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CHLAMYDIA
ANTIGEN SUBSTRATE SLIDE

NOTE: Changes highlighted

PRODUCT AVAILABILITY

The following Chlamydia Antigen Substrate Slides are available individually from Bion Enterprises, Ltd. (Bion):

Antigen Substrate Slide	Code No. REF
Chlamydia (LGV-1)	CH-4112
Σ Number of Tests	12-Well

INTENDED USE

The Bion CHLAMYDIA ANTIGEN SUBSTRATE SLIDES may be used as the antigenic substrate in indirect fluorescent antibody assays for the qualitative and/or semi-quantitative determination of Chlamydia antibodies in human serum. Bion CHLAMYDIA ANTIGEN SUBSTRATE SLIDES are intended for use as an aid in the diagnosis of primary infection or reinfection and as a determination of immunological experience with Chlamydia.

SUMMARY AND EXPLANATION

The Chlamydiae are a large group of obligate intracellular parasites of eukaryotic cells. There are four species: Chlamydia psittaci, primarily an animal pathogen (although it can cause pneumonia in humans); Chlamydia trachomatis, primarily a human pathogen[1]; and recently established Chlamydia pneumoniae consisting of TWAR and TWAR-like isolates.[2,3] Originally thought to be a C. psittaci strain[4,5] it now appears to be a human pathogen with no evidence of bird or animal hosts.[3,6] A fourth species, Chlamydia pecorum, has been described but its role as a pathogen is not clear.[7]

The major human chlamydial infections are pneumonia, Lymphogranuloma venereum, trachoma, inclusion conjunctivitis, and genital tract infection.[8] C. trachomatis has become a major etiologic agent of sexually transmitted diseases.[9] In men, Chlamydiae are associated with nongonococcal urethritis (NGU), post gonococcal urethritis, and epididymitis.[10,11] Chlamydial infections do not respond to short courses of penicillin used to treat gonorrhea; therefore, urethritis may persist despite eradication of the gonococcal infection. In women, Chlamydiae are associated with nonspecific genital infections which may include cervicitis, urethritis and acute salpingitis.[12,13] C. trachomatis has also been implicated in pelvic inflammatory disease which includes inflammation of the cervix, uterus, fallopian tubes and ovaries.[9] Pathology ranges from asymptomatic to quite severe.[14,15] A high rate of neonatal infections is seen in deliveries from pregnant women genitally infected with Chlamydia trachomatis, especially infant pneumonia and conjunctivitis.[16,17] C. pneumoniae causes acute respiratory tract diseases such as pharyngitis, sinusitis, bronchitis and is one of the major causes of atypical pneumonia often confused with Mycoplasma pneumoniae and Legionella.[3]

All Chlamydiae share a common antigen, but they include a variety of microorganisms with different biological, serological and pathogenic properties. Trachoma, inclusion conjunctivitis and genital tract infections may be diagnosed by detecting a significant rise in titer between paired acute and convalescent sera particularly in psittacosis. However, it is often difficult to demonstrate rising antibody titers, particularly in the sexually active population of individuals with many chronic or repeat infections. It is also difficult to demonstrate rising IgG titers with LGV as the patient usually sees the physician after the acute stage; however, active cases usually have relatively high levels of IgM. High levels of IgM are also associated with C. pneumoniae in neonates as detection of IgG antibodies are less useful as these infants have high levels of maternal acquired IgG from their infected mothers.[7]

Commonly, diagnosis of Chlamydia involves demonstrating typical intracytoplasmic inclusions identified by immunofluorescence, iodine or Giemsa staining of cycloheximide treated cell cultures. Because cell cultures are relatively demanding, establishment of serological tests is preferred. The genus specific complement fixation (CF) test is widely used for diagnosing Chlamydial infections, particularly psittacosis and LGV but is relatively insensitive in diagnosing trachoma, inclusion conjunctivitis, related genital tract infections and neonatal chlamydial infections. Both the micro-IF and indirect immunofluorescent (IFA) methods are more sensitive and can be used to measure both IgG and IgM antibodies. EIA techniques have not yet been readily applied to the detection of IgM antibody. The micro-IF test can be antigen specific with a single serovar, but it is usually run with a mixture of all of the 15 or more antigenic types. The IFA test with whole chlamydia inclusions as the substrate is capable of detecting both group (genus) and most type specific IgG and IgM antibodies to C. trachomatis, C. psittaci or C. pneumoniae.[7,18]

