

Lab Name:

Procedure #:

Procedure: CLIA Complexity: Moderate

Prepared By	Date Adopted	Supersedes Procedure #

Review Date	Revision Date	Signature

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This Procedural Bulletin is intended to provide a ready outline reference for performance of the assay. These abbreviated directions for use are not intended to replace the complete package insert. It is the obligation of every manufacturer of medical devices labeled FOR *IN VITRO* DIAGNOSTIC USE to provide a complete package insert in accordance with FDA labeling regulation (21CFR 809.10). Prepared in accordance with the guidelines recommended by the Clinical and Laboratory Standards Institute, Wayne, PA 19087; CLSI Document GP2-A2.

American Screening provides CLSI procedures for your use. The procedures are required to include the same information as listed in the package insert. Any modifications to this document are the sole responsibility of the Laboratory.

Lab Name:

QUICKVUE[®]
Chlamydia TEST

CLIA Complexity: MODERATE

INTENDED USE

The QuickVue Chlamydia test is a lateral flow immunoassay intended for the rapid, qualitative detection of chlamydia directly from endocervical swab and cytology brush specimens. The test is intended for use as an aid in the presumptive diagnosis of chlamydial infection.

SUMMARY AND EXPLANATION

Chlamydia trachomatis is the most common cause of sexually transmitted venereal infection in the world, with an incidence estimated at 3 to 4 million cases per year in the United States.¹ Chlamydia are composed of elementary bodies (the infectious form) and reticulate or inclusion bodies (the replicating form) and comprise 15 known serovars.²

Chlamydia trachomatis has both a high prevalence and asymptomatic carriage rate, with frequent serious complications in both women and neonates. Complications of chlamydial infection in women include cervicitis, urethritis, endometritis, pelvic inflammatory disease (PID) and increased incidence of ectopic pregnancy and infertility.³ Vertical transmission of the disease during parturition from mother to neonate can result in inclusion conjunctivitis and pneumonia.⁴

Various methods are available for the diagnosis of chlamydial infection. Conventional isolation of *Chlamydia trachomatis* involves culturing the organism in a suitable cell line.⁵ The culture may be stained for visual examination with Giemsa, iodine or fluorescein-conjugated antibodies after 48–72 hours. More recently, rapid immunoassays using antibodies to chlamydia antigens have also been developed. These methods include direct fluorescence assays and enzyme immunoassays.

PRINCIPLE OF THE TEST

To perform the test, an endocervical clinical specimen is obtained and placed into a tube containing Reagent A (Extraction Solution); after 2 minutes, Reagent B (Neutralization Solution) is added to the tube. After extraction and neutralization, 3 drops of extracted sample is added to the Test Cassette Sample Well.

If the extracted sample contains chlamydial antigen, a faint-to-dark red Test Line along with a blue Control Line will appear indicating a positive result. If chlamydial antigen is not present or present at very low levels, only a blue Control Line will be visible. If the blue Control Line does not develop, the test result is considered invalid.

Lab Name:

REAGENTS AND MATERIALS SUPPLIED

- Individually-wrapped Test Cassettes (25)
Murine monoclonal antibodies to chlamydia (Test Line) and a control rabbit polyclonal antibody capable of binding the blue-colored control label (Control Line).
- Reagent A (1) 0.2N sodium hydroxide.
- Reagent B (1) 0.1N hydrochloric acid; <0.05% Thimerosal
- Tubes, Tips and Disposable Droppers (25)
- Transport Tubes each with 2 sterile Swabs (on solid plastic shafts) (25)
 - Swab is sterile if package is intact.
- Positive Control (1) ca. 350 IFU/test formalin-inactivated chlamydia, 0.02% sodium azide
- Negative Control (1) ca. 10⁶ CFU/test heat-inactivated group B *Streptococcus*, 0.02% sodium azide
- Package Insert (1)
- Procedure Card (1)

Warnings and Precautions

- For in vitro diagnostic use.
- Do not use kit contents after the expiration date printed on the outside of the kit.
- Use appropriate precautions in the collection, storage, handling and disposal of patient samples and used kit contents.
- Use of Nitrile or Latex gloves is recommended when handling patient samples.⁶
- Dispose of containers and unused contents in accordance with Federal, State, and Local requirements.
- The Test Cassette must remain sealed in the protective foil pouch until just prior to use. All kit components must be at room temperature prior to use.
- Reagent A contains sodium hydroxide - a basic solution; Reagent B contains hydrochloric acid - an acidic solution. If either of the reagents contact the skin or eye, flush with large volumes of water.

