

# Chlamydia MIF IgA (OUS)

**REF** IF1250A

Rev. S

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

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**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) IgA is intended for the qualitative detection and semi-quantitation of human serum IgA antibodies to *Chlamydia pneumoniae* and *Chlamydia trachomatis*. The assay is not intended for self-testing.

## SUMMARY AND EXPLANATION OF TEST

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 uninfected 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 (RB) (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.<sup>1</sup>

The EB 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. This protein 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.<sup>9</sup>

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.<sup>2</sup> Most *C. trachomatis* infections are asymptomatic.<sup>3</sup> 65% of babies born vaginally to infected mothers become infected with *C. trachomatis*.<sup>3</sup> *Chlamydia psittaci* is represented by many serotypes which are responsible for human psittacosis, an acute zoonotic disease associated with infected birds<sup>4</sup>. The newly recognized species, *Chlamydia pneumoniae*, is associated with acute respiratory disease and pneumonia. *C. pneumoniae* antibody is detectable in 25% to 45% of adults tested and is responsible for approximately 10% of atypical pneumonia cases.<sup>7,8</sup> *C. pneumoniae* infections may be subclinical.

Culture, direct fluorescent antibody (DFA) testing, and serology can be used to diagnose chlamydia infections. Positive culture and DFA are the most definitive. However, specimen collection and transport difficulties and procedure complexity are associated with DFA and culture.<sup>4,10</sup> As a result, serologies including complement fixation (CF), indirect immunofluorescent assays (IFA) and enzyme immunoassays (EIA) are used for routine diagnosis.<sup>3</sup> Serologies may be used for presumptive diagnosis of the some chlamydia infections, including: LGV, infant pneumonia due to *C. trachomatis*, psittacosis and pneumonia due to *C. pneumoniae*. Serology is not useful for routine genital infections caused by *C. trachomatis*. 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.<sup>4</sup> Commercially available EIAs employ a broadly cross-reactive antigen usually derived from an LGV serotype, however only group antibody responses are detected.<sup>9</sup>

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.<sup>11</sup> The other available IFA, a micro-immunofluorescent assay (MIF) introduced in the 1970s,<sup>9</sup> 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.<sup>9</sup>

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 auscultatory 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*.

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 four fold rise in IgG is diagnostic. When *C. pneumoniae* or *C. psittaci* is suspected, detectable IgM is highly diagnostic.<sup>12</sup> 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.<sup>12</sup>

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<sup>13</sup> and a weak or absent IgA response.<sup>18</sup> After acute *C. pneumoniae* infection, IgM antibodies are usually lost within 2 to 6 months,<sup>13</sup> IgG antibody titers rise and usually decrease slowly; whereas IgA antibodies tend to disappear rapidly.<sup>19</sup>

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 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<sup>14</sup>.

*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 chlamydial 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<sup>15</sup>.

Micro-immunofluorescent assays (MIF) is considered the serological “gold standard”<sup>20</sup>. 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<sup>17</sup>. 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<sup>16</sup>.

In a study<sup>15</sup>, 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 one strain of *C. pneumoniae*, two strains of *C. psittaci* and eight 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 twelve wells; on each well are four 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 is diluted in PBS, added to appropriate slide wells in contact with the substrate, and incubated. Following incubation, the slide is washed in buffered saline which removes unbound serum antibodies. In the second stage, each antigen well is overlaid with fluorescein-labeled antibody to IgA. The slide is incubated allowing antigen-antibody complexes to react with the fluorescein-labeled anti-IgA. 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**

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.

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

**IgA Conjugate-Dual Species, 3.5mL**

One vial of fluorescein-labeled goat anti-human IgA, alpha-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 with 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.

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**Polyvalent Detectable Control, 0.3mL**

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.**

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**Non-Detectable Control, 0.25mL**

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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. **Do not dilute.**

**Mounting Medium, 2.5 mL**

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One dropper bottle containing PBS-buffered glycerol at a pH 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**

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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**

- 24 x 50 mm coverslips
- Test tubes and rack, microcentrifuge tubes or microtiter plate for serum dilutions
- Clinical centrifuge
- 35 to 37°C incubator or water bath for slide incubation
- 2 to 8°C refrigerator
- Plastic wash bottle
- Calibrated pipettes or piston-type pipettors with disposable tips
- Coplin jars or slide staining dish with slide holder
- Clean volumetric flask or graduated cylinder, 1 liter
- Humid chamber for incubation of slides
- Distilled or purified water
- Timer
- Absorbent paper for blotting slides
- 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**