PRINCIPLE OF THE IFA PROCEDURE

The Bion CHLAMYDIA ANTIGEN SUBSTRATE SLIDES may be utilized in the indirect fluorescent antibody assay method first described by Weller and Coons[19] and further developed by Riggs, et al.[20] The procedure is carried out in two basic reaction steps:

Step 1 - Human serum is reacted with the antigen substrate. Antibodies, if present, will bind to the antigen forming stable antigen-antibody complexes. If no antibodies are present, the complexes will not be formed and serum components will be washed away.

Step 2 - Fluorescein labeled antihuman IgG (or IgM) antibody is added to the reaction site which binds with the complexes formed in step one. This results in a positive reaction of bright apple-green fluorescence when viewed with a properly equipped fluorescence microscope. If no complexes are formed in step one, the fluorescein labeled antibody will be washed away, exhibiting a negative result.

REAGENTS

Bion CHLAMYDIA ANTIGEN SUBSTRATE SLIDES are individually foil-wrapped twelve well slides with Chlamydia, LGV Type 1 (clinical specimen) infected L929 cells fixed onto each well. Each well contains an average of 10-50% infected cells per 200X field.

STORAGE AND STABILITY

The Bion CHLAMYDIA ANTIGEN SUBSTRATE SLIDES are stable in sealed foil pouches at 8°C or lower until labeled expiration date.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use. Thus, only staff trained in methods of in vitro diagnsotics may perform the test. IVD
2. Substrate slides are for single use only and must not be used more than once.
3. Use with non-Bion reagents could result in erroneous results.
4. Care should be taken when handling substrate slides due to sharp edges.
5. The antigenic substrates have been fixed in acetone and contain no detectable live chlamydial agents. However, they should be handled and disposed of as any potentially biohazardous laboratory material.
6. Do not remove slides from pouches until ready for testing. Do not use if pouch has been punctured, as indicated by a flat pouch.
7. Antigen substrate slides should be brought to room temperature (20-25°C) prior to use.
8. Abnormal test results may be seen if the antigen substrate slides are allowed to dry during the staining procedure.
9. Refrigeration (2-8°C) of antigen substrate slides immediately upon arrival will insure stability until labeled expiration date.
10. Antigen substrate slides should not be used beyond stated expiration date.
11. Avoid microbial contamination of all reagents involved in the testing procedure or incorrect results may occur.
12. Incubation times or temperatures other than those specified may give erroneous results.
13. Reusable glassware must be washed and thoroughly rinsed free of detergents.
14. Care should be taken to avoid splashing or generation of aerosols.
15. Previously frozen specimens after thawing should be thoroughly mixed prior to testing. It is recommended that sera is freeze thawed no more than one time. If repeated testing is required, it is suggested that specimen be aliquoted.
16. Patient samples, as well as all materials coming into contact with them, should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the CDC/NIH manual “Biosafety in Microbiological and Biomedical Laboratories”, 1984 Edition. Never pipette by mouth. Avoid contact with skin and mucous membranes.

SPECIMEN COLLECTION

Blood should be collected fasting or at least one hour after meals to avoid lipemic serum, as excess lipids may produce a “film” over the substrate. Aseptically collect 5-8 ml of blood by venipuncture. Allow the blood to clot at room temperature (20-25°C) before separating serum to avoid hemolysis which could interfere with test results. Specimens should be stored refrigerated at 2-8°C and tested within one week of collection. Long term storage should be at -20°C in aliquots to avoid repeated freezing and thawing. Do not store in self-defrosting freezer.

Avoid using contaminated sera as they may contain proteolytic enzymes which will digest the substrate. It is unnecessary to heat inactivate serum specimens prior to testing; however, sera that have been heat inactivated may be used.

When testing paired samples to look for evidence of recent infection, the acute specimen should be obtained as soon as possible after onset of illness and the convalescent specimen obtained 7-14 days later. Acute and convalescent specimens must be tested simultaneously, in the same assay, looking for a significant change in antibody titer between the paired sera. If the first specimen is obtained too late during the course of the infection, a significant rise in the antibody titer may not be detected.

