

NOTE: Changes highlighted

PRODUCT AVAILABILITY

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

Antigen Substrate Slide	Code No. REF
Adenovirus	AD-3112
Number of Tests	12-Wells

INTENDED USE

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

SUMMARY AND EXPLANATION

Adenovirus infections are primarily associated with respiratory and ocular disease symptoms in humans. Except in military recruits, Adenoviruses cause little significant respiratory disease in adults. However, in children under six years of age they make a relatively important contribution to febrile upper respiratory illness, such as pharyngitis, pharyngoconjunctival fever, and influenza-like diseases, as well as croup, bronchiolitis, and pneumonia.¹ Epidemic keratoconjunctivitis is the most serious ocular disease associated with Adenoviruses. It typically begins with redness of the conjunctiva, followed by involvement of the cornea. Healing proceeds without sequelae, and recurrent infections have not been reported.¹

Adenovirus infections are predominantly enteric, may be abortive or invasive, and are followed by persistent intermittent excretion for periods as long as 18 months after initial infection.² Following the initial infection, the viruses may persist as a latent infection in the lymphoid tissues of the nasopharynx and enteric tract and be reactivated by some mechanism such as infection with another agent, e.g., Bordetella pertussis.³ Adenoviruses, like other latent viruses, are a problem and can be fatal in immunosuppressed patients with transplants, cancer chemotherapy and those with AIDS.^{4,5} Adenovirus capsid antigen (hexon) is shared by all 41 human Adenovirus serotypes. This allows any Adenovirus serotype to be used for serological testing.¹

PRINCIPLE OF THE IFA PROCEDURE

The Bion ADENOVIRUS ANTIGEN SUBSTRATE SLIDES may be utilized in the indirect fluorescent antibody assay method first described by Weller and Coons⁶ and further developed by Riggs, et al.⁷ 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 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 ADENOVIRUS ANTIGEN SUBSTRATE SLIDES are individually foil-wrapped twelve well slides with Adenovirus, type 5 (CDC Strain) infected and uninfected HEp-2 cells fixed onto each well. Each well contains an average of 10-50% infected cells per 200X field.

STORAGE AND STABILITY

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

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use. Thus, only staff trained in methods of *in vitro* diagnostics may perform the test. IVD
- Substrate slides are for single use only and must not be used more than once. ②
- Use with non-Bion reagents could result in erroneous results.
- Care should be taken when handling substrate slides due to sharp edges.
- The antigenic substrates have been fixed in and contain no detectable live Adenovirus. However, they should be handled and disposed of as any potentially biohazardous laboratory material.
- Do not remove slides from pouches until ready for testing. Do not use if the pouch has been punctured, as indicated by a flat pouch.
- Antigen substrate slides should be brought to room temperature (20-25°C) prior to use.
- Abnormal test results may be seen if the antigen substrate slides are allowed to dry during the staining procedure.
- Refrigeration (2-8°C) of the antigen substrate slides immediately upon arrival will insure stability until labeled expiration date.
- Antigen substrate slides should not be used beyond the stated expiration date.
- Avoid microbial contamination of all reagents involved in the testing procedure or incorrect results may occur.
- Incubation times or temperatures other than those specified may give erroneous results.
- Reusable glassware must be washed and thoroughly rinsed free of detergents.
- Care should be taken to avoid splashing and generation of aerosols.
- Previously frozen specimens after thawing should be thoroughly mixed prior to testing. It is recommended that sera be freeze thawed no more than one time. If repeated testing is required, it is suggested that specimen be aliquoted.
- 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.^{8,9,10}

MATERIALS PROVIDED

BionADENOVIRUSANTIGEN SUBSTRATE SLIDES.

Lot Number provided on label. **LOT**

MATERIALS AVAILABLE FROM Bion

1. Fluorescent Antibody Conjugate with 0.01% Evans Blue counterstain
2. Phosphate Buffered Saline (PBS)
3. 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 a mercury or tungsten-halogen light source, a 390-490 nm excitation filter and 515-520 nm barrier filter, and optics to give a total magnification of 200X or 250X. The excitation wavelength of FITC is 490 nm and the emission wavelength is 520 nm.

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 patient's serum to 0.45 ml of PBS.

Semi-quantitation:

Serum dilutions are utilized to measure antibody titer. Each laboratory should establish its own titrating 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.

2. SLIDE PREPARATION

Remove reagents and as many substrate 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 the 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.

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 the 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 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.

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. COVERSIP

Remove slides one at a time from 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:¹¹

- 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 ADENOVIRUS 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 ADENOVIRUS ANTIGEN SUBSTRATE SLIDES, the infected cells should exhibit well defined specific fluorescent staining at an intensity of 3+ or greater. The Adenovirus fluorescent staining pattern is both nuclear and cytoplasmic with more apparent intranuclear inclusions seen at the periphery of the nucleus. Approximately 10-50% of the cells should exhibit this staining pattern with the uninfected cells staining 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 reaction, do not report test results.

