydial infections occur annually [CDC, 1993]. In humans, *C. trachomatis* species are responsible for infections of the cervix, urethra, and upper genital tract in women; infections of the urethra and epididymitis in men; and conjunctivitis and pneumonia in newborns [Schacter, J. and M. Grossman, 1981]. The *Chlamydia trachomatis* species is comprised of 15 serovars. Human isolates of *C. trachomatis* are grouped into trachoma (serovars A-K) and LGV biovars (serovars LGV I, LGV II, and LGV III). The serovars D through K are the major cause of genital chlamydial infection in men and women [Schacter, J. 1978].

Historical and Conventional Methods

Tissue culture methods remain the gold standard for diagnosing infection with these organisms, and they are the reference methods to which new nonculture assays are compared. Tissue culture methods include isolation in cell culture and demonstration of typical intracytoplasmic inclusions by appropriate immunofluorescence, iodine, or Giemsa staining procedures. Culture has several merits. For *C. trachomatis*, culture is considered 100% specific because the organism is directly observed. As well, specimen adequacy can be assessed by microscopically examining a sample of the culture transport medium. Culture is the sole method recommended in forensic cases. Despite its merits, culture has several disadvantages. *C. trachomatis* culture is expensive, time and labor intensive, and very dependent on the training and skill level of the technicians involved.

Specimen transport issues and the requirement for organism viability are the main disadvantages of *C. trachomatis* culture.

Non-culture diagnostic tests for *C. trachomatis* make more aggressive screening and prevention strategies possible because of their ease of use, shorter time-to-result, and absence of viability requirements. Nonculture methods for *C. trachomatis* detection include direct fluorescent antibody staining (DFA), enzyme immunoassay (EIA), nonamplified nucleic acid hybridization (probe assays), polymerase chain reaction (PCR), ligase chain reaction (LCR), and transcription mediated amplification (TMA), which is the most recently developed target-amplified nucleic acid test for *C. trachomatis*.

Target nucleic acid-based amplification assays are the newest non-culture methods available [Wolcott, M.J., 1992]. Amplification technology allows for detection of lower numbers of organisms as compared to other nonculture methods due to the higher sensitivity inherent in the technology. This allows for detection of infection in individuals harboring much lower levels of organisms than are detected by nonamplified nonculture methods. Because of their high sensitivity, all target-amplified nucleic acid assays for *C. trachomatis* are capable of detecting very low levels of nucleic acid, including that from nonviable cells. However, because *C. trachomatis* is a pathogen, the presence of any detectable nucleic acid indicates a biological condition that requires treatment. Amplified assays cannot be used, however, to monitor therapeutic progress because they detect nonviable organisms.

Patient Care and Public Health Implications

Screening for the presence of chlamydial infections is the cornerstone for prevention strategies. A large number of infections may be asymptomatic or have symptoms that are not specific; this makes reduction of the prevalence of chlamydial infections difficult. Accurate and prompt diagnoses of these infections is important to ensure appropriate patient management, to prevent disease complications and their associated medical costs, and to control transmission to uninfected partners.

Intended Use

The GEN-PROBE AMPLIFIED Chlamydia Trachomatis Assay (AMP CT) Kit is a target-amplified nucleic acid probe test used for the *in vitro* diagnostic qualitative detection of rRNA from *Chlamydia trachomatis* in endocervical and male urethral swab specimens and in female and male urine specimens.

Summary of Technological Characteristics

The AMP CT assay kit incorporates the technologies of *in vitro* nucleic acid amplification and target amplicon hybridization with an acridinium ester-labeled DNA probe to specifically detect *C. trachomatis* ribosomal ribonucleic acid (rRNA) in clinical specimens. Gen-Probe's proprietary technologies are combined in this product to allow qualitative detection of *C. trachomatis* rRNA in quantities as minute as those contained in a single organism. The presence of *C. trachomatis* rRNA provides evidence of the organisms's presence in a clinical specimen in the same way as does the observation of *C. trachomatis* on a coverslip from tissue culture.