PROCEDURE

Detailed descriptions of indirect immunofluorescence techniques may be found in the references listed in the bibliography.[21,22,23]

MATERIALS PROVIDED

Bion CHLAMYDIA ANTIGEN SUBSTRATE SLIDES.
Lot Number provided on label. LOT

MATERIALS AVAILABLE FROM Bion

1. Fluorescent Antibody Conjugate with 0.01% Evans Blue counterstain
2. Chlamydia Positive Human Control Serum
3. Chlamydia Negative Human Control Serum
4. Phosphate Buffered Saline (PBS)
5. Mounting Medium

MATERIALS REQUIRED BUT NOT PROVIDED

1. Disposable test tubes (12 x 75 mm or comparable) and rack
2. Disposable serological pipettes
3. Calibrated pipettes to deliver 50 µl, 100 µl and 200 µl with disposable pipette tips
4. Pasteur pipettes and bulbs
5. Moist chambers
6. Plastic squeeze wash bottle
7. Coplin jars or staining dishes with slide racks
8. 24 x 60 mm #1 coverslips
9. Felt tip marking pen
10. Fluorescence microscope equipped with mercury or tungsten-halogen light source, a 390-490nm excitation filter and 515-520nm barrier filter, and optics to give a total magnification of 200X or 250X. The excitation wavelength of FITC is 490nm and the emission wavelength is 520nm.

TEST PROCEDURE

1. SPECIMEN PREPARATION

Screening:
Each laboratory should establish its own protocol for the preparation of serum screening dilutions. Most indirect fluorescent antibody staining procedures utilize a 1:10 dilution of each patient's serum which is prepared by adding 0.05 ml (50 µl) of the patient's serum to 0.45 ml of PBS.

NOTE: If testing for IgM specific antibodies using an IgM specific fluorochrome conjugate, each patient serum specimen must be pre-treated to remove any IgG interference by separating the IgM from the IgG, and then running the screening test on the IgM eluate. Suggested methodologies are ion exchange chromatography,[24] or IgG immunoprecipitation.[25,26]

Semi-quantitation:
Serum dilutions are utilized to measure antibody titer. Each laboratory should establish its own titering protocol. The selection of either twofold or fourfold dilution procedures depends upon the experience level and training of the individual(s) reading the fluorescent antibody assay.

The following fourfold serial titration is suggested for IgG testing:

a. Prepare a 1:10 dilution of each patient's serum by adding 0.05 ml (50 µl) of patient's serum to 0.45 ml of PBS in tube #1.
b. Add 0.3 ml PBS to tubes #2, #3, #4, and #5.
c. Using a 100 µl pipette, transfer 0.1 ml (100 µl) from tube #1 to tube #2. Mix. Using a new tip for each dilution, transfer 0.1 ml (100 µl) from the second tube to the third, from the third tube to the fourth, and from the fourth tube to the fifth, mixing after each transfer.

The following twofold titration is suggested for IgM testing:

a. Prepare a 1:10 dilution of each patient's serum using one of the treatment methodologies mentioned in the "Screening NOTE" above. This will be designated as tube #1.
b. Add 0.2 ml PBS to tubes #2, #3, #4, and #5.
c. Using a 200 µl pipette, transfer 0.2 ml (200 µl) from tube #1 to tube #2. Mix. Using a new tip for each dilution, transfer 0.2 ml (200 µl) from the second tube to the third, from the third tube to the fourth, and from the fourth tube to the fifth, mixing after each transfer.

These titrations will have the following dilutions:

Fourfold	Twofold
Tube #1 = 1:10	Tube #1 = 1:10
Tube #2 = 1:40	Tube #2 = 1:20
Tube #3 = 1:160	Tube #3 = 1:40
Tube #4 = 1:640	Tube #4 = 1:80
Tube #5 = 1:2560	Tube #5 = 1:160

2. SLIDE PREPARATION

Remove reagents and as many slides as are required from the refrigerator or freezer and allow to equilibrate to room temperature (20-25°C) for at least five minutes. Remove slides from sealed foil pouches being careful not to touch the antigen surface. Identify each slide using a felt tip marking pen.

3. SPECIMEN APPLICATION

Using separate Pasteur pipettes, apply one drop (20-30 µl) of the positive control, one drop (20-30 µl) of the negative control and one drop (20-30 µl) of each patient serum dilution to individual wells of the slide. Do not touch the antigen surface with the pipette while dropping. Do not allow drops to mix, as cross contamination of samples between wells could cause erroneous results.

