Chlamydia MIF IgG
(OUS)

REF IF1250G

Rev. S

Micro-immunofluorescent assay (MIF) for the detection of human serum IgG antibodies to *Chlamydia pneumoniae* and *Chlamydia trachomatis*

This package insert is for export only and not for distribution in the United States.

Outside of the United States:
For *in vitro* Diagnostic Use.

INTENDED USE

Focus Diagnostics' Chlamydia micro-immunofluorescent assay (MIF) IgG is intended for the qualitative detection and semi-quantitation of human serum IgG antibodies to *Chlamydia pneumoniae* and *Chlamydia trachomatis.* In conjunction with the Focus Diagnostics' Chlamydia MIF IgM, the assay is indicated for testing pneumonia patients as an aid in diagnosing community-acquired pneumonia caused by *Chlamydia pneumoniae* and infantile pneumonia caused by *Chlamydia trachomatis.* In conjunction with the Focus Diagnostics' Chlamydia MIF IgA, the assay is indicated for testing sexually active adults as an aid for diagnosing *Chlamydia trachomatis* infections, which may lead to pelvic inflammatory disease (PID). The assay is not intended for self-testing.

SUMMARY AND EXPLANATION OF TEST

Pneumonia is the leading cause of death due to infectious disease in the United States. There are an estimated 4 million cases of community-acquired pneumonia (CAP) in the United States annually, resulting in a million hospitalizations. Pneumonia is an acute respiratory infection, and is accompanied by an infiltrate detected by chest radiograph or ausculatatory observations consistent with pneumonia (e.g., altered breathing sounds). Emerging pathogens are complicating the diagnosis and management of CAP. Chlamydia, Mycoplasma, and Legionella are now among the most common causes of atypical pneumonia. The term "typical" pneumonia is a somewhat dated term, left over from when the vast majority of pneumonia was caused by *Streptococcus pneumoniae.*

Some CAP pathogens trigger differential public health responses. For example, a Legionella outbreak may warrant conducting an expensive environmental investigation to determine the source(s) of Legionella species, and decontaminating the site if found.

Chlamydia are obligate intracellular organisms that cause acute and chronic disease in mammalian and avian species. The chlamydial life cycle can be divided into 2 distinct phases: a non-replicating extracellular infectious stage and an obligate intracellular uninfectious replicating stage. The infectious form, or elementary body (EB), attaches to the target cell membrane and enters the cell via phagocytosis. Following cell entry the elementary body reorganizes into reticulate particles (forming inclusion bodies) and binary fusion begins. After 18 to 24 hours the reticulate particles condense to form elementary bodies which are released to begin another infection cycle.[1]

The genus *Chlamydia* is represented by 3 separate species. *Chlamydia trachomatis* is comprised of 12 individual serotypes (A-L) and is the etiological agent associated with trachoma, lymphogranuloma venereum (LGV), pelvic inflammatory disease, and infantile pneumonia.[2] Most *C. trachomatis* infections are asymptomatic.[3] 65% of babies born vaginally to infected mothers become infected with *C. trachomatis.*[3] *Chlamydia psittaci* is represented by many serotypes which are responsible for human psittacosis, an acute zoonotic disease associated with infected birds.[4] The newly recognized species, *C. pneumoniae*, is associated with pneumonia. *C. pneumoniae* antibody is detectable in 25% to 45% of adults tested and is responsible for approximately 10% of pneumonia cases.[5,6]

The chlamydial elementary body possesses genus (group) specific, species specific, and serotype specific antigens. The group antigens are most closely associated with the lipopolysaccharide (LPS) of the outer membrane. This extractable LPS is commonly used to produce group reactive antigens for serological assays. The major outer membrane protein (MOMP) contains species and serotype specific antigens and constitutes approximately 60% of the organism's outer membrane. Several other structural proteins are associated with the chlamydial outer membrane; however, the LPS and MOMP dominate the induction of the human immune response.[7]