Principles of the AMP CT Technology

The AMP CT assay utilizes Transcription Mediated Amplification (TMA) and the Hybridization Protection Assay (HPA) to qualitatively detect *C. trachomatis* (rRNA). TMA is an RNA transcription-dependent amplification technology, in which RNA strands serve as templates for the synthesis of DNA intermediates. These DNA intermediates are then used for the transcription of multiple copies of RNA amplicon. The RNA amplicon can then serve as templates for further synthesis of DNA intermediates, which in turn are used for further transcription of copies of RNA amplicon. The AMP CT assay uses TMA to amplify *C. trachomatis* rRNA in clinical specimens to easily detectable levels. HPA then is used to detect the amplicon.

Mechanism of the Hybridization Protection Assay (HPA)

The key aspect of HPA is the steric protection of the acridinium ester chemiluminescent label, linked to the Detection Probe, from a hydrolysis reaction that destroys the chemiluminescence of the label. This protection occurs only when the Detection Probe hybridizes with the complementary amplicon sequence. Thus, when a selection reagent is added to a solution containing a mixture of hybridized and unhybridized Detection Probes, only the hybridized probes will maintain their chemiluminescent properties. Following this "differential hydrolysis" step, Detection Reagents are added to the solution and the acridinium ester molecules that are attached to the hybridized Detection Probes emit photons. Thus, photons are emitted only if the target nucleic acid is present, and this light emissions constitutes a positive result [Arnold, L. J., 1989 and Nelson, N.C., 1992].

Potential Adverse Effects of the Device on Health

When this *in vitro* device is used as indicated, there are no know adverse effects on health. Failure of this device would produce either a false positive result or a false negative result. A false positive result occurs when the test result is above the test cut-off even though *C. trachomatis* rRNA is not present. A false negative result occurs when the

test result is below the test cut-off even though rRNA specific to *C. trachomatis* is present.

A false positive result could lead to misdiagnosing a patient's medical status, resulting in the administration of unnecessary therapy. A false negative result could delay the correct diagnosis of *C. trachomatis* infection. Misdiagnosis of a patient who has a *C. trachomatis* infection could result in a delay of antibiotic therapy. In addition, the sexual partners of an infected patient might not be notified.

False positive or false negative results can be caused by incorrect specimen collection and storage, sample misidentification, presence of low numbers of *C. trachomatis* in the specimen, specimen inhibition, interfering substances, use of the test by unqualified personnel, procedural errors and deviations, insufficient mixing, carryover contamination, transcription errors, or inappropriate test result reporting.

Summary of Non-Clinical (Analytical Laboratory) Performance Data

Determination and Rationale for Establishing the Cut-Off

A total of 130 *C. trachomatis* positive and 982 negative clinical samples were tested using the AMP CT assay to establish the assay cut-off. A total of 431 swabs (45 positive and 386 negative) and 681 urine samples (85 positive and 596 negative) were tested. Samples were considered positive based on culture and DFA. The data, analyzed by Receiver Operating Characteristic (ROC) and Decision Level (DL) curves, indicated a cut-off value between 10,000 RLU and 50,000 RLU was appropriate. A cut-off of ≥50,000 RLU was selected for both sample types to minimize false positive and false negative results. The selection of this cut-off value was validated through the clinical trial.

Limits of Detection (Analytical Sensitivity)

To establish the limits of detection in terms of rRNA copies, a purified stock of *C. trachomatis* rRNA was serially diluted and assayed in pure system (transport medium) and in swab and urine specimens. The data indicated that the AMP CT assay was capable of reliably detecting as few as 20 copies (0.05 fg) of rRNA. Twenty (20) copies of rRNA is equivalent to 1/100 of the rRNA in a *C. trachomatis* organism.