4. INCUBATION 1

Incubate in a moist chamber at room temperature (20-25°C) for 30 minutes. THE ANTIGEN MUST NOT BE ALLOWED TO DRY DURING ANY OF THE FOLLOWING STEPS. Nonspecific binding may occur if the reagent is allowed to dry on the slide.

NOTE: For IgM testing, incubate the substrate slides in a moist chamber at 35-37°C for 90 minutes.

5. RINSE 1

Remove slides from moist chamber and rinse GENTLY with PBS using a squeeze wash bottle. Do not focus the PBS stream directly onto the wells. To prevent cross contamination tilt slide first toward wells 1-6 and, running a PBS stream along the midline of the slide, allow the PBS to run off the top edge of the slide. Then, tilt the slide toward wells 7-12 and repeat this procedure, allowing the PBS to run off the bottom edge of the slide.

6. WASH 1

Place slides in Coplin jars or staining dishes and wash in two changes of PBS for not less than five minutes or more than ten minutes each, agitating gently at entry and prior to removal.

7. CONJUGATE APPLICATION

Remove slides from the wash one at a time, shake off excess PBS, dry around outside edges if necessary and return each slide to the moist chamber. Apply one drop of an appropriate fluorescent antibody (IgG or IgM) conjugate with counterstain (diluted to its predetermined proper working dilution) to each well of each slide, making sure that each well is completely covered.

8. INCUBATION 2

Incubate in a moist chamber at room temperature (20-25°C) for 30 minutes. Protect slides from excessive light.

NOTE: For IgM testing, incubate in a moist chamber at 35-37°C for 60 minutes.

9. RINSE 2

Remove slides from moist chamber and rinse GENTLY with PBS using a squeeze wash bottle. As suggested in step 5., do not focus PBS stream directly onto the wells.

10. WASH 2

Place slides in Coplin jars or staining dishes and wash in two changes of PBS for not less than five minutes or more than ten minutes each, agitating gently at entry and prior to removal.

11. COVERSLIP

Remove slides one at a time from the last PBS wash, shake off excess PBS and immediately add two to four drops of mounting medium across the slide. Tilt slide and rest the edge of the coverslip against the bottom of the slide allowing the mounting medium to form a continuous bead between the coverslip and slide. Gently lower the coverslip from the bottom of the slide to the top, being careful to avoid air bubbles. Drain excess mounting medium by holding the edge of the slide against absorbent paper. Wipe off back of slide.

12. READ

Examine stained slides as soon as possible using a properly equipped fluorescence microscope. It is recommended that slides be examined on the same day they are stained. If any delay is anticipated, store slides in the refrigerator (2-8°C) away from direct light and read the following day. Do not allow mounting medium to dry between slide and coverslip. If drying should occur, add additional mounting medium or recoverslip slide.

FLUORESCENT INTENSITY GRADING

Fluorescent intensity may be semi-quantitated by following the guidelines established by the Centers for Disease Control, Atlanta, Georgia:[27]

4+ = Maximal fluorescence; brilliant yellow-green.
3+ = Less brilliant yellow-green fluorescence.
2+ = Definite but dull yellow-green fluorescence.
1+ = Very dim subdued fluorescence.

The degree of fluorescent intensity is not clinically relevant and has only limited value as an indicator of titer. Differences in fluorescence microscope optics, filters and light sources may result in differences of 1+ or more fluorescent intensity when observing the same slide using different microscopes.

QUALITY CONTROL

SPECIFICITY CONTROL

Both a positive and negative antibody control must be included with each run. These controls must be examined prior to reading test samples and should demonstrate the following results:

Negative Control
Using a negative control serum on Bion CHLAMYDIA ANTIGEN SUBSTRATE SLIDES, the infected cells should exhibit less than 1+ fluorescence and appear reddish-orange due to the counterstain.

Positive Control
Using a positive control serum on Bion CHLAMYDIA ANTIGEN SUBSTRATE SLIDES, the infected cells should exhibit well defined specific fluorescent staining at an intensity of 3+ or greater. The Chlamydia fluorescent staining pattern consists of well defined intracytoplasmic inclusion bodies found near the cell nucleus. Pinpoint elementary bodies may often be observed both on and between the cells. The uninfected cells stain reddish-orange due to the counterstain.