Lab Name:

- Thimerosal is used as a preservative. Incidental contact with or ingestion of Reagent B can lead to increased hypersensitivity reactions including irritation to the skin, eyes or mouth. Seek medical attention if symptoms are experienced.
- Use **ONLY** the sterile swabs supplied in the kit, or cytology brushes to obtain endocervical specimens.
- To obtain accurate results, you must follow the Package Insert instructions.

KIT STORAGE AND STABILITY

Store kit at room temperature 59–86°F (15–30°C) out of direct sunlight. Kit contents are stable until the expiration date printed on the outer box. Do not freeze.

SPECIMEN COLLECTION AND STORAGE

The quality of the specimen obtained is of extreme importance.⁷ Detection of chlamydia requires a vigorous and thorough collection technique which provides cellular material rather than just body fluids.

A first swab should be taken to remove excess mucus from the exocervix. The second swab should be inserted into the endocervical canal, past the squamocolumnar junction, until most of the tip is no longer visible. This will permit acquisition of columnar or cuboidal epithelial cells which are the main reservoir of chlamydia organisms. Firmly rotate the swab for 15–20 seconds. The swab should be withdrawn without contamination with exocervical or vaginal cells.

Alternatively, endocervical specimens can be collected with a cytology brush (caution: do not use cytology brushes with pregnant patients). After cleaning the exocervix with the swab, insert the cytology brush into the endocervical canal past the squamocolumnar junction. Leave in place two to three seconds. Rotate the cytology brush two full turns; withdraw the brush without touching any vaginal surface.

The specimen may be tested immediately or returned to the provided transport tube for storage or transport. Do not place the specimen in any transport device containing medium since transport medium interferes with the assay and viability of the organisms is not required for the assay. Specimens may be stored for 6 hours at room temperature (59–81°F, 15–27°C) or 72 hours refrigerated (36–46°F, 2–8°C). It is recommended that specimens be processed as soon as possible after collection.

Lab Name:

QUALITY CONTROL

Built-in Control Features

The QuickVue Chlamydia test contains built-in control features. The two color result format provides a clear-cut readout for positive and negative results. The appearance of a blue Control Line next to the letter "C" provides several forms of control. First, detection components for the specimen and internal control are processed concurrently using identical procedures; therefore, the appearance of the Control Line ensures that functional activity is maintained for both components. Secondly, the appearance of the Control Line also ensures that the foil pouch integrity has been maintained and that the Test Cassette has been stored in such a manner as not to compromise its functionality. Third, the appearance of the Control Line indicates that proper volume of fluid entered the Test Cassette and capillary flow occurred. This would indicate that the Test Cassette was assembled properly, by acting as a check for all membrane interfaces and proper positioning of components. Lastly, if the Control Line does not develop within 10 minutes, the test result is invalid. As a negative control, the background color in the Result Window area should be white to light pink within 10 minutes and not interfere with the reading of the test result. If background color remains in the Result Window which interferes with your ability to read the test result, your result may be invalid. In this case, contact Quidel Technical Support.

Positive and Negative Quality Control

External control materials may also be used to assure that the reagents and assay procedure are performing properly. For this purpose Positive and Negative Control solutions are supplied in the kit.

For Best Recovery of Chlamydia Antigen:

- 1)** Invert or shake the Positive Control several times before use to evenly suspend antigen in solution.
- 2)** Add **two drops** of the Positive or Negative Control solution to a sterile swab; allow the drops to be absorbed into the swab.
- 3)** Squeeze the tube tightly between the thumb and forefinger while extracting the chlamydia antigen from the swab.

Continue with the assay as described in the **Test Procedure** using these swabs in place of a patient specimen.

The QuickVue Positive and Negative Control solutions should be tested with each new lot or shipment of test materials once for each 25-test kit and as otherwise required by your laboratory's standard quality control procedures.