To establish the limits of detection in terms of cultured *C. trachomatis*, the 15 serovars were grown in McCoy cell monolayers and harvested to yield stocks. The stocks were diluted in a buffered salt solution to yield low numbers of infective units. Equal volume aliquots of each dilution were used to infect 2 fresh McCoy cell monolayers and to inoculate Gen-Probe transport medium. Culture IFU counts were made by DFA-staining the monolayers. Dilutions of the last culture-positive dilution were also made in order to determine the lower limit of detection for the AMP CT assay. The AMP CT assay was

able to detect fractional equivalents of IFU for all serovars. However, because 1 IFU is the lowest clinically relevant detection point, the AMP CT limit of detection was set at 1 IFU per assay in the AMP CT the labeling.

Analytical Specificity

A total of 115 culture isolates were evaluated using the AMP CT assay. These isolates included 72 organisms that may be isolated from the urogenital tract and 43 additional organisms that represent a phylogenetic cross-section of organisms. The tested organisms included bacteria, fungi, yeast, and parasites. In addition, the following viruses were tested: (1) Herpes Simplex I, (2) Herpes Simplex II, (3) Cytomegalovirus, and (4) Human Papilloma Virus Type 16. Culture isolates of *C. trachomatis* (15 serovars), *C. psittaci* and *C. pneumoniae* were tested. The bacterial and fungal organisms were tested at concentrations of 2.5×10^5 cells/assay; 5 organisms were tested using purified rRNA (2.4 to 23.8 ng/assay) because cell lysates were not available. Cytomegalovirus was tested using infected culture cells at a concentration of 4.8×10^5 cells/assay. ATCC stock preparations of the viruses Herpes Simplex I and Herpes Simplex II were tested at the supplied concentrations of 2.5×10^4 $TCID_{50}$ and 6.0×10^4 $TCID_{50}$, respectively. Purified DNA (2.0×10^6 copies/assay) was used to test Human Papilloma Virus Type 16. Only the *C. trachomatis* samples produced positive results in the AMP CT assay. Urine specimens from 89 *Chlamydia* culture negative patients reporting symptoms of urinary tract infections (UTI) were also tested. No cross-reactions were observed in the AMP CT assay.

Interference Studies

To determine whether non-target organisms would interfere with the AMP CT assay, *C. trachomatis* rRNA (0.5-500 fg/assay) was tested in the presence and absence of *E. coli, N. gonorrhoeae, G. vaginalis,* which are organisms that can be found in urogenital specimens. Specimens from patients with UTI symptoms were also tested since they likely contained a wide range of organisms naturally encountered in clinical specimens. All positive AMP CT reactions remained positive even in the presence of 240,000 cells/assay of the non-target organisms and in the presence of the naturally occurring UTI organisms.

In laboratory studies, the presence of up to 12% (v/v) blood in swab specimens and up to 0.2% (v/v) blood in urine specimens did not interfere with the AMP CT assay. In the clinical studies, even grossly bloody swab and urine specimens did not yield assay interference. Mucus, gynecological lubricants, and spermicides in swab specimens did not interfere with the assay. A hemorrhoidal anesthetic and a body oil also did not interfere when tested at elevated concentrations of 10% (w/v); loss of positive signal recovery occurred for both agents when a vast excess (50% w/v) was tested. Protein, glucose, ketones, bilirubin, nitrite, urobilinogen, vitamins, minerals, and over-the-counter pain relievers did not result in high background or prevent recovery of signal in urine specimens when present at normal or elevated levels. The assay tolerated a wide range

of urine pH (i.e., 4 to 9). Leucocytes at 7 x 10^6/mL (1.2 x 10^6/assay) [highest level tested] and excessive levels of cellular debris in urine specimens did not interfere.

Talcum powder and feminine hygiene spray interfered with the assay by elevating the background signals and reducing positive signal. These agents should not be used prior to specimen collection. The effects of tampon use and douching were not assessed.