Each control must demonstrate the expected reaction in order to validate the test. If the controls fail to appear as described above, the test results should not be reported and the test should be repeated. If upon repeat testing the controls still fail to show the proper staining reaction, do not report the test results.

SENSITIVITY CONTROL

A titered control included with each run tests substrate sensitivity, as well as, checks technique, conjugate quality and the microscope optical system. The endpoint titer of this control must be determined and there must not be more than a twofold difference (+/-) in titer from this determined endpoint. Each run should include the endpoint dilution, one twofold or fourfold dilution above and one twofold or fourfold dilution below the endpoint dilution. The more concentrated dilution should be positive and the less concentrated dilution negative. If the control does not behave as described, the test results are invalid and the tests should be repeated. If the control again fails to show the proper reaction upon repeat testing, do not report the test results.

READING OF TEST RESULTS

NEGATIVE

A serum dilution is considered to be negative for Chlamydia antibodies if the cells exhibit less than 1+ fluorescence and appear reddish-orange due to the counterstain, or if the fluorescence observed is not the specific staining pattern of Chlamydia.

A sample is considered negative for Chlamydia antibodies if it exhibits less than 1+ fluorescence at a serum dilution of 1:10 and all greater dilutions, or if the fluorescence observed is not the specific staining pattern of Chlamydia.

... Negative samples may exhibit fluorescent staining slightly greater than the negative control, but less than 1+.

... Nonspecific staining of all cells observed in some sera at low dilutions is most likely due to the presence of autoantibodies against cellular components in either the nucleus or cytoplasm.

... Staining of areas other than the Chlamydia infected cells should be interpreted as negative, and attention should be directed to specific steps in the staining method (e.g., RINSE and WASH steps).

POSITIVE

A serum dilution is considered positive for Chlamydia (IgG or IgM) antibodies if fluorescent staining of well defined intracytoplasmic inclusion bodies found near the cell nucleus and pinpoint elementary bodies on and between the tissue culture cells are observed at an intensity of 1+ or greater. The number of cells exhibiting a positive staining reaction and the type of fluorescent staining should closely approximate that seen with the positive control.

A sample is considered positive for Chlamydia antibodies if it exhibits the characteristic staining pattern with a fluorescent intensity of 1+ or greater at a serum dilution of 1:10 or greater.

NOTE: Each field should contain cells that exhibit no apple-green fluorescence. Should most of the cells in the patient test wells fluoresce apple-green in the nucleus and/or the cytoplasm an autoimmune staining reaction due to the presence of autoantibodies should be considered.[28,29] It is recommended that such samples be diluted beyond the interference for better interpretation. It is possible that autoantibody staining may mask specific staining such that a specific interpretation cannot be made. Should this occur, test results should be reported as "Unable to interpret due to the presence of interfering antibodies."

TITRATION

If a semi-quantitative titration is performed, the result should be reported as the reciprocal of the last dilution in which 1+ apple-green fluorescent intensity of the specific staining pattern is detected. When reading fourfold serial dilutions, endpoints can be extrapolated where necessary.

EXAMPLE OF ENDPOINT EXTRAPOLATION:
1:10 = 4+
1:40 = 3+
1:160 = 2+
1:640 = +/-
The extrapolated endpoint is reported as 320.

TROUBLESHOOTING

Possible solutions to problems that may occur in immunofluorescent assays are discussed in an accompanying brochure titled "TROUBLESHOOTING IN IMMUNOFLUORESCENCE".

INTERPRETATION OF RESULTS

Detection of the presence of Chlamydia antibodies indicates current or previous infection with Chlamydia. A significant (fourfold or greater) increase in titer between acute and convalescent serum samples and/or a positive test for IgM specific antibody usually indicates evidence of a recent or active infection.