Polymerase chain reaction (PCR), culture, direct fluorescent antibody (DFA) testing, and serology can be used to diagnose chlamydial infections. PCR, positive culture and DFA are the most definitive. However, specimen collection and transport difficulties and procedure complexity are associated with DFA and culture.[4,8] As a result, serologies including complement fixation (CF), indirect immunofluorescent assays (IFA) and enzyme immunoassays (EIA) are used for routine diagnosis.[4] Chlamydial CF tests became available in the 1940s. This assay utilizes an enriched LPS antigen for the detection of group antibody. Complement fixation assays are technically difficult to perform and are inherently insensitive.[4] Commercially available EIAs employ a broadly cross-reactive antigen usually derived from an LGV serotype. Consequently, as with the CF test, only group antibody responses are detected.[7] Experts recognize MIF as the method of choice for diagnosing infantile pneumonia infections caused by *Chlamydia trachomatis*.[3]

Two forms of the indirect immunofluorescent assay are available. One IFA utilizes infected cells which express whole chlamydial inclusion bodies as the substrate. Reticulate bodies, which comprise the inclusion bodies, express genus specific epitopes and, therefore, do not allow the differential detection of chlamydial species-specific antibody reactions.[9] The other available IFA, a micro-immunofluorescent assay (MIF) introduced in the 1970s,[7] utilizes purified Elementary Bodies (EB) as the substrate. By removing the genus-reactive LPS antigen, the purified EBs can be used to detect species and serovar specific chlamydial antibody reactions. EBs from all chlamydial species and serotypes can be purified and pooled, or used as individual substrate spots.[7]

The primary immune response to chlamydia is an IgM class antibody which appears early in the infection. The IgG and IgA antibody response follows the initial IgM response closely. The early IgG, IgM and IgA antibody response is to chlamydial group specific antigens as well as species specific antigens. In primary chlamydial infections a 4-fold rise in IgG is diagnostic. When *C. pneumoniae* or *C. psittaci* is suspected, detectable IgM is highly diagnostic.[10] However, the presence of IgM in *C. trachomatis* patients is less predictive of current *C. trachomatis* infection: the IgM response is detectable in only 28% to 33% of patients with current *C. trachomatis* infections and may be detectable in patients without active chlamydial infection. [10]

The primary *C. pneumoniae* infection is characterized by a predominant IgM response within 2 to 4 weeks, a delayed IgG response within 6 to 8 weeks[11] and a weak or absent IgA response.[12] After acute *C. pneumoniae* infection, IgM antibodies are usually lost within 2 to 6 months,[11] IgG antibody titers rise and usually decrease slowly; whereas IgA antibodies tend to disappear rapidly. [13]

There are an estimated 92 million new cases of *Chlamydia trachomatis* that occur each year worldwide (WHO, 2001). Each year, an estimated four million new cases occur in the United States and industrialized countries. Of those infected, up to 70% of women and 50% of men are asymptomatic. *C. trachomatis* often causes asymptomatic genital tract infections in both men and women and the bacteria may remain infectious in the host for months. This leads to a high number of unrecognized infected individuals who spread the infection to other men and women via sexual contact. Men are typically less likely than women to seek diagnosis and most reported infections occur in the 15- to 24-year-old age group. Although this leads to an overall underestimated number of cases, it makes *C. trachomatis* one of the most common curable sexually transmitted pathogens of humans. This has public health significance because *C. trachomatis* can have

serious long-term consequences especially in women. It is a well-established cause of pelvic inflammatory disease (PID) which can lead to infertility, ectopic pregnancy, and chronic pain. These conditions can have major lifetime consequences for the affected individual and can be expensive to treat. In addition, ophthalmia neonatorum and pneumonitis can occur in children who are born to women infected by Chlamydia.

PID is a complex syndrome that includes a wide range of inflammatory diseases such as endometriosis, salpingitis, and tubo-ovarian abscess. These diseases can be caused by a variety of different organisms. Unlike treatment for other specific sexually transmitted organisms, there is no single therapeutic regimen of choice for persons with PID. Several antimicrobial regimens have been proven highly effective in achieving clinical cure for persons with PID. Therapeutic choices for patients with PID have been designed to provide flexibility. PID therapy regimens typically provide broad-spectrum coverage of likely etiologic pathogens. The selection criteria for a treatment regimen should consider microbial etiology as well as institutional availability, cost-control efforts, patient acceptance, and regional differences in antimicrobial susceptibility. This broad-spectrum treatment for persons with PID will continue until more definitive studies are performed. Any regimen used should cover *C. trachomatis, N. gonorrhoeae*, anaerobes, gram-negative rods, and streptococci[17].

