



## SeroFIA™ *C. psittaci*

An immunofluorescence assay for the detection of specific IgG, IgA and IgM antibodies to *C. psittaci* in human serum

### Instruction Manual

Test kit for 105 determinations  
Catalog No. 570-01

For In Vitro Diagnostic Use.  
For professional use only.  
Store at 2-8°C. **Do not freeze.**



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### Intended Use

The SeroFIA™ *C.psittaci* is a semi-quantitative immunofluorescence assay for the differential determination of *C.psittaci* specific IgG, IgA and IgM antibodies in a single human serum sample.

### For In Vitro Diagnostic Use.

### Introduction

Chlamydia, a highly specialized Gram-negative bacteria, is composed of four species: *C. trachomatis*, *C. pneumoniae* (TWAR), *C. psittaci* and *C. pecorum*.

*C. trachomatis* includes 15 serotypes sharing immunogenic epitopes at various degrees. *C.trachomatis* is a major sexually transmitted disease and is associated with nongonococcal urethritis (NGU) and epididymitis in men, cervicitis, urethritis and pelvic inflammatory disease in women, Reiter's syndrome in HLA-B27 haplotype individuals and neonatal conjunctivitis and pneumonia in the newborn (2-6).

*C. pneumoniae* is an important respiratory pathogen in humans and causes up to 10% of community-acquired pneumonia cases. It has been associated with acute respiratory diseases, pneumonia, asthma, bronchitis, pharyngitis, acute chest syndrome of sickle cell disease, coronary heart disease, and Guillain-Barre syndrome (7-9).

*C.psittaci* infects a diverse range of host species from molluscs to birds to mammals and also causes severe pneumonia.

Serological testing is routinely used for diagnosing chlamydial infections. They serve as a non-invasive tool in identification of both distal and chronic chlamydial infections (10, 11), where

direct detection methods are rarely efficient. Furthermore, the presence of certain antibody types may also indicate the state of the disease.

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

Chlamydial re-infections are characterized by absence of IgM response and prompt IgG and IgA responses (9). IgA antibodies have been shown to be a reliable immunological marker of primary, chronic and recurrent infections. These antibodies usually decline rapidly to baseline levels following treatment and eradication of the chlamydial infection (1-6, 10, 11).

The persistence of elevated IgA antibody titers is generally considered as a sign of chronic infection (13). In a study conducted on elderly patients with respiratory infections it was estimated that one fifth of *C. pneumoniae* cases would have been missed without an IgA determination (14). IgG antibodies persist for long periods and decline very slowly. Therefore, the presence of IgG antibodies is mainly indicative of a chlamydial infection at an undetermined time. However, a four-fold rise in IgG or high levels of IgG antibodies may indicate an on-going chronic or systemic infection.

The SeroFIA™ *C.psittaci* test is a micro-IF assay based on the principles of MIF. SeroFIA™ *C.psittaci* uses as antigen purified elementary bodies of *C.psittaci* (SZ-1).

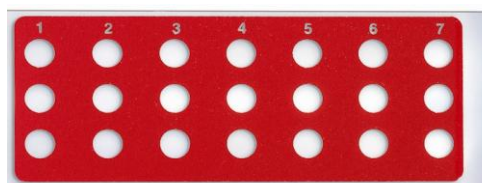
### Principle of the Test

- Purified elementary bodies (Ebs) of *C.psittaci*, are fixed onto the SeroFIA™ slide wells.
- Diluted patients sera are incubated for 30 minutes at 37°C with the antigen.
- Unbound serum components are removed by washing.
- Fluorescein-conjugated anti-human IgG, IgA or IgM is added and incubated for 30 minutes at 37°C.
- Unbound conjugate is removed by washing.
- Slides are dried and mounted by adding 3 drops of Mounting Fluid.
- Slides are examined using fluorescence microscopy. Positive reactions appear as bright apple-green fluorescent Ebs against a dark background.
- Qualitative determination is achieved by a single dilution of sera. Semi-quantitative results are achieved by endpoint titrations.

### Kit Contents

1. **Reaction Slides (3x7 wells/unit):** Slides coated with *C.psittaci* antigen. Each slide is packed in an aluminum pouch containing silica gel packet.