LIMITATIONS OF THE PROCEDURE

1. Chlamydia antibody test results should be used in conjunction with information available from clinical evaluation and other diagnostic information.
2. A single serological IgG antibody titer to Chlamydia should not be used as the only criterion for diagnosis. Paired serum samples (acute and convalescent) and testing for IgM specific Chlamydia antibodies may provide more meaningful data.
3. A negative test result does not necessarily rule out current or recent infection. The specimen may have been collected too early in the disease before demonstrable antibody is present.
4. Lack of significant rise in titer does not exclude the possibility of recent infection but may indicate an acute phase specimen was obtained too late.
5. In some instances, high IgG or IgM antibody levels in the first of paired specimens may prevent the detection of increases in total antibody, resulting in apparently stationary total antibody titer.
6. Test results on specimens from immunosuppressed patients and pregnant women may be difficult to interpret.
7. Positive test results from cord blood or neonates should be interpreted with caution. The presence of Chlamydia IgG antibodies in cord blood is usually the result of passive transfer from mother to the fetus. The presence of Chlamydia IgM antibodies in cord blood may be due to contamination of cord blood with maternal IgM. It is therefore prudent to confirm positive IgM antibody results on cord blood samples by testing a follow-up specimen from the infant, preferable within the first five days of life.[30] A negative test, however, may be useful in excluding possible infection.[31]
8. Positive test results may not be valid in persons who have received blood transfusions or various blood products within the past several months.
9. Antinuclear antibodies (ANA) present in serum may interfere with the Chlamydia IFA test. They can be differentiated from Chlamydia staining in that ANAs stain the nuclei in all cells; whereas, Chlamydia antibodies exhibit staining only in the 10-50% infected cells.[28]
10. Cytoplasmic fluorescence in the majority of the cells may be due to the presence of antimitochondrial antibodies (AMA) often seen in primary biliary cirrhosis.[29] They can be differentiated from the specific antigen staining in that AMA will stain the cytoplasm of all cells; whereas, Chlamydia antibodies exhibit staining in only the 10-50% infected cells.
11. Endpoint reactions may vary between laboratories due to differences in type or condition of fluorescence microscope employed, diluting apparatus, IgG/IgM separation methods, as well as the experience level of personnel performing the assay.
12. If both the positive and negative control substrate cells are not visible when viewed using the fluorescence microscope, it may be necessary to replace or realign the light source and check the specific filters.
13. Cell culture substrate slides may exhibit nonspecific fluorescence due to contamination of antibodies or PBS rinse-wash solutions with bacteria or fungi. It is very important that personnel reading the staining results have experience in fluorescence microscopy.
14. If testing for IgM specific antibodies, the presence of Rheumatoid Factor (RF) in serum may cause a false positive reaction if pathogen specific IgG is also present. Routine RF tests may not be sensitive enough to detect small amounts of RF which exist within the normal range, but are sufficient to cause a false positive reaction in the more sensitive IFA technique.[32] Therefore, all sera should be treated by ion exchange chromatography[24] or IgG immunoprecipitation[25,26] before testing to eliminate possible RF interference.
15. If testing for IgM specific antibodies, high titers of specific IgG when present in the patient serum may compete with the pathogen specific IgM for the antigen sites resulting in a false negative IgM reaction.[32] Therefore, all sera should be treated by ion exchange chromatography[24] or IgG immunoprecipitation[25,26] before testing to avoid this possible problem.

LIMITATION OF THE PROCEDURE (continued)

16. Two methods such as immunoprecipitation and ion exchange chromatography have been commonly used for neutralizing or removing possibly interfering IgG antibodies prior to testing for specific IgM antibodies in IFA tests. Immunoprecipitation neutralizes all classes of IgG while not affecting the IgM levels; however, high levels of IgG may need to be treated with proportionally increased amounts of the precipitating reagent. Ion exchange chromatography will only eliminate IgG subclasses 1, 2 and 3 with subclass 4 (usually less than 5% of the total IgG) remaining in the fraction with the IgM. Also, only a portion of the IgM antibodies can be recovered.[38]
17. IgM serology performed by IFA is very attractive since it combines specificity with sensitivity and in most cases only a single serum sample is required. However, the need for careful interpretation of the significance of positive IgM tests in relationship to patient's clinical situations must be emphasized. It is essential to have an awareness of understanding of the many problems associated with IgM testing to avoid the many pitfalls that can trap the most experienced of workers. IgM results must always be interpreted with caution.[38]