SENSITIVITY CONTROL

A titrated 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 Adenovirus 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 Adenovirus.

A sample is considered negative for Adenovirus 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 Adenovirus.

- ... Negative samples may exhibit fluorescent staining of the infected cells 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 viral 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 Adenovirus antibodies if well defined specific fluorescent staining is observed in the Adenovirus infected cells at an intensity of 1+ or greater. The Adenovirus fluorescent staining pattern is both nuclear and cytoplasmic with more apparent intranuclear inclusions seen at the periphery of the nucleus. The number of cells exhibiting a positive staining reaction and the type of fluorescent staining pattern should closely approximate that seen in the positive control.

A sample is considered positive for Adenovirus antibodies if it exhibits the characteristic Adenovirus 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 cytoplasm, an autoimmune staining reaction due to the presence of autoantibodies should be considered.^{12,13} 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 entitled "TROUBLESHOOTING IN IMMUNOFLUORESCENCE".

INTERPRETATION OF RESULTS

Detection of the presence of Adenovirus antibodies indicates a current or previous infection with Adenovirus. A significant (fourfold or greater) increase in titer between the acute and convalescent serum samples usually indicates evidence of a recent or active infection.

LIMITATIONS OF THE PROCEDURE

1. Adenovirus 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 Adenovirus should not be used as the only criterion for diagnosis. Paired serum samples (acute and convalescent) 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 that an acute phase specimen was obtained too late.
5. In some instances, high IgG 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 of specimens from immunosuppressed patients and pregnant women may be difficult to interpret.
7. Positive test results may not be valid in persons who have received blood transfusions or various blood products within the past several months.
8. Antinuclear antibodies (ANA) present in serum may interfere with the Adenovirus IFA test. They can be differentiated from Adenovirus staining in that ANAs will stain the nuclei of all cells; whereas, Adenovirus antibodies will exhibit staining only in the 10-50% infected cells.¹²

LIMITATIONS OF THE PROCEDURE (continued)

9. Cytoplasmic fluorescence in the majority of the cells may be due to the presence of antimitochondrial antibodies (AMA) often seen in primary biliary cirrhosis.¹³ They can be differentiated from the specific antigen staining in that AMA will stain the cytoplasm of all cells; whereas, Adenovirus antibodies will exhibit staining in only the 10-50% infected cells.
10. Positive test results from cord blood or neonates should be interpreted with caution. The presence of IgG antibodies in cord blood is usually the result of passive transfer from mother to the fetus. A negative test, however, may be useful in excluding possible infection.¹⁴
11. Endpoint reactions may vary between laboratories due to differences in type or condition of fluorescence microscope employed, diluting apparatus, 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.

SPECIFIC LIMITATIONS OF THE ADENOVIRUS ASSAY

1. Due to the fact that Adenoviruses have been identified as latent viruses, recrudescence of virus can be accompanied by a significant rise in antibody titer.¹ Also, heterotypic anamnestic responses may occur.⁵ Therefore, a certain amount of caution must be applied to the significance of antibody response when associated with specific clinical symptoms. Evidence of Adenovirus infection may be coincidental to a disease caused by infection with a different agent.⁵
2. False negative results have been reported to occur due to the poor (or slow) serologic responses in infants and children. However, this may be explained by the fact that most of the serological data was collected from CF test results which has the disadvantage of low sensitivity.⁵
3. Indirect fluorescent antibody procedures used to detect the immunoglobulin G (IgG) antibody response are known to detect the common antigen of all Adenovirus types.^{5,15}

EXPECTED VALUES

Most healthy individuals already have IgG antibodies to viruses such as Adenovirus. Diseases caused by the many serotypes of this virus are common and constant exposure is reflected in measurable levels of IgG in nearly 100% of individuals. Titer levels as high as 1:320 may commonly be found in the majority of these individuals. For IFA test results to be significant a fourfold, or greater, rise in IgG titer is required to be indicative of an acute or current infection.

SPECIFIC PERFORMANCE CHARACTERISTICS

Bion ADENOVIRUS ANTIGEN SUBSTRATE SLIDES have been evaluated for the presence of specific Adenovirus antigen using commercial monoclonal antisera. A positive reaction was identified with the infected cell cultures when stained with its corresponding antisera.¹⁶ Also, there was no cross-reactivity with other specific viral or chlamydial monoclonal antisera and the Adenovirus antigen.¹⁶

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

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Bion Enterprises, Ltd.
455 State Street, Suite 100
Des Plaines, IL 60016 USA
Phone: +1-847-544-5044
Fax: +1-847-544-5051

EC REP

Obd RepS BV,
Groenenborgerlaan 16,
2610 Wilrijk, Belgium