- 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.
- 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, 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 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.<sup>2,21,24</sup>
- Evan's Blue is a carcinogen; however this product is below the reportable threshold (less than 0.1%).
- Do not substitute or mix reagents from different kit lots or from other manufacturers.
- Use only protocols described in this insert.
- 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.
- Bacterial contamination of serum specimens or reagents can produce erroneous results. Use aseptic techniques to avoid microbial contamination.
- Perform the assay at room temperature (approximate range 20 to 25°C).
- Use proper pipetting techniques, maintaining the pipetting pattern throughout the procedure to ensure optimal and reproducible values.
- 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**

- Kits are stable through the end of the month indicated in the expiration date when stored at 2 to 8°C.
- Do not use test kit or reagents beyond their expiration dates.
- Do not expose reagents to strong light during storage or incubation.
- 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, 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.<sup>21</sup> Allow blood samples to clot at room temperature prior to centrifugation. Aseptically transfer serum to a tightly closing sterile container for storage at 2 to 8°C. If testing is to be delayed longer than 5 days, the sample should be frozen 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 the screening dilution.

**TEST PROCEDURE (Incubation at 37°C)**

- Remove slides from cold storage. To avoid condensation, allow slides to reach room temperature before opening slide packets.
- Apply 25 µL of **Detectable Control**, as bottled, to the appropriate slide well.
- 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  $\mu$ L 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  $30 \pm 2$  minutes at  $35$  to  $37^{\circ}\text{C}$ .
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 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.
8. Add approximately 25  $\mu$ L **IgA Conjugate-Dual Species** to each slide well.
9. Incubate slides in a humid chamber for  $30 \pm 2$  minutes at  $35$  to  $37^{\circ}\text{C}$ .
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  $8^{\circ}\text{C}$  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  $\mu$ L of **Detectable Control**, as bottled, to the appropriate slide well.
3. Apply 25  $\mu$ L of **Non-Detectable Control**, as bottled, to the appropriate slide well.
4. For each patient sample to be tested, add approximately 25 $\mu$ L 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  $60 \pm 2$  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  $\mu$ L **IgA Conjugate-Dual Species** to each slide well.
9. Incubate slide(s) for  $30 \pm 2$  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  $8^{\circ}\text{C}$  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 elementary bodies and negligible reactivity with yolk sac control.
2. The Non-Detectable Control should show no reactivity with elementary bodies.

**If controls do not exhibit these results, 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 endpoint titer.

At lower dilutions the specimens may exhibit cross reaction to all 3 chlamydial 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.

$\geq 1:16$	A single specimen <i>C. pneumoniae</i> IgA serum endpoint titer $\geq 1:16$ should be considered evidence of infection at an undetermined time. Further interpretation will depend upon the clinical presentation of the patient and IgG and IgM results. Elevated IgA titers in the absence of IgM titers may indicate reinfection or chronic infection; whereas positive IgA titers in the presence of positive IgM titers may indicate primary infection.
$< 1:16$	No IgA antibody detected.

### 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 have 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. pneumoniae*-specific reaction will have 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. Interpretation of IgA results are dependent upon patient presentation: it is essential that all results from chlamydial serologies correlate with clinical history and other data available to the physician.
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 matrices other than serum. Test only indicated specimen types.
4. The performance of this assay has not been established for monitoring therapy.
5. A negative result does not rule out a present active 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.
6. The *psittaci* spot includes two *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.
7. The predictive value of a positive or negative result depends on the population's prevalence and the pretest likelihood of infection.
8. During a Chlamydia infection the antibody response may crossreact with multiple Chlamydia species. Crossreactivity may also occur due to exposure to more than one Chlamydia species.
9. Species specific antibodies may be detected due to past exposure or cross reactivity from other chlamydial-species infections.
10. Serological results cannot be used to determine the site of infection.
11. Serological testing may be negative in a chlamydia infected individual, due to different immunogenetics 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.<sup>22</sup> Reported cases of *C. psittaci* infection in the U.S. runs typically less than 50 per year, with 16 cases reported in 1999.<sup>23</sup> Sources of human *C. psittaci* infection other than infected birds have been identified and may be more common than currently recognized.<sup>22</sup> The prevalence of *C. trachomatis* infections in adolescent women usually exceeds 10%, and in some populations can reach 40%.

### PERFORMANCE CHARACTERISTICS

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

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