Lab Name:

If controls do not perform as expected, do not use the test results. Repeat the test or contact Quidel Technical Support.

TEST PROCEDURE

All reagents, Test Cassettes and clinical specimens must be at room temperature before beginning the assay.

Extraction Procedure:

- Add 5 DROPS of REAGENT A to a clean tube.

- Insert the patient swab into the tube containing Reagent A.
 - Compress the bottom of the tube between the thumb and forefinger and twirl the swab 10 times.

- WAIT 2 MINUTES
 - Compress the bottom of the tube between the thumb and forefinger and twirl the swab 10 times.

- Fill a clean disposable dropper to the bottom of the bulb with REAGENT B. With the swab shaft to the side, add REAGENT B to the tube. Discard the dropper.
 - Compress the bottom of the tube between the thumb and forefinger and twirl the swab 10 times.

- Express the liquid from the swab by compressing the middle of the tube and pulling the swab up through it. Discard the swab.

- Insert a tip onto the tube.

Test Procedure:

- Remove the Test Cassette from the foil pouch and place it on a clean, dry, level surface.

- Add 3 DROPS of the extracted sample from the tube to the round Sample Well on the Test Cassette.

- READ RESULTS AT 10 MINUTES. Some positive results may be seen earlier.

Lab Name:

INTERPRETATION OF RESULTS

Refer to the Procedure Card for visual color interpretation of the Test and Control Lines.

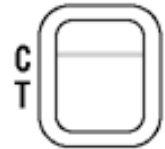
Positive Result:

The appearance of **any** faint-to-dark red Test Line along with a blue Control Line indicates a positive result. A positive QuickVue result indicates that the specimen is positive for the presence of chlamydial antigen.



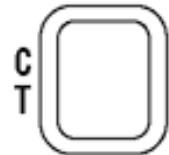
Negative Result:

The Appearance of only the blue Control Line indicates a negative result. A negative QuickVue result indicates that the specimen is a presumptive negative for the presence of chlamydial antigen.



Invalid Result:

The test result is considered invalid if the blue Control Line is not visible within 10 minutes. If this occurs, retest using 3 drops of the remaining extract solution and a new QuickVue Test Cassette or contact Quidel Technical Support.



LIMITATIONS

The QuickVue Chlamydia test has been tested using endocervical swab and cytology brush clinical specimens for the qualitative detection of chlamydia antigen. Performance with other specimens has not been assessed.

The test employs genus-specific monoclonal antibodies and will not specifically differentiate *C. trachomatis*, *C. pneumonia* or *C. psittaci*.

Detection of chlamydia is dependent on the number of organisms present in the specimen. This may be affected by specimen collection methods and patient factors such as age, history of STD, presence of symptoms, etc. The minimum detection level of this test may vary according to serovar.

Test results should be interpreted in conjunction with other laboratory and clinical data available to the physician. Standard chlamydial cell culture methods should be used in the evaluation of suspected sexual abuse and for other medicolegal cases where diagnosis could lead to adverse psychosocial impact.

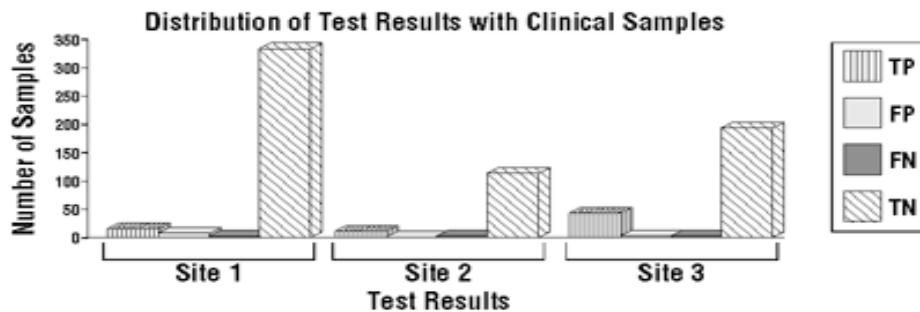
Lab Name:

Spermicides, gynecological lubricants and talcum powder were evaluated in the test and did not affect the expected results at concentrations up to 4.5 mg/test. The presence of mucus and whole blood in cervical specimens does not have an inhibitory effect on the test. However, grossly bloody specimens (approximately 100µL whole blood/test) may interfere with your ability to read the test result and therefore invalidate the result.