C. trachomatis screening programs should aim to detect and treat a significant proportion of asymptomatic infections and thereby result in a reduction of morbidity associated with chlaymidial infections along with the incidence and prevalence of infection. Although serology can never replace methods aiming at the direct detection of *Chlamydia trachomatis*, there are situations in which reliable serological tests can be helpful. Indeed, urogenital infections with these bacteria are frequently unapparent. Therefore, determination of antibodies to *C. trachomatis* antigens may be useful in determining whether a patient has had a previous infection encounter. For example, in chronically infected patients in whom the bacteria are no longer detectable locally, a positive serological test may be the only indication of chlamydial involvement[18].

Micro-immunofluorescent assays (MIF) is considered the serological "gold standard". MIFs have been shown to detect acute phase antibodies against *C. trachomatis* more often in pelvic inflammatory disease (PID) patients using serology in comparison with polymerase chain reaction (PCR) or tissue culture on samples of cervix and urethra obtained from the same infected patients[19]. Several other tests can be used to detect antibodies to *C. trachomatis* in human serum samples, including complement fixation, EIA, and radioimmunoassay. While the antigens used in these tests vary considerably, the tests all measure antibodies against different antigenic determinants of Chlamydia species. Most women with acute pelvic inflammatory disease have serum antibodies to *C. trachomatis*, often in high titers. Since serum antibodies persist for several years after the acute infection, it is difficult to use serum antibody testing in the diagnosis of acute chlamydial PID. However, some studies suggest that specific short-lived immunoglobulin A (IgA) antibodies may be a potential marker of active chlamydial infection[20].

In a study[18], Western blot analysis of serum samples from healthy blood donors and *C. trachomatis*-infected patients were used to determine antibody responses to whole *C. trachomatis* antigens. The results of the Western blot analysis showed that the IgM response appeared to be limited to a small number of antigens while the IgA, and IgG in particular, recognized a high number of antigens. The Western blot analysis showed that serum from *C. trachomatis*-infected patients increased in the number of IgG, IgM, and IgA responses to whole Chlamydia antigens in comparison to sera from healthy blood donors. *C. trachomatis*-infected patients had significantly more IgG to LPS, MOMP, hsp60, and pgp3 and more IgA to LPS and MOMP than healthy blood donors when the percentage of individuals with serum antibodies to synthetic proteins or recombinant antigens were examined.

The Focus Diagnostics Chlamydia MIF assay utilizes 1 strain of *C. pneumoniae*, 2 strains of *C. psittaci* and 8 serotypes (D-K) of *C. trachomatis.* The Chlamydial elementary bodies have been treated with a proprietary process to remove interfering LPS and diluted in 3% yolk sac to add contrast to the background. Each slide contains 12 wells; on each well are 4 individual spots. Each well contains separate spots for each species and a separate yolk sac control.

TEST PRINCIPLE

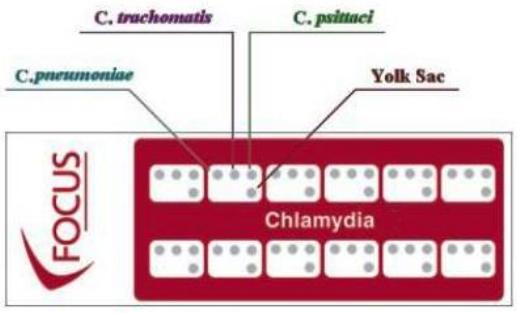
The micro-immunofluorescent antibody (MIF) assay is a 2-stage "sandwich" procedure. In the first stage, the patient sera are diluted in PBS. The diluted sera are added to appropriate slide wells in contact with the substrate, and incubated. Following incubation, the slide is washed in phosphate buffered saline which removes unbound serum antibodies. In the second stage, each antigen well is overlaid with fluorescein-labeled antibody to IgG. The slide is incubated allowing antigen-antibody complexes to react with the fluorescein-labeled anti-IgG. After the slide is washed, dried, and mounted, it is examined using fluorescence microscopy. Positive reactions appear as bright apple-green fluorescent EBs with a background matrix of yolk sac. Semi-quantitative endpoint titers are obtained by testing serial dilutions of positive specimens.