**5 units**



2. **Concentrated Wash Buffer (x20):** A PBS-Tween buffer, (pH 7.4-7.6) which contains NaCl, Na<sub>2</sub>HPO<sub>4</sub>, KH<sub>2</sub>PO<sub>4</sub> and Tween 20.

**1 bottle, 100ml**

3. **Serum Diluent:** A PBS buffer. Contains gelatin, bovine serum albumin, MgCl<sub>2</sub> and <0.1% sodium azide  
**1 bottle, 80ml**
4. **Negative Control:** Human serum negative for IgG, IgA, and IgM antibodies to *C. pneumoniae*, *C. trachomatis*, *C. psittaci*. Contains <0.1% sodium azide. Ready to use.  
**1 vial, 0.5 ml**
5. **IgG Positive Control:** Human serum positive for IgG antibodies to *C.psittaci*. Contains <0.1% sodium azide. Ready to use.  
**1 vial, 0.2ml**
6. **IgA Positive Control:** Human serum positive for IgA antibodies to *C.psittaci*. Contains <0.1% sodium azide. Ready to use.  
**1 vial, 0.2ml**
7. **IgM Positive Control:** Human serum positive for IgM antibodies to *C.psittaci*. Contains <0.1% sodium azide. Ready to use.  
**1 vial, 0.2ml**
8. **IgG FITC-Conjugate:** Fluorescein-labelled rabbit anti-human IgG ( $\gamma$  - chain specific). Ready to use.  
**1 vial, 3.3ml**
9. **IgA FITC-Conjugate:** Fluorescein-labelled rabbit anti-human IgA ( $\alpha$  - chain specific). Ready to use.  
**1 vial, 3.3ml**
10. **IgM FITC-Conjugate:** Fluorescein-labelled rabbit anti-human IgM ( $\mu$  - chain specific). Ready to use.  
**1 vial, 3.3ml**
11. **IgG Inactivation Reagent:** anti-human IgG containing <0.1% sodium azide. **(For IgM Only)**  
**1 bottle, 4ml**
12. **Mounting Fluid:** Contains <0.1% sodium azide.  
**1 dropper bottle, 1.5ml**
13. **Cover slips:** **1 unit**
14. **Instruction manual:** **1**

#### Materials Required But Not Supplied

1. Clean microtiter plates or tubes for patients' sera dilutions.
2. Clinical centrifuge.
3. Adjustable micropipettes (5-50, 50-200, 200-1000 microliters ranges) and disposable tips.
4. Volumetric cylinder (1 liter).
5. Vortex mixer.
6. A 37°C water bath with a lid, or a moisture chamber placed in a 37°C incubator.
7. Plastic tray for slide incubation.
8. Distilled or double deionized water for the dilution of Concentrated Wash Buffer.
9. Squeeze type plastic wash bottle.
10. Slide carrier and staining jar.
11. Timer.
12. Fluorescence microscope with filters appropriate for reading FITC fluorescence and with magnifications of 400x and 1000x.

#### Warnings and Precautions

##### For In Vitro Diagnostic Use.

1. This kit contains human sera that have been tested by FDA - approved techniques, and found to be negative for HBsAg, and for antibodies to HCV and HIV 1&2. However, since no known method can offer complete assurance that products derived from human blood do not transmit infection, all human blood components supplied in this kit must be

- handled as potentially infectious serum or blood, in a manner identical with or similar to the recommendations published in the CDC/NIH manual "Biosafety in Microbiological and Biomedical Laboratories", 1988.
2. The chlamydial antigenic material coating the slides has been inactivated and contains no detectable live organisms. However, since no known method can offer complete assurance that products derived from pathological organisms do not transmit infection, the slides should be handled and disposed of as would any potentially bio hazardous material, in a manner identical with or similar to the recommendations published in the CDC/NIH manual, "Biosafety in Micro - biological and Biomedical Laboratories" 1988.
3. Sodium azide has been reported to form explosive lead or copper azides in laboratory plumbing. To prevent the accumulation of these compounds, flush sink and plumbing with large quantities of water after disposing of azide-containing solutions.
4. Do not pipette by mouth.
5. Avoid skin contact with any of the kit reagents.
6. Wear disposable gloves while handling sera and performing the test. Wash hands thoroughly after removing gloves.
7. Any equipment, liquids and other substances coming into direct contact with human sera should be considered potentially contaminated. They should be sterilized or inactivated after use and before disposal or cleaning. Inactivation can be accomplished by autoclaving at 121°C for at least one hour, or by treating with a solution of sodium hypochlorite of 5% final concentration (household bleach) for at least 30 minutes.
8. The FITC-Conjugate contains Evans Blue that is a carcinogen. Avoid contact with skin and eyes.
9. Mounting Medium contains corrosive ingredients. Avoid contact with skin and do not inhale. In case of contact with skin and eyes rinse immediately with plenty of water.

