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DIRECT IDENTIFICATION

ANTIGEN CONTROL SLIDE

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NOTE CHANGES:
Fluorescent Staining Patterns section - removed Respiratory Panel (Ad, Inf A & B, Para 1, 2, 3, RSV)
Product Availability section - removed product code QRP-6314, Respiratory Panel Antigen Control Slide

PRODUCT AVAILABILITY

For Bion Enterprises, Ltd. (Bion) Product Availability for Direct Identification Antigen Control Slides, see back page.

INTENDED USE

The Bion DIRECT IDENTIFICATION ANTIGEN CONTROL SLIDES are intended for use as quality control reagents. These slides are used to establish specificity of viral, chlamydial, or any other antigen reagents used in direct or indirect immunoassay identification systems to identify the presence or absence of a specific microbial antigen and as an indicator of fluorescence microscope performance.

SUMMARY AND EXPLANATION

Immunofluorescence techniques are increasingly being used in diagnostic virology because of the current importance of rapid results and the improved availability of commercial reagents. Either direct or indirect immunofluorescence is frequently utilized to detect viral or chlamydial antigens early in tissue culture isolation, or directly from the clinical specimens. One advantage of this method is that viral or chlamydial antigens may be demonstrated late in infection after infectious virus or chlamydial agent is no longer present or is neutralized by the patient's antibodies.[1]

It is important that the laboratory establish an appropriate method for controlling the reagents being used to identify the presence of viral or chlamydial antigens. This procedure should include the use of cells infected with the specific antigen under consideration, along with uninfected cells, if used, of the same type.[2,3,4]

Methods used for the detection of viral antigens in clinical specimens include Electron Microscopy (EM) and Immunoelectron Microscopy (IEM), Counterimmunoelectrophoresis (CIE), Immunofluorescence (IF) and Immunoperoxidase (IP) staining, Radioimmunoassays (RIA) and Enzyme-linked immunoassays (EIA), and DNA probe techniques.[5,6] Of these, EM and IEM require expensive instrumentation, considerable time and have a low sensitivity. CIE is less expensive and timely, but also has a low sensitivity. IF and IP are rapid, fairly sensitive and are best suited for the detection of antigens localized on the surface of, or within, infected cells. RIA, EIA and DNA probes, although very sensitive and used in research studies, have not been widely applied to routine diagnoses because of the limited availability of suitable commercial reagents.