MATERIALS SUPPLIED

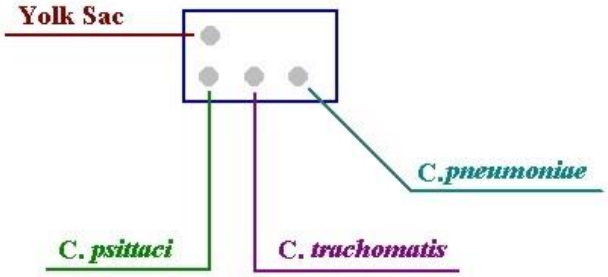
Focus Diagnostics' Test kit contains sufficient materials to perform 120 determinations.

Chlamydia MIF Substrate Slide | **REF** | **IF1201** | **Ag**

Ten slides of 12 wells each. Each well contains 4 spots: 1 yolk sac control spot and 3 individual antigen spots consisting of EBs suspended in a yolk sac matrix. Store sealed slide packets at 2 to 8°C. The sealed slides are stable until the date stated on the slide packet labels. To avoid condensation, allow the slides to warm to room temperature before opening the sealed packets.



Note: The majority of fluorescent microscopes invert the image of the slide. When viewed through the microscope the antigens will appear as below.



IgG Conjugate-Dual Species, 3.5mL | REF | IF0011 | CONJ | IgG

One vial of fluorescein-labeled goat anti-human IgG, gamma-chain specific, blended with a fluorescein-labeled goat anti-mouse IgG. The anti-mouse IgG has been standardized to provide specific antigen control. Contains Evan's Blue counterstain, protein stabilizer and preservatives. Ready for use. Stable at 2 to 8°C until the date stated on the label. Do not use if cloudiness, discoloration or other indications of bacterial contamination are present. Allow to warm to room temperature before use.

Polyvalent Detectable Control, 0.3mL | REF | IF1214 | CONTROL | >

One vial of mouse monoclonal antibodies bottled at screening dilution. Contains preservatives. Stable at 2 to 8°C until the expiration date stated on the label. Do not use if cloudiness, discoloration or other indications of bacterial contamination are present. Allow to warm to room temperature before use. **Do not dilute.**

Non-Detectable Control, 0.25mL | REF | IF1213 | CONTROL | <

One vial of human serum bottled at screening dilution. Contains preservatives. Stable at 2 to 8°C until the expiration date stated on the label. Do not use if cloudiness, discoloration or other indications of bacterial contamination are present. Allow to warm to room temperature before use. Repeated freezing and thawing is deleterious and should be avoided. **Do not dilute.**

Mounting Medium, 2.5 mL | REF | IF0007 | REAG | MONT

One dropper bottle containing PBS-buffered glycerol at a pH of 7.2 ± 0.1. Contains preservatives. Stable at 2 to 8°C until the expiration date stated on the bottle label. Allow to warm to room temperature before use.

PBS | REF | IF0005 | BUF

One vial of phosphate buffered saline (PBS) powder. Reconstitute with 1 liter distilled (or purified) water. The reconstituted solution is a 0.01 M buffer at pH 7.2 ± 0.1. Before and after reconstitution, store PBS at 2 to 8°C. Allow to warm to room temperature before use. Do not use if cloudiness, discoloration, or other indications of bacterial contamination are present.

MATERIALS REQUIRED, BUT NOT SUPPLIED

1. 24 x 50 mm coverslips
2. Test tubes and rack, microcentrifuge tubes or microtiter plate for serum dilutions
3. Clinical centrifuge
4. 35 to 37°C incubator or water bath for slide incubation
5. 2 to 8°C refrigerator
6. Plastic wash bottle
7. Calibrated pipets or piston-type pipettors with disposable tips
8. Coplin jars or slide staining dish with slide holder
9. Clean volumetric flask or graduated cylinder, 1 liter
10. Humid chamber for incubation of slides
11. Distilled or purified water
12. Timer
13. Absorbent paper for blotting slides
14. Fluorescence microscope, recommended parameters

Excitation Filter	470-490 nm
Barrier Filter	520-560 nm
Light Source	HBO 100W, mercury
Objective	20-40X, fluorescence quality, high dry