#### Storage and Shelf Life of Reagents

All the materials supplied should be stored at 2-8°C. If kept at 2-8°C the test reagents are stable until the expiry date indicated on the kit pack. Do not use the kit components beyond their expiration dates. Exposure of kit components to ambient temperature for a few hours will not cause damage to the reagents.  
**Do not expose reagents to strong light. Do not freeze the reagents.**

#### Collection and Preparation of Samples

##### Serum Collection

Prepare sera from aseptically collected samples using standard techniques. Heat inactivated sera should not be used. The use of lipemic, turbid or contaminated sera is not recommended. Particulate material and precipitates in sera may cause erroneous results. Such specimens should be clarified by centrifugation or filtration prior to the test.

##### Storage

Specimens should be stored at 2-8°C and tested within 7 days (adding of 0.1% Sodium Azide is highly recommended). If a longer storage period is anticipated, aliquot and store the specimens below -20°C. Avoid repeated thawing and freezing.

### Validity of the Test

For the test to be valid the following criteria must be met. If these criteria are not met the test should be considered invalid and should be repeated.

1. Positive Control exhibits moderate to intense apple green fluorescence staining of Chlamydia EB particles of the *C.psittaci*.
2. Negative Control shows negligible reactivity (staining) with *C.psittaci*.

### Interpretation and Significance of Results

It is recommended to read control wells first to ensure correct interpretation of test results.  
Read fluorescence of clinical samples and grade as follows:

+	Moderate to intense, sharp or diffused, apple green fluorescence of elementary bodies.
±	Definite but dim fluorescence of elementary bodies. Should be considered as the endpoint titer of the serum. The end point titer of a given serum is defined as the last dilution that still gives a noticeable staining. The next dilution will display a picture identical to a negative serum.
-	No fluorescence or faint background fluorescence with no clear chlamydia morphology

### Assay Procedure for IgG

#### Sample Preparation

For initial screening dilute sera 1:64 in Serum Diluent, by adding 10µl of serum to 630µl of Serum Diluent. To determine endpoint titers serially dilute sera in Serum Diluent starting from 1:64.

#### Note:

For each test run, testing of one well of the Negative Control and one well of the IgG Positive Control is recommended.

**Positive Control can be used as an end-point titer control if diluted at 1:64.**

1. Bring all components and clinical samples to be tested to room temperature.
2. Dilute Concentrated Wash Buffer 1:20 by adding 50ml of Concentrated Wash Buffer to 950ml of distilled or double deionized water. Diluted buffer can be stored at 2-8°C for up to 2 weeks.
3. Pipette 10µl of controls and diluted sera into the appropriate wells.
4. Incubate slides in a moist chamber at 37°C for 30 minutes.
5. Remove slides from the moist chamber and gently rinse each slide with a stream of diluted Wash Buffer using a squeeze type wash bottle. Wash slides by submersing the slides into a staining jar containing diluted Wash Buffer. Leave immersed for 10 minutes. Dip washed slides in double distilled water. Remove and air dry.
6. Pipette 10µl of FITC-Conjugate into each well.
7. Incubate at 37°C for 30 minutes.
8. Repeat rinsing and washing of slides as in step 5.

9. Place 3 drops of Mounting Fluid along the center of each slide. Cover with provided cover slip. Remove air bubbles by pressing gently on the cover slip.
10. Read results on a fluorescence microscope at a magnification of 400x or 1000x. For best results, read the slides on the same day the assay is performed. If this is not possible, mounted slides can be stored in the dark at 2-8°C for up to 3 days.