Therapeutic failure or success cannot be determined as antigen may persist following appropriate antimicrobial therapy.

EXPECTED VALUES

In high-risk populations, such as women attending STD (Sexually Transmitted Disease) clinics, sexually active women less than 25 years of age, women confirmed with *Neisseria gonorrhoea* infection, or those exposed to a partner with a sexually transmitted disease, the prevalence of chlamydia infection has been reported to be between 10 and 20%. In a low risk population, such as those patients attending obstetrics and gynecology practices, the prevalence of chlamydia infection is estimated to be approximately 5% or less. The prevalence of chlamydial infection by culture in the multi-center clinical study presented below ranged from 4.4% to 17.9%. A histogram showing the distribution of 723 clinical specimens is shown below. Description of this study with explanations of interpretation of test results and result resolution with Direct Fluorescent Antibody (DFA) is included in Performance Characteristics.



PERFORMANCE CHARACTERISTICS

Clinical Sensitivity, Specificity, and Accuracy

A total of 723 endocervical specimens, obtained from women attending sexually transmitted disease (STD) clinics, Family Planning and OB/GYN clinics, were evaluated in a multi-center clinical study. Testing was performed by users with various levels of work experience and educational backgrounds. Clinical performance characteristics were determined for the QuickVue Chlamydia test relative to culture techniques, both before and after resolution of QuickVue positive/culture negative specimens by DFA analysis.

Lab Name:

Seventy-three (73) endocervical specimens were positive by culture and 67 of these were also positive by QuickVue Chlamydia test. Of the 73 culture positive specimens, the QuickVue test correctly identified 81% (13/16) of the 1+ cultures corresponding to approximately ≤ 100 IFU/mL; 91% (21/23) of the 2+ cultures corresponding to 100–1,000 IFU/mL; 97% (28/29) of the 3+ cultures corresponding to 1,000–10,000 IFU/mL; and 100% (5/5) of the 4+ cultures corresponding to $>10,000$ IFU/mL.

The results summarized in Table 1 contain a comparison of the QuickVue test to culture and DFA. Of the 11 QuickVue positive/culture negative specimens, 2 were found to be positive upon analysis by DFA and an additional 3 were positive by PCR*, indicating that the 5/11 QuickVue false positive results were, in fact, true positives that were missed by culture.

*FDA-Cleared AmpliCor Polymerase Chain Reaction (PCR) Test

**TABLE 1
Performance Summary**

Culture		POS	NEG	NEG	POS	NEG				
QuickVue Test		POS	POS	POS	NEG	NEG				
DFA**		ND	POS	NEG	ND	ND				
(Interpretation)***	Prevalence %	(TP)	(TP)	(FP)	(FN)	(TN)	Sensitivity* %	Specificity* %	PPV* %	NPV* %
Total Asymptomatic (3 Sites)	9.1	43	1	4	3	454	93.6 (82.5-98.7)	99.1 (97.8-99.8)	91.2	99.4
Total Symptomatic (3 Sites)	12.4	24	1	5	3	185	89.3 (71.8-97.7)	97.4 (94.0-99.1)	82.9	98.5
Total Sites Combined	10.1	67	2	9	6	639	92.0 (85.9-98.1)	98.6 (97.4-99.4)	88.1	99.1

* Compared to Culture and DFA (95% Confidence Intervals)

** ND: Not Done Unless QuickVue Positive/Culture Negative

*** Interpretation of Results as Compared to Culture/DFA:

TP = True Positive; FP = False Positive; FN = False Negative;

TN = True Negative; PPV = Positive Predictive Value;

NPV = Negative Predictive Value

Performance Summary: Cytobrush Studies

An additional 500 endocervical specimens, collected on cytology brush, were evaluated in the QuickVue Chlamydia test at two STD clinical sites. Clinical data are presented below, both before and after resolution of discrepant specimens by DFA analysis. Of the 3 QuickVue positive/culture negative specimens, 1 was found to be positive upon analysis by DFA, indicating that 1/3 QuickVue false positive results was a true positive that was missed by culture.