WARNINGS AND PRECAUTIONS

1. This package insert is for export only and not for distribution in the United States. Outside of the United States, product regulatory status is for *in vitro* diagnostic use.
2. All blood products should be treated as potentially infectious. Source materials from which this product (including controls) was derived have been screened for Hepatitis B surface antigen, Hepatitis C antibody and HIV-1/2 (AIDS) antibody by FDA-approved methods and found to be negative. However, as no known test methods can offer 100% assurance that products derived from human blood will not transmit these or other infectious agents, all controls, serum specimens and equipment coming into contact with these specimens should be considered potentially infectious and decontaminated or disposed of using proper biohazard precautions. CDC and the National Institutes of Health recommend that potentially infectious agents be handled at the Biosafety Level 2.[2,14,23]
3. Evan's Blue is a carcinogen; however this product is below the reportable threshold (less than 0.1%).
4. Do not substitute or mix reagents from different kit lots or from other manufacturers.
5. Use only protocols described in this insert. Incubation times or temperatures other than those specified may give erroneous results.
6. Cross-contamination of patient specimens on a slide can cause erroneous results. Add patient specimens and handle slide carefully to avoid mixing of sera from adjoining wells.
7. Bacterial contamination of serum specimens or reagents can produce erroneous results. Use aseptic techniques to avoid microbial contamination.
8. Perform the assay at room temperature (approximate range 20 to 25°C).
9. Use proper pipetting techniques, maintaining the pipetting pattern throughout the procedure to ensure optimal and reproducible values.
10. Mounting Medium contains 30 to 60 % glycerol which may cause irritation upon inhalation or skin contact. Upon inhalation or contact, first aid measures should be taken.

SHELF LIFE AND HANDLING

1. Kits are stable through the end of the month indicated in the expiration date when stored at 2 to 8°C.
2. Do not use test kit or reagents beyond their expiration dates.
3. Do not expose reagents to strong light during storage or incubation.
4. Allow reagents to warm to room temperature before use.

SPECIMEN COLLECTION AND PREPARATION

Serum is the preferred specimen source. No attempt has been made to assess the assay's compatibility with other specimens. Hyperlipemic, hemolyzed, heat inactivated, or contaminated sera may cause erroneous results; therefore, their use should be avoided.

Specimen Collection and Handling

Collect blood samples aseptically using approved venipuncture techniques by qualified personnel.[14] Allow blood samples to clot at room temperature prior to centrifugation. Aseptically transfer serum to a tightly closing sterile container for storage. Separated serum/plasma should remain at 22°C for no longer than 8 hours. If the assay will not be completed within 8 hours, refrigerate the sample at 2 to 8°C. If the assay will not be completed within 48 hours, or for shipment of samples, freeze at –20°C or colder. Thaw and mix samples well prior to use.

Specimen Preparation

The serum screening dilution is 1:16 in **PBS**. To determine endpoint titers, use PBS to serially dilute beyond the screening dilution.

TEST PROCEDURE (Incubation at 37°C)

1. Remove **Slides** from cold storage. To avoid condensation, allow slides to reach room temperature before opening slide packets.
2. Apply 25 µL of **Detectable Control**, as bottled, to the appropriate slide well.
3. Apply 25 µL of **Non-Detectable Control**, as bottled, to the appropriate slide well.
4. For each patient sample to be tested, add approximately 25 µL of the **diluted sample** (see Specimen Preparation, above) to an appropriate slide well. Make notations to later identify each well when reading the results.
5. Incubate slide(s) in a humid chamber for 30 ± 2 minutes at 35 to 37°C.
6. Remove slides from the humid chamber and gently rinse each slide with a stream of **PBS**. Do not aim stream of PBS directly at the slide wells. Rinse one row at a time to avoid mixing of specimens. Wash slides by submersing the rinsed slides into Coplin or slide staining jars containing PBS for 10 minutes.
7. Dip the washed slides briefly in distilled or purified water, and allow the slides to air dry.
8. Add approximately 25 µL **IgG Conjugate** to each slide well.
9. Incubate slides in a humid chamber for 30 ± 2 minutes at 35 to 37°C.
10. Repeat wash steps 6 and 7.
11. Place a few drops of **Mounting Medium** on the slide and cover with a 24 x 50 mm coverslip. Remove any air bubbles and excess Mounting Medium with absorbent paper.
12. View wells at a final magnification of 400X on a properly equipped fluorescence microscope. For optimum fluorescence, read slides the same day the assay is performed. If this is not possible, store in the dark at 2 to 8ºC for up to 24 hours.