Fluorescence appearance at 1:64 titer	Interpretation
+	<b>Presence of specific IgG antibodies to <i>C.psittaci</i>.</b> End point titration is required for determination of current, recent or past infection <sup>1</sup> .
±	<b>Indication of possible exposure to <i>C.psittaci</i>.</b> Second sample testing required after 2-4 weeks <sup>2</sup>
-	<b>Negative.</b> No detectable IgG antibodies to <i>C.psittaci</i> .

1. Titer of  $\geq 1:64$  shows evidence of current or recent infection of *C.psittaci*.
2. When testing a second sample, both the first and the second sample should be tested simultaneously. If borderline result is repeated, specimen must be considered negative.

**Note:** In rare cases, a clear and dense staining of very small particles (smaller than the EB's) might be observed on the antigen. This may represent reactivity due to LPS. Testing IgA and IgM antibodies or testing a second serum sample, taken after 2-3 weeks, should be performed. If IgM and IgA are negative and IgG results are repeated, sample should be considered negative.

### Assay Procedure for IgA

#### Sample Preparation

For initial screening dilute sera 1:32 in Serum Diluent, by adding 10µl of serum to 310µl of Serum Diluent. To determine endpoint titers serially dilute sera in Serum Diluent starting from 1:32.

#### Note:

For each test run, testing of one well of the Negative Control and one well of the IgA Positive Control is recommended.

**Positive Control can be used as an end-point titer control if diluted at 1:128.**

1. Bring all components and clinical samples to be tested to room temperature.

- Dilute Concentrated Wash Buffer 1:20 by adding 50ml of Concentrated Wash Buffer to 950ml of distilled or double deionized water. Diluted buffer can be stored at 2-8°C for up to 2 weeks.
- Pipette 10µl of controls and diluted sera into the appropriate wells.
- Incubate slides in a moist chamber at 37°C for 30 minutes.
- Remove slides from the moist chamber and gently rinse each slide with a stream of diluted Wash Buffer using a squeeze type wash bottle. Wash slides by submersing the slides into a staining jar containing diluted Wash Buffer. Leave immersed for 10 minutes. Dip washed slides in double distilled water. Remove and air dry.
- Pipette 10µl of FITC-Conjugate into each well.
- Incubate at 37°C for 30 minutes.
- Repeat rinsing and washing of slides as in step 5.
- Place 3 drops of Mounting Fluid along the center of each slide. Cover with provided cover slip. Remove air bubbles by pressing gently on the cover slip.
- Read results on a fluorescence microscope at a magnification of 400x or 1000x. For best results, read the slides on the same day the assay is performed. If this is not possible, mounted slides can be stored in the dark at 2-8°C for up to 3 days.

Fluorescence appearance at 1:32 titer	Interpretation
+	<b>Presence of specific IgA antibodies to <i>C.psittaci</i>.</b> A titer of $\geq 1:32$ is considered presumptive evidence of infection.
±	<b>Indication of possible exposure to <i>C.psittaci</i>.</b> Second sample testing required after 2-4 weeks <sup>1</sup> .
-	<b>Negative.</b> No detectable IgA antibodies to <i>C.psittaci</i> .

- When testing a second sample, both the first and the second sample should be tested simultaneously. Those patients with current *C.psittaci* infection will exhibit a rise in the antibody titer. If borderline result is repeated, specimen must be considered negative.

**Note:** In rare cases, a clear and dense staining of very small particles (smaller than the EB's) might be observed on the antigen. This may represent reactivities due to LPS. Testing IgG and IgM antibodies or testing a second serum sample, taken after 2-3 weeks, should be performed. If IgM and IgG are negative and IgA results are repeated, sample should be considered negative.

### Assay Procedure for IgM

#### Sample Preparation

- Add 5µl of each patient serum to 45µl of IgG Inactivation Reagent. Vortex gently.

- To each of the above 50µl treated sera add 50µl of Serum Diluent. Final sera dilution obtained at this step is 1:20.

To determine endpoint titers serially dilute sera in Serum Diluent starting from 1:20.

**Note:**

For each test run, testing of one well of the Negative Control and one well of the IgM Positive Control is recommended.