SPECIFIC LIMITATIONS OF THE CHLAMYDIA ASSAY

1. At least fifteen serotypes of C. trachomatis have been identified and characterized.[33,34,35] Serotypes A, B, and C are usually associated with hyperendemic blinding trachoma; D through K associated with oculogenital disease, and L_1, L_2 and L_3 are Lymphogranuloma venereum serotypes.[35]
2. Interrelationships between the serotypes have been identified, and it is known that type L_1 (LGV-7) cross reacts with serotypes L_2 and L_3; as well as some minor cross reactivity with D, E, Ba, and K.[33,35]
3. The Chlamydia IFA test with whole chlamydia inclusions as the substrate is capable of detecting both group (genus) and most type specific IgG and IgM antibodies to C. trachomatis, C. psittaci or C. pneumoniae.
4. Serodiagnosis is helpful in Lymphogranuloma venereum. However, at least 90 percent of men attending STD clinics are reported to have existing IgG antibody to the Chlamydia organisms at the time of diagnosis. Most studies indicate that women have higher absolute levels of antibody as well as higher rates of seropositivity. Studies have demonstrated that 99 percent of women with active Chlamydial infection have detectable IgG antibody levels.[35]
5. Individuals with systemic infections have higher antibody levels than those with superficial mucous membrane infections. Highest levels of antibody are seen in patients with Lymphogranuloma venereum.[35]
6. Many Chlamydial infections are asymptomatic or minimally symptomatic. Lack of an abrupt onset of symptoms often results in patients being seen when IgM antibody or rising or falling IgG antibody titers cannot be identified.[36]
7. Diagnostic significance of single high titers of antichlamydial antibodies of any immunoglobulin is of questionable value due to the tendency of most STD clinic patients (60-80 percent) already having demonstrable antibodies to C. trachomatis.[34]

EXPECTED VALUES

In a study, 1231 human sera samples from different groups were tested for Chlamydia antibodies using the indirect fluorescent antibody technique and the following results were obtained. In the group of 193 men with non-gonococcal urethritis (NGU) and positive Chlamydia isolation, 38 (20%) had titers of 128-1024, 104 (54%) had titers of 16-64, and 51 (26%) had titers less than 8. In the group of 394 men with NGU and negative Chlamydia isolation, 66 (17%) had titers of 128-1024, 154 (39%) had titers of 16-64, and 126 (82%) had titers less than 8. With the 152 men in the control group, 2 (1%) had titers of 128-1024, 24 (16%) had titers of 16-64, and 126 (83%) had titers less than 8.[37]

In the group of 58 women with positive Chlamydia isolation, 27 (47%) had titers of 128-1024, 24 (41%) had titers of 16-64, and 7 (12%) had titers less than 8. In the group of 91 women with NGU partners and negative Chlamydia isolation, 21 (23%) had titers of 128-1024, 28 (31%) had titers of 16-64, and 42 (46%) had titers of less than 8. With the 160 women in the control group, 15 (8%) had titers of 128-1024, 48 (26%) had titers of 16-64, and 120 (66%) had titers less than 8.[37]

In addition, of 160 children from age 1 to 15 years, 0 (0%) had titers of 128-1024, 6 (4%) had titers of 16-64, and 154 (96%) had titers less than 8.[37]

Some studies using immunofluorescence assays have identified antibody titers in the range of 1:8 to 1:256 in patients with genital tract infections of urethritis and cervicitis. Men with non-gonococcal urethritis who were originally seronegative, but later developed IgG antibody had titers between 1:8 and 1:32 in 60 percent of the cases evaluated, and between 1:64 and 1:256 in 40 percent. High antibody titers in adults (1:256 or greater) have been reported in women with salpingitis; higher titers (1:1024 or greater) have been found in adults with Lymphogranuloma venereum.[35]

A study was conducted on 99 normal human serum samples from the Mid-Central United States. Using BION CHLAMYDIA ANTIGEN SUBSTRATE SLIDES the following results were obtained. Ten (10.1%) specimens were less than 1:10, eighty specimens (80.8%) had a titer of 1:10 to 1:160, and nine specimens (9.1%) had a titer of 1:320 or greater.[38]

SPECIFIC PERFORMANCE CHARACTERISTICS

Bion CHLAMYDIA ANTIGEN SUBSTRATE SLIDES have been evaluated for the presence of specific Chlamydia antigen using commercial monoclonal antisera. A positive reaction was identified with the infected cell cultures when stained with its corresponding antisera.[38] Also, there was no cross-reactivity with other specific monoclonal antisera and the Chlamydia antigen.[38]

Each laboratory should determine its own performance characteristics using all reagents assembled to perform the IFA test.

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