TEST PROCEDURE (Incubation at Room Temperature)

1. Remove **slides** from cold storage. To avoid condensation, allow slides to reach room temperature before opening slide packets.
2. Apply 25 µL of **Detectable Control**, as bottled, to the appropriate slide well.
3. Apply 25 µL of **Non-Detectable Control**, as bottled, to the appropriate slide well.
4. For each patient sample to be tested, add approximately 25µL of the **diluted sample** (see Specimen Preparation, above) to an appropriate slide well. Make notations to later identify each well when reading the results.
5. Incubate slide(s) for 60 ± 2 minutes at Room Temperature, covered.
6. Gently rinse each slide with a stream of **PBS**. Do not aim stream of PBS directly at the slide wells. Rinse 1 row at a time to avoid mixing of specimens. Wash slides by submersing the rinsed slides into Coplin or slide staining jars containing PBS for 10 minutes.
7. Dip the washed slides briefly in distilled or purified water, and allow the slides to air dry. **Note: If using an instrument to automate the wash process, it may not be possible to allow the slides to air dry prior to addition of conjugate.**
8. Add approximately 25 µL **IgG Conjugate** to each slide well.
9. Incubate slide(s) for 30 ± 2 minutes at Room Temperature, covered.
10. Repeat wash steps 6 and 7.
11. Place a few drops of **Mounting Medium** on the slide and cover with a 24 x 50 mm coverslip. Remove any air bubbles and excess Mounting Medium with absorbent paper.
12. View wells at a final magnification of 400X on a properly equipped fluorescence microscope. For optimum fluorescence, read slides the same day the assay is performed. If this is not possible, store in the dark at 2 to 8°C up to 24 hours.

QUALITY CONTROL

Each run (each time a slide, or group of slides, is processed) should include both Detectable and Non-Detectable Controls.

Assay Validity

1. The Detectable Control should exhibit 2+ to 4+ fluorescence with all antigen spots and negligible reactivity with yolk sac control.
2. The Non-Detectable Control should be non-reactive with all spots.

If the controls are not within these parameters, patient test results should be considered invalid and the assay repeated.

The Detectable and Non-Detectable Controls are intended to monitor for substantial reagent failure. The Detectable Control is made using mouse antibodies and not human antibodies. The Detectable Control only ensures reagent functionality. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

Sample Validity

Background Fluorescence. Occasionally a specimen may react with the yolk sac diluent. When this occurs the assay will not be interpretable if the anti-yolk sac titer is equal to or greater than the anti-Chlamydia titer. This anti-yolk sac reaction can be confirmed by examining the yolk sac control spot. Fluorescence in this spot indicates non-specific (non-Chlamydia) reactivity. Continue to examine all dilutions of patient sera. If the anti-yolk sac titer is greater than or equal to any anti-Chlamydia titer the result is not interpretable and should not be reported.

INTERPRETATION OF TEST RESULTS

Microscope optics, light source condition and type will determine overall fluorescent intensity and endpoint titers. Read control wells first during every run to ensure correct interpretation.

Reading the Slides

Read fluorescent intensity of the elementary bodies (see **Elementary Bodies vs. Green Fluorescent Particles** note below), and grade the fluorescence as follows:

2 to 4+	Moderate to intense apple-green fluorescence.
1+	Definite, but dim fluorescence.
Negative	No fluorescence or fluorescence equal to that observed in the corresponding yolk sac control spot or in the Non-Detectable Control well.

Elementary Bodies vs. Green Fluorescent Particles. Read only the fluorescence of the elementary bodies. Elementary bodies have consistent size, with an even distribution density throughout the antigen spot. Unevenly distributed and irregular green fluorescent particles (when present) should not be interpreted as positive reactivity.

Interpreting the Patient Specimens Results

The reciprocal of the highest serum dilution that gives definite (1+) apple-green fluorescence is termed the serum endpoint titer.

At lower dilutions the specimens may cross-react to all three Chlamydia species. To determine the chlamydial species which elicits the predominant immune response, serially dilute specimens to endpoint titer. Combine serological results with clinical evidence to aid in diagnosis.

≥ 1:16	A single specimen endpoint titer ≥ 1:16 indicates a presumed past infection at an undetermined time. A second specimen drawn 3 to 8 weeks after the original draw should be tested in parallel with the first. If the second specimen exhibits a 4-fold increase over that of the initial specimen, then current (acute) infection is indicated. Unchanging titers suggest past infection.
≥ 1:512	A single specimen endpoint titer ≥ 1:512 indicates a possible acute infection.
< 1:16	IgG endpoint titers less than 1:16 suggest that the patient does not have a current infection. This may be found in patients with either no history of Chlamydial infection or those with past infection whose antibody levels have dropped below detectable levels.