Lab Name:

Cytobrush Performance Summary Before Discrepant Resolution

	Culture +	Culture -
QuickVue +	46	3
QuickVue -	10	441

Sensitivity: 82.1% (95% Con.Int. 72.1-92.2)

Specificity: 99.3% (95% Con.Int. 98.0-99.9)

Accuracy: 97.4%

PPV: 93.7%

NPV: 97.8%

Cytobrush Performance Summary After Discrepant Resolution

	Culture +	Culture -
QuickVue +	47	2
QuickVue -	10	441

Sensitivity: 82.5% (95% Con.Int. 72.6-92.3)

Specificity: 99.5% (95% Con.Int. 98.4-99.9)

Accuracy: 97.6%

PPV: 95.5%

NPV: 97.8%

Analytical Sensitivity

The analytical sensitivity of the QuickVue Chlamydia test was determined by testing serial dilutions of cultures of known infectivity. The Chlamydia trachomatis serovars A, B, Ba, C, D, E, F, G, I, K, L1 and L3 ranged from ≤ 200 to 2,000 IFU/test; serovars H, L2 and J ranged from 2,000 to 20,000 IFU/test. In addition, C. pneumonia strain TWAR was 300 IFU/test.

Analytical Specificity

A total of 48 culture isolates were evaluated in the test: 32 were organisms that may be isolated from the urogenital tract. Organisms tested at $\square 10^6$ CFU/test produced negative results in the QuickVue Chlamydia test.

Reproducibility Studies

The within-run, between-run, between-day, between-site performance of the QuickVue Chlamydia test was evaluated by following the methods contained in the NCCLS EP5-T2 guideline. The studies were conducted at two outside reference laboratories using a blind-coded panel of samples prepared from C. trachomatis Serovar D infected McCoy cell suspensions. The panel contained two low positive samples (150 and 200 IFU/test), two moderately positive samples (500 and 1000 IFU/test) and Positive and Negative Controls. Testing was performed by the laboratory personnel twice per day in replicates

Lab Name:

of three each level over three days.

All qualitative results obtained at each laboratory site were 100% in agreement with the expected results.

Physician's Office Laboratory (POL) Studies

An evaluation of the QuickVue Chlamydia test was conducted at three Physician's Offices using a panel of coded specimens. Testing was performed by physician's office personnel with diverse educational backgrounds and work experience at three different locations. The proficiency panel contained blind-coded negative, moderate positive and high positive specimens. Each specimen level was tested in replicates of five at each site over a period of three days.

The results obtained at each site ranged from 95–98% agreement with the expected results. No significant differences were observed within run (five replicates), between runs (three different assay days) or between sites (three POL sites).

ASSISTANCE

If you have any questions regarding the use of this product, please call **American Screening Corporation** at 318-227-4994.

REFERENCES

1. U.S. Department of Health and Human Services, Centers for Disease Control, Center for Prevention Services, Division of Sexually Transmitted Diseases. Chlamydia trachomatis infection. Policy guideline for prevention and control. Atlanta: August 1985.
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Lab Name:

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Covered by U.S. Patents 4,943,522, 5,763,262, 5,773,234, 5,766,961, and 5,770,460; European Patents 0 260 965, 0 296 724 and 0 566 695; other patents pending.

REF

0B006 - QuickVue Chlamydia 25 Test Kit

IVD

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EC REP

MDSS
Burckhardtstrasse 1
30163 Hannover,
Germany



To Order Please Contact:
American Screening Corporation
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www.americanscreeningcorp.com

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Lab Name:

LOG SHEET



Record Built-in Procedural Controls on the first patient tested each day.

	Date	Patient Name	Negative Procedural Control (Clear Bkgd)	Positive Procedural Control (Blue Line)	Test Results At 10 minutes	Lot Number/Exp. Date	Technician
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							

Lab Name:

LOG SHEET



Need to run external Positive and Negative Controls once per lot or shipment of material per CLIA package insert requirements.

	Date/Date Received	External Positive Control	External Negative Control	Lot Number and Exp. Date	Technician
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					