CE 0483

Chlamydia IgA SeroFIA™ IVD

An immunoflourescence assay for the detection of specific IgA antibodies to *C. pneumoniae, C. trachomatis* and *C. psittaci* in human serum

Instruction Manual

Test kit for 3 X 105 determinations
Catalog No. 513-01

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

Savyon® Diagnostics Ltd.
3 Habosem St. Ashdod 77610
ISRAEL
Tel.: +972.8.8562920
Fax: +972.8.8523176
E-mail: support@savyondiagnostics.com.

Intended Use
The Chlamydia IgA SeroFIA™ is a semi-quantitative immunofluorescence assay for the differential determination of *C. pneumoniae*, *C. trachomatis* and *C. psittaci* specific IgA 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 Savyon Chlamydia SeroFIA™ test is a micro-IF assay based on the principles of MIF. SeroFIA™ uses as antigen purified elementary bodies of *C. pneumoniae* (TW-183), *C. trachomatis* (L2) and *C. psittaci* (SZ-1). Each SeroFIA™ reaction slide contains 3 rows of 7 wells, each row containing *C. pneumoniae*, *C. trachomatis* or *C. psittaci* antigens. This separation between each of the chlamydial antigens prevents possible confusion between the different chlamydial species and makes interpretation of results simple and error free.

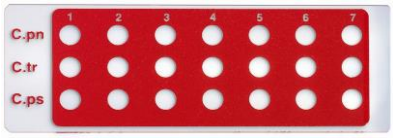
Principle of the Test

- Purified elementary bodies (Ebs) of *C. pneumoniae* (C.pn), *C. trachomatis* (C.tr) and *C. psittaci* (C.ps) are fixed onto the SeroFIA™ slide wells, each in a different row of the slide.
- Diluted patients sera are incubated for 30 minutes at 37ºC in each row with the respective antigens.
- Unbound serum components are removed by washing.
- Fluorescein-conjugated anti-human IgA is added and incubated for 30 minutes at 37ºC.
- 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. pneumoniae*, *C. trachomatis*, and *C. psittaci* antigens, each in a different row. Each slide is packed in an aluminum pouch containing silica gel packet.

15 units



2. Concentrated Wash Buffer (x20): A PBS-Tween buffer, (pH 7.4-7.6) which contains NaCl, Na_2HPO_4, KH_2PO_4 and Tween 20.

1 bottle, 100ml

3. Serum Diluent: A PBS buffer. Contains gelatin, bovine serum albumin, $MgCl_2$ 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. *C. trachomatis* Positive Control: Human serum positive for IgA antibodies to *C. trachomatis*. Contains <0.1% sodium azide. Ready to use.

1 vial, 0.2ml

6. *C. pneumoniae* Positive Control: Human serum positive for IgA antibodies to *C. pneumoniae***.** Contains <0.1% sodium azide. Ready to use.

1 vial, 0.2ml

7. *C. psittaci* Positive Control: Human serum positive for IgA antibodies to *C. psittaci*. Contains <0.1% sodium azide. Ready to use.

1 vial, 0.2ml

8. FITC-Conjugate: Fluorescein-labelled rabbit anti-human IgA (α - chain specific). Ready to use.

1 vial, 3.3ml

9. Mounting Fluid: Contains <0.1% sodium azide.

1 dropper bottle, 1.5ml

10. Cover slips: **1 unit**

11. 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 37ºC water bath with a lid, or a moisture chamber placed in a 37ºC 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 40x and 100x.

Warnings and Precautions

For *In Vitro* Diagnostic Use.

1. This kit contains human sera that have been tested by FDA and CE 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 Micro-biological 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 2-8ºC. If kept at 2-8ºC 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.

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.

Assay Procedure

Note*:*
For each test run, testing of one well of the Negative Control and one well of the Positive Controls of each C.pneumoniae, C.trachomatis and C.psittaci on the respective rows 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.
2. Dilute Concentrated Wash Buffer 1:20 by adding 50ml of Concentrated Wash Buffer to 950ml of double deionized or distilled water. Diluted buffer can be stored at 2-8ºC for up to 2 weeks.
3. Pipette 10µl of controls and diluted sera into the appropriate wells of each of the three rows.
4. Incubate slides in a moist chamber at 37ºC 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 37ºC 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 2-8ºC for up to 3 days.

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 Controls exhibit moderate to intense apple green fluorescence staining of Chlamydia EB particles of the respective chlamydia species.

2. Negative Control shows negligible reactivity (staining) with each of the chlamydia species.

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

Fluorescence appearance at 1:32 titer			Interpretation
C.pn	C.tr	C.ps	
+	-	-	Presence of specific IgA antibodies to C.pn, C.tr or C.ps respectively. A titer of 1:32 is considered presumptive evidence of infection.
-	+	-	
-	-	+	
+	+	-	Presence of IgA antibodies to chlamydia. May represent multiple infections or inter-species cross-reactions. In order to determine the predominant chlamydia species, endpoint titration should be performed.
-	+	+	
+	-	+	
+	+	+	
-	-	-	Negative. No detectable IgA antibodies to either C.pn, C.tr, or C.ps.

Note: In rare cases, a clear and dense staining of very small particles (smaller than the EB's) might be observed on one or more of the antigens. 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.

Significance of Endpoint Titers

If semi-quantitative results are required or if reactivity (fluorescence staining) with more than one chlamydia species is detected the antigen demonstrating the highest endpoint titer) by at least four fold (indicates the chlamydia species assumed responsible for 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. Reactivity of serum with multiple chlamydia species may be due to exposure to more than one Chlamydia species or due to cross reactive antibodies.
4. 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. trachomatis*, *C. pneumoniae* or *C. psittaci*.

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

MIF / SeroFIA™	Positive	Negative	Total
Positive	11	6	17
Negative	2	52	54
Total	13	58	71

Sensitivity: 11/13 x 100 = 84.6%
Specificity: 52/58 x 100 = 89.7%
Overall Agreement: 63/71 x 100 = 88.7%

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

MIF / SeroFIA™	Positive	Negative	Total
Positive	39	0	39
Negative	0	74	74
Total	39	74	113

Sensitivity: 39/39 x 100 = 100%
Specificity: 74/74 x 100 = 100%
Overall Agreement: 113/113 x 100 = 100%

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

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

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

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Glossary of Symbols:

Symbols for IVD components and Reagents			
	Manufacturer	IVD	In vitro diagnostic medical device
EU REP	EU Authorized representative		Consult instructions for use
Σ n	Contains Sufficient for <n> tests		Temperature limitation
REF	Catalogue Code		Use by Date
LOT	Batch Number	CE	CE mark

CE 0483

EU REP

European Authorized Representative:
MedNet EC-REP GmbH
Borkstrasse 10,
48163 Muenster, Germany