Diagnosis of acute infection based on a single IgG result is not recommended. If single IgG results only, then report with caution. There is no test for validating chronic infection.

Intra-genus Reactivity

The chlamydia MOMP contains both species- and genus-specific antigens, and serological cross reactions may be seen in both acute and convalescent samples.

The *C. psittaci* and the *C. trachomatis* antigen spots, provided as chlamydia speciation controls, are intended as an aid in interpreting the specificity of the *C. pneumoniae* serological reaction. When cross reactions are observed, interpret the specificity of the immune response with caution. In most cases, a *C. pneumoniae*-specific reaction will endpoint titers two fold or greater than the titers observed with the other two chlamydia antigen spots. To determine whether *C. pneumoniae* elicits the predominant immune response, serially dilute specimens to endpoint titer. Combine serological results with clinical evidence to aid in diagnosis.

The *C. psittaci* and the *C. pneumoniae* antigen spots, provided as chlamydia speciation controls, are intended as an aid in interpreting the specificity of the *C. trachomatis* serological reaction. When cross reactions are observed, interpret the specificity of the immune response with caution. In most cases, a *C. trachomatis*-specific reaction will endpoint titers two fold or greater than the titers observed with the other two chlamydia antigen spots. To determine whether *C. trachomatis* elicits the predominant immune response, serially dilute specimens to endpoint titer. Combine serological results with clinical evidence to aid in diagnosis.

LIMITATIONS

1. All results from this and other serologies must be correlated with clinical history, epidemiological data, and other data available to the attending physician in evaluating the patient.
2. The performance of this assay has not been established for the general population, pediatrics or geriatrics.
3. The performance of this assay has not been established for diagnosing *Chlamydia psittaci* infections.
4. The performance of this assay has not been established for diagnosing non-pneumonia diseases caused by *Chlamydia pneumonia*, for example, bronchitis, sinusitis, and otitis.
5. The performance of this assay has not been established for diagnosing non-pneumonia diseases associated with *Chlamydia pneumonia*, for example, coronary heart disease, asthma, and multiple sclerosis.
6. The performance of this assay has not been established for diagnosing chronic infections.
7. The performance of this assay has not been established for matrices other than serum. Test only indicated specimen types.
8. The performance of this assay has not been established for monitoring therapy.
9. A negative result does not rule out a present acute infection. Absence of MIF antibodies in culture positive persons has been reported. This is rare in adults, but may be more common in young children. Also, samples obtained too early during primary infection may not contain detectable antibodies. If chlamydial infection is suspected, a second sample should be obtained 10 to 21 days later and tested in parallel with the original sample.
10. A positive result does not always indicate a present acute infection. Anti-chlamydia antibodies can persist in some patients for several months or more. Also, during a primary Chlamydia infection the early antibody response may cross-react with multiple Chlamydia species. Cross-reactivity may also occur due to exposure to more than one Chlamydia species.
11. The *psittaci* spot includes 2 *C. psittaci* strains (parrot and parakeet), but does not include all *C. psittaci* serovars. So some *C. psittaci* infections may not be detected. However, very few psittacosis cases are reported (usually < 50 per year in the U.S.), and most cases are caused by parrot/parakeet strains. Sera from suspected cases of psittacosis should also be screened by CF for detection of Chlamydial group antigens.
12. The predictive value of a positive or negative result depends on the population's prevalence and the pretest likelihood of infection.
13. Serological results cannot be used to determine the site of infection.
14. Serological testing may be negative in a chlamydia infected individual, due to different immunogenicities of strains and the site of infections.

EXPECTED VALUES

Approximately 40% to 60% of adult populations around the world have antibodies to *C. pneumoniae*, which suggests that the infection is extraordinarily prevalent, and re-infection is common.[15] Reported cases of *C. psittaci* infection in the U.S. runs typically less than 50 per year, with 16 cases reported in 1999.[16] Sources of human *C. psittaci* infection other than infected birds have been identified and may be more common than currently recognized.[15] The prevalence of *C. trachomatis* infections in adolescent women usually exceeds 10%, and in some populations can reach 40%.

PERFORMANCE CHARACTERISTICS

For customers outside of the United States, the product performance characteristics in English are supplied as a separate sheet.

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