**Positive Control can be used as an end-point titer control if diluted at 1:64.**

- Bring all components and clinical samples to be tested to room temperature.
- Dilute Concentrated Wash Buffer 1:20 by adding 50ml of Concentrated Wash Buffer to 950ml of distilled or double deionized water. Diluted buffer can be stored at 2-8°C for up to 2 weeks.
- Pipette 10µl of controls and diluted sera into the appropriate wells.
- Incubate slides in a moist chamber at 37°C for 90 minutes.
- Remove slides from the moist chamber and gently rinse each slide with a stream of diluted Wash Buffer using a squeeze type wash bottle. Wash slides by submersing the slides into a staining jar containing diluted Wash Buffer. Leave immersed for 10 minutes. Dip washed slides in double distilled water. Remove and air dry.
- Pipette 10µl of FITC-Conjugate into each well.
- Incubate at 37°C for 30 minutes.
- Repeat rinsing and washing of slides as in step 5.
- Place 3 drops of Mounting Fluid along the center of each slide. Cover with provided cover slip. Remove air bubbles by pressing gently on the cover slip.
- Read results on a fluorescence microscope at a magnification of 400x or 1000x. For best results, read the slides on the same day the assay is performed. If this is not possible, mounted slides can be stored in the dark at 2-8°C for up to 3 days.

Fluorescence appearance at 1:20 titer	Interpretation
+	<b>Presence of specific IgM antibodies to <i>C.psittaci</i>.</b> Titers of $\geq 1:20$ are considered presumptive evidence of infection.
±	<b>Indication of possible exposure to <i>C.psittaci</i>.</b> Second sample testing required after 2-4 weeks <sup>1</sup> .
-	<b>Negative.</b> No detectable IgM antibodies to <i>C.psittaci</i> .

- In case of borderline results a second serum sample should be taken 2-4 weeks later and tested together with the first sample. If borderline result is repeated, specimen must be considered negative.

**Significance of IgM Endpoint Titers**

If semi-quantitative results are required endpoint titration should be performed.

**In order to achieve a more comprehensive antibodies' profile, IgM, IgG and IgA should also be tested**

**Interpretation of results based on the combination of IgG, IgA and IgM antibodies.**

Levels of <i>C.psittaci</i> antibodies			Interpretation of Results
IgM	IgG	IgA	
Negative	Negative	Negative	No indication of <i>C.psittaci</i> infection
Positive	Negative or Positive	Negative or Positive	Indication of current infection
Negative	Positive	Negative	Indication of past or current infection.
Negative	Positive or Negative	Positive	Indication of current or chronic infection

**Test Limitations**

1. No single serological test should be used for a final diagnosis. All clinical and laboratory data should be taken into account.
2. Samples obtained too early during primary infection may not contain detectable antibodies. If chlamydial infection is suspected, a second sample should be obtained 2-3 weeks later and tested in parallel with the original sample.
3. Microscope optics and light source conditions and type may affect determination of the overall fluorescent intensity and endpoint titers.

**Performance Characteristics**

The study was carried out at an independent medical center on patients suspected of having *C. psittaci*.

***C. psittaci* results obtained by IgG SeroFIA™ vs. Reference MIF**

	MIF	Positive	Negative	Total
SeroFIA™				
Positive		18	1	19
Negative		2	10	12
Total		20	11	31

Sensitivity:  $18/20 \times 100 = 90\%$   
 Specificity:  $10/11 \times 100 = 91\%$   
 Overall Agreement:  $28/31 \times 100 = 90\%$

***C. psittaci* results obtained by IgA SeroFIA™ vs. Reference MIF**

	MIF	Positive	Negative	Total
SeroFIA™				
Positive		1	4	5
Negative		1	24	25
Total		2	28	30

Specificity:  $24/28 \times 100 = 88\%$   
 Overall Agreement:  $25/30 \times 100 = 83\%$   
 Note: Sensitivity cannot be determined due to a low number of positive samples.

***C. psittaci* results obtained by IgM SeroFIA™ vs. Reference MIF**

	MIF	Positive	Negative	Total
SeroFIA™				
Positive		3	2	5
Negative		0	26	26
Total		3	28	31

Sensitivity:  $3/3 \times 100 = 100\%$   
 Specificity:  $26/28 \times 100 = 93\%$   
 Overall Agreement:  $29/31 \times 100 = 94\%$

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