Precision

A panel of 10 clinical swab and urine specimens, consisting of 2 negatives, 4 low positives (avg. ≤10 IFU in culture), and 4 high positives (avg. ≥50 IFU in culture), were tested. All samples were natural positives or negatives with the exception of 1 low and 1 high positive, these samples were prepared by adding purified *C. trachomatis* IFU to culture- and amplification-negative specimens. The panel was tested in a random order in triplicate twice a day for 3 days. Positive and negative amplification controls were included in each run. All samples yielded the expected results for each replicate in each run; no false positive or false negative results occurred.

Reproducibility/Proficiency

As part of the clinical studies, a proficiency panel consisting of 3 positive swab, 3 positive urine, 2 negative swab, and 2 negative urine samples was tested in triplicate by 2 or 3 operators at each of 5 sites on 3 consecutive days. The operators were trained on the assay just prior to starting proficiency runs. Of the 12 operators, 11 operators generated 100% correct panel results. One (1) operator at 1 site had 1 false result on Day 3 testing; all results were correct after the operator repeated the entire 3 day panel.

Clinical Study Design Summary

A multicenter clinical study was conducted to assess the performance characteristics of the GEN-PROBE AMPLIFIED Chlamydia Trachomatis (AMP CT) Assay Kit in identifying *C. trachomatis* in endocervical and male urethral specimens, and in female and male urine specimens, as compared to the Predicate Device (i.e., tissue culture media and components using standard laboratory culture methods for detection of *C. trachomatis*). Resolution of AMP CT apparent false positive results included reculture, direct immunofluorescence staining (DFA) of the culture transport media, or DFA of urine (urine discrepants only).

The AMP CT assay was evaluated using 1,897 swab and 2,139 urine specimens from 2,295 patients (both symptomatic and asymptomatic) at 5 geographically distributed sites. This total included 1,510 females and 785 males. Of the females evaluated, 489 (327 symptomatic and 162 asymptomatic) represented high prevalence populations, and 1,021 (245 symptomatic and 776 asymptomatic) represented low prevalence populations. Males evaluated included 530 symptomatic patients and 255 asymptomatic patients.

Prevalence of *C. trachomatis* by culture ranged from 4.0% to 13.6% with an overall prevalence of 6.8%.

Patients enrolled in the study represented all patients evaluated for *C. trachomatis* using tissue culture media and components at the participating clinical facilities during the study period, providing the inclusive study criteria were satisfied.

Symptomatic patients were defined as patients reporting symptoms consistent with a possible *C. trachomatis* infection at the time of the clinic visit. Asymptomatic patients were defined as: (i) patients not reporting symptoms at the time of the clinic visit, but known to be a sexual partner or contact of a person with a confirmed or suspected *C. trachomatis* infection or (ii) patients undergoing screening evaluations for possible *C. trachomatis* infection. Any clinical signs of an infection found by the clinician at the time of examination were recorded, but did not alter the classification of the patient as asymptomatic.

The AMP CT assay protocol in the Package Insert was followed. Positive controls and negative controls were included in each run. AMP CT specimen results that were within the range of 15,000 to 200,000 RLU were retested in duplicate at the study site to evaluate the need for an assay equivocal range (retest zone).

The clinical performance of the AMP CT assay was characterized by sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). Sensitivity and specificity were evaluated based on the pre-selected 50,000 RLU cut-off.

Clinical Study Results Summary

Receiver Operating Characteristic (ROC) and Decision Level (DL) curves of the clinical data supported the validity of the pre-selected cut-off of 50,000 RLU using the clinical data. The results of the equivocal zone testing indicated that a equivocal (retest) zone was not warranted for the AMP CT assay.

The sensitivity and specificity of the AMP CT assay were determined by comparing the assay results to *C. trachomatis* tissue culture results. Specimens that were AMP CT positive/culture negative were recultured, tested by DFA of the culture transport medium, and assayed by urine DFA (urine discrepants only).

AMP CT Assay Performance Summary Compared to Culture and DFA Testing

		AMP CT:	Pos	Pos	Pos	Pos	Neg	Neg		
		Culture:	Pos	Neg	Neg	Neg	Pos	Neg		
		DFA:	N/A	Pos	Neg	Neg	N/A	N/A		
		Urine DFA:	N/A	N/A	Pos	Neg	N/A	N/A	Sensitivity (95% CI)	Specificity (95% CI)
Sample Type by Population		N								
Female Endocervical	Symptomatic	563	35	17	N/A	9	1	501	98.1% (52/53) (93.5-100)	98.2% (501/510) (97.0-99.5)
	Asymptomatic	934	44	9	N/A	11	0	870	100% (53/53) (99.0-100)	98.8% (870/881) (98.0-99.5)
	TOTAL	1,497	79	26	N/A	20	1	1,371	99.1% (105/106) (96.7-100)	98.6% (1,371/1,391) (97.9-99.2)
Female Urine	Symptomatic	529	30	14	5	6	6	468	89.1% (49/55) (79.9-98.2)	98.7% (468/474) (97.6-99.8)
	Asymptomatic	844	32	6	1	7	9	789	81.3% (39/48) (69.2-93.3)	99.1% (789/796) (98.4-99.8)
	TOTAL	1,373	62	20	6	13	15	1,257	85.4% (88/103) (78.1-92.7)	99.0% (1,257/1,270) (98.4-99.6)
Male Urethral	Symptomatic	237	30	14	N/A	12	1	180	97.8% (44/45) (92.4-100)	93.8% (180/192) (90.1-97.4)
	Asymptomatic	163	7	4	N/A	5	0	147	100% (11/11) (95.5-100)	96.7% (147/152) (93.5-99.9)
	TOTAL	400	37	18	N/A	17	1	327	98.2% (55/56) (93.9-100)	95.1% (327/344) (92.6-97.5)
Male Urine	Symptomatic	516	52	24	6	17	5	412	94.3% (82/87) (88.8-99.7)	96.0% (412/429) (94.1-98.0)
	Asymptomatic	250	12	4	3	5	2	224	90.5% (19/21) (75.5-100)	97.8% (224/229) (95.7-99.9)
	TOTAL	766	64	28	9	22	7	636	93.5% (101/108) (88.4-98.6)	96.7% (636/658) (95.2-98.1)
Total Specimens		4,036	242	92	15	72	24	3,591	93.6% (349/373)	98.0% (3,591/3,663)

Hypothetical positive and negative predicative values (PPV and NPV) for different prevalence rates were calculated using the AMP CT overall clinical sensitivity and specificity of 93.6% and 98.0%, respectively.

AMP CT Assay Hypothetical Predictive Values at Different Prevalence Rates

Prevalence Rate (%)	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
5	93.6	98.0	71.5	99.7
10	93.6	98.0	84.1	99.3
15	93.6	98.0	89.4	98.9
20	93.6	98.0	92.2	98.4

Conclusions from Non-Clinical and Clinical Data

The nonclinical and clinical performance data generated for the GEN-PROBE AMPLIFIED Chlamydia Trachomatis Assay supports the statement of **SUBSTANTIAL EQUIVALENCE** of the GEN-PROBE AMPLIFIED Chlamydia Trachomatis Assay Kit to tissue culture media and components for accurately detecting *C. trachomatis* in clinical specimens.

The results of this clinical study demonstrate reasonable evidence that when the GEN-PROBE AMPLIFIED Chlamydia Trachomatis Assay kits are labeled as proposed, the GEN-PROBE AMPLIFIED Chlamydia Trachomatis Assay is safe and effective for its stated Intended Use.

The GEN-PROBE AMPLIFIED Chlamydia Trachomatis Assay, as a diagnostic tool, provides information that measurably contributes to a diagnosis of *C. trachomatis* infection by being substantially equivalent to tissue culture media in terms of Safety and Effectiveness.

Contraindications and Cautions

There are no contraindications or cautions.

References

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