CE 0483

IPAzyme™ Chlamydia IgG/IgA

Indirect Immunoperoxidase Assay (IPA) for the detection of specific IgG antibodies and specific IgA antibodies to ***Chlamydia*** in human serum

Instruction Manual

Test kit for 144 determinations
(Catalog No. 011-01)

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

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Intended Use

The Savyon Chlamydia IPAzyme assay is intended for the detection and titration of anti Chlamydia specific IgG antibodies and anti Chlamydia specific IgA antibodies in human serum.

For ***In Vitro*** Diagnostic Use Only

Introduction

Chlamydia is an energy parasitic bacterium with a genome of 660 x 10^6 daltons (1-5). The genus Chlamydia comprises three species: Chlamydia *trachomatis*, Chlamydia *psittaci* and recently described Chlamydia *pneumoniae* (TWAR) strain (6, 7). All members of Chlamydia share a common genus specific antigen which is a glycolipid (8). C.*pstitaci* is recognized as the causative agent of psittacosis (12). Chlamydia *trachomatis* serovars share species and type specific antigens to a varying degree. Four of 15 serovars (A, B, A, Ba, and C) cause endemic binding trachoma; eight serovars (D through K) are responsible for sexually transmitted Chlamydia infections; three serovars (L_1, L_2 and L_3) are responsible for lymphogranuloma venereum (LGV) (9). It is now evident that Chlamydia *trachomatis* (serovars D through K) is the major cause of sexually transmitted infections, such as non-gonococcal urethritis (NGU), post gonococcal urethritis (PGU), epididymitis, cervicitis, endometritis, salpingitis, perihepatitis, peritonitis, Reiter's syndrome, conjunctivitis and pneumonia (reviewed by Ladany and Sarov (10).
Anti Chlamydia antibody titers correlate with the severity of the tubal inflammation (as shown by laparoscopy) and the duration of lower abdominal pains before medical attention is sought (11,14). Furthermore, previous infection (mainly subclinical) with CT is related to approximately one-third to two-thirds of tubal infertility. Procedures used in the diagnosis of Chlamydia infections include isolation in cell culture, detection of Chlamydia directly in smears by an immunofluorescence test, enzyme linked immunosorbent assay (ELISA), and/or serological methods for the detection of antibodies to CT, such as complement fixation (CF) assay, the microimmunofluorescence (MIF) test, enzyme immunoassay (EIA) or radioimmunoassay (reviewed by Evans and Woodland (15) and by Treharne et al (16)).
One of the problems in isolation of CT as a standard diagnostic test , is the fact that this cannot be done in all CT infections. For instance, in the case of salpingitis and mechanical infertility due to CT, the agent cannot usually be isolated from fallopian tube washings. In a certain percentage of the patients, CT can be identified from biopsy specimens(14, 30). The possibility has been raised in a number of recent studies (11,14, 17-23) that elevated titers of specific IgG and IgA anti Chlamydia antibodies may serve as markers for early detection of active CT infection. Particularly elevated anti CT IgG antibody titers were found in chronic or systemic infections such as salpingitis, mechanical infertility, perihepatitis, (Fitz-Hugh-Curtis Syndrome), and pneumonitis (11-14, 17-21, 24, 25, 33). It has been demonstrated that the presence of anti CT IgA antibodies is significantly higher in non-gonococcal urethritis (NGU) patients (positive isolation) in women with acute salpingitis and in women with mechanical infertility, when compared with matched controls (21, 26-28). The availability of serologic markers for CT infections reduce the necessity for invasive procedures in diagnosis. Genital infections caused by CT are often asymptomatic, and early detection of these infections is of real value since effective treatments exist (e.g. tetracycline, erythromycin) which might prevent CT from either reaching or damaging the upper genital tract. It is possible that total population screening may be cost efficient if a reduction in infertility treatment expenses is taken into consideration. Both anti CT IgG and anti CT IgA antibodies can be used for efficient monitoring of the clinical situation of patients subjected to clinical treatment of CT infections.
Savyon Diagnostics Ltd., proposes an indirect immunoperoxidase assay comprising CT serotype L_2 infected cells as antigen for detection of anti CT specific IgG antibodies and anti CT specific IgA antibodies. Serotype L_2 has been found to have a broad reacting antigen and has been estimated to react with 95.5% of human sera positive of anti CT antibodies by single antigen indirect immunofluorescence assay (IFA) . This test can be performed in almost any clinical laboratory, since it is economical and easy to perform.

Principle of Indirect Immunoperoxidase Assay

The steps of the procedure are as follows:
In step one, the human serum to be tested is brought into contact with the antigenic material (***Chlamydia trachomatis*** infected cells). Antibody if present in the serum, will attach to the antigen, forming an antigen-antibody complex. If the serum being examined contains no antibody for this particular antigen, no complex is formed and all the serum components are washed away in the rinse phase. The second step involves the addition of horseradish peroxidase (HRP) conjugate of anti-

human IgG (gamma chain specific) or anti-human IgA (alpha chain specific) to the test. If an antigen-antibody complex was formed in step one, the peroxide labeled antibody will bind to the antibody moiety of the complex in step two. A positive reaction, a blue to deep blue precipitate inside the infected cells can be seen with the aid of an ordinary light microscope following the enzymatic reaction of the peroxidase moiety with hydrogen peroxide and a suitable chromogen reactant.

Summary of Steps

1. Human Serum (Ab_1) + Chlamydia *trachomatis* infected cells (Ag)

   ↓

   Ab_1Ag complex

2. Ab_1Ag complex + HRP con. Anti human IgG/IgA (Ab_2)

   ↓

   Ab_1AgAb_2 complex

3. Ab_1AgAb_2 complex + Substrate / Chromogen

   ↓

   Insoluble colored precipitate

Notes:

1. **Warning:** THE ANTIGENIC MATERIAL IN THIS KIT HAS BEEN FIXED AND CONTAINS NO DETECTABLE LIVE ORGANISMS. HOWEVER, SLIDES SHOULD BE HANDLED AND DISPOSED OF AS WOULD ANY POTENTIALLY BIO-HAZARDOUS LABORATORY MATERIAL.
2. **Precaution:** The human serum components have been tested by FDA approved methods and found to be negative for Hepatitis B Surface Antigen (HBsAg) and Human Immunodeficiency Virus (HIV) antibody. This does not ensure the absence of HBsAg or HIV. Therefore, human serum components and patients' serum specimens should be handled and disposed of as would any potentially biohazardous laboratory material.
3. All components in this kit have been tested and standardized as a unit. Do not mix components from different kit lots or other manufacturers' kits.
4. Do not use reagents beyond the stated expiry date when the components are still originally stoppered. The shelf life of all kit components except mounting medium, slide cover slips and blotting paper strips is limited to sixty days after opening.
5. For *in vitro* use only.

Materials Supplied

1. 12 x 12 Well Slides with Chlamydia *Trachomatis* Infected Cells.
2. 1 x 0.5ml Vial Positive Control – Human Serum Positive for IgA antibodies. Ready for Use
3. 1 x 0.5ml Vial Positive Control – Human Serum Positive for IgG antibodies. Ready for Use
4. 1 x 0.5ml Vial Negative Control – Human Serum Negative for IgA, IgG and IgM Anti Chlamydia Antibodies. Ready for Use
5. 1 x 1.0ml Vial HRP Conjugated Anti-Human IgA (alpha chain specific) Ready for Use
6. 1 x 1.0ml Vial HRP Conjugated Anti-Human IgG (gamma chain specific) (rabbit) Ready for Use
7. 1 x 2.0ml Vial Substrate/Chromogen Ready for Use
8. 1 x 4.0ml Mounting Medium. Ready for Use
9. 1 x 100ml Concentrated IPAzyme Buffer (x20)
10. 3 Blotting paper strips
11. 15 Slide Cover Slips
12. Control Sera Data form
13. Instructions For Use

Materials Required but not Supplied

1. Test tubes and rack for preparation of patient's and/or control serum dilutions.
2. Micropipettes (10, 20, 60 and 300 microliter) and safety pipetting devices.
3. A 37ºC water bath with a lid, or a moist chamber placed in a 37ºC incubator.
4. Slide holder tray.
5. light microscope, **10X20 magnification**, with blue filter.
6. 1 liter Erlenmeyer flask for concentrated IPAzyme Buffer dilution.
7. Wash bottle.
8. Double deionized or distilled water for the dilution of Concentrated IPAzyme Buffer.

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Storage and Shelf-Life of Reagents

All materials supplied, except for Slide Cover Slips and blotting paper strips, should be stored at **2º - 8ºC**. However, an exposure of a few hours to an ambient temperature will not cause any damage to reagents. **Do not freeze**. If a constant storage temperature (2º-8ºC) is maintained, reagents in vials originally stoppered will be stable until the expiry date stated on each label. Matched reagents in a kit will be stable until the dated indicated on the kit package. When a kit is in use, the shelf life to consider is sixty days from the first day of use. After sixty days all matched reagents of a particular kit batch should be discarded.

Specimen Collection

Serum specimens should be collected aseptically and stored at 2º - 8ºC with 0.2% sodium azide (NaN_3) as preservative, if they are to be tested within a few days. For longer periods aliquots of serum specimens should be stored at -20ºC.
Although hemolysis and/or turbidity effects on the assay have not been elucidated, testing of clear non-hemolytic serum specimens is highly recommended.

Assay Procedure

Notes:

1. The person performing the assay should be familiar with laboratory practices and routines.
2. All reagents should reach room temperature before use.

3. Positive and Negative Controls must be run each time the test is performed (sufficient on one slide).
4. Control Sera Data form enables a self control of the performance of the test.
5. Use disposable tips. Avoid cross contamination by replacing tips between different reagents.

Procedure

1. Remove the required number of slides from their foil pouches and place them in a slide-holder tray.

2. Prepare the following dilutions of each patient serum with IPAzyme Buffer:

   1:16 – (20 microliter of patient serum + 300 microliter IPAzyme Buffer.)

   1:64 – (20 microliter of 1:1:16 diluted serum + 60 microliter of IPAzyme Buffer.)

   1:128 – (20 microliter of 1:64 diluted serum + 20 microliter of IPAzyme Buffer.)

   Use 1:16 for IgA
   Use 1:64 and 1:128 for IgG

3. Pipette into each well 10 microliter of either a control serum or a dilution of a patient's serum specimen.

4. Place the slides in a moist chamber or in a covered water bath* and incubate at 37°C for 45 minutes

   *Special attention should be paid to proper closer of the incubation chamber or bath, otherwise the pipette reagents will dry during the incubation. Avoid dripping condensed water drops from the lid on to the slides.

5. Rinse the slides thoroughly, either with a light stream of IPAzyme Buffer using a wash bottle, or in a staining reservoir full of IPAzyme Buffer, by moving the slides up and down.

6. Dry the slides by blotting over the blotting paper strips as follows: press the well surface of the slide briefly and gently over the paper (do not rub). Blot twice, each time on a dry part of the paper, and then turn the slide surface upwards. If still not completely dry, repeat this step. If preferred, the slides may be dried by compressed air rather than by blotting.

7. Pipette 10 microliter of HRP conjugate into each well. Place the slides in a moist chamber or in a covered water bath and incubate at 37°C for 45 minutes.

8. Repeat steps 5 and 6.

9. Pipette 10 microlitersof chromogen/substrate solution into each well. Incubate for 15 minutes at room temperature.

10. Repeat steps 5 and 6.

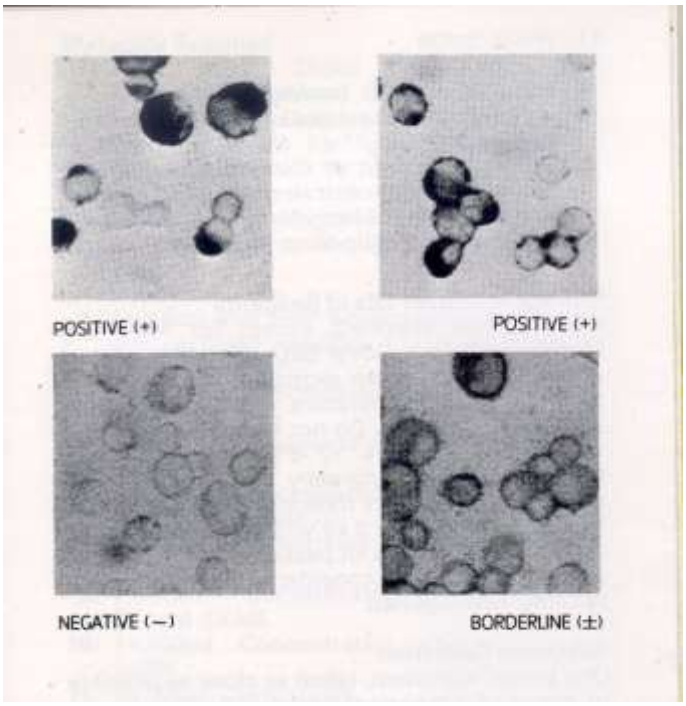
11. Place four small drops of mounting medium on each slide and cover with a cover slip. Avoid entrapping air bubbles between the slide and the cover slip.

12. Read the slides within the same day at a **magnification of 10 x 20.** It is recommended to use a blue filter when observing the results.

Notes:
1. It is recommended to determine final results at a **magnification of 10 x 20** or at any close magnification. **Other magnifications may mislead**.
2. In the event of mounting medium spilling on to the microscope, it is advisable to clean immediately with a damp cloth.

Display of Results

The presence of a blue precipitate in the infected cells indicates a positive reaction. The absence of a blue precipitate in the infected cells indicates a negative reaction in this test. No blue precipitate should develop in uninfected cells. Ay blue color in the infected cells when compared to the uninfected cells should be considered positive.



Interpretation of Results

Assay	Dilution	Possible Results							
		1	2	3	4	5	6	7	8
IgG	1:64	+	+	+	+	+	+	-	-
IgG	1:128	+	+	-	-	±	-	-	-
IgA	1:16	+	-	+	±	-	-	+	-

Significance of Results:

1. Indication of active infection.
2. Indication of active infection.
3. Indication of active infection.
4. Borderline or active infection
5. Positive
6. Positive
7. Rare possibility: repeat the test.
8. Negative (or low positive beyond the sensitivity of this test)

Notes:

If the result falls into the category 5, 6, 7 or 8, it is recommended to test another serum sample after 10 days.
If the results fall into category 4, repeat the IgA test at 1:8 dilution. The possible results are shown in the following table

	IgA 1:8		IgA :16 (±)	Category
If	**+**	**then**	**+**	**3**
If	**-**	**then**	**-**	**6**
If	**±**	**then**	**-**	**6**

Remarks:

A. These interpretations are based on a study of hospitalized pelvic inflammatory disease (PID) patients and controls in Israel. It is recommended to reconfirm the significance of the results based on a single specimen, by determination of the normal antibody level in the geographical area and specific population being tested.
B. It is recommended to use this test for PID patients. However, it is conceivable that similar results might be obtained in mechanical infertility, in systemic CT infections (pneumonia, perihepatitis) and other chronic infections.
C. In the case of superficial mucosal infection (e.g., non-gonococcal urethritis) a different immune response might be generated.
D. For sero-epidemiological studies or population screening titer of 1:32 of CT antibodies should also be considered.

Limitations

1. Should all the cells exhibit a positive reaction, the test result is not valid. In IPA performed with IPAzyme Chlamydia, this phenomenon is rare and serum related, possibly caused by a disease state unrelated to or in addition to Chlamydia infections, e.g. antinuclear antibody, antimitochondrial antibody or unexplained nonspecific reaction (31, 32).
2. Interpretation of results in PID is based on a limited number of serum dilutions. Performance of further series of serum dilutions is encouraged.
3. The test is a single server inclusion (L_2) immunoperoxidase assay. L_2 contains antigenic determinants existing in the other serovars of C.*trachomatis* as well as the group antigen. Although the cells have been infected by Chlamydia *trachomatis* of the L_2 serovar, antibodies against C. *psittaci*, and Acinebacter calcoaceticus may be revealed by this IPA.
4. If serial dilutions are carried out, the titer of either IgG antibodies or IgA antibodies can be determined. However, no single serological testing should be used as the sole criterion for diagnosis. All clinical and laboratory data must be taken into account including the chemotherapeutic profile of patient already subjected to a medication regime.
5. Excess lipids in the serum may produce a "filming" reaction, which is caused by lipids which stick nonspecifically to the glass and are difficult to remove. This reaction, although rare, can be observed along the edges of wells where the serum comes into contact with the slide coating. This reaction, being nonspecific, should be ignored. Only the reaction observed in the inner area of the well should be considered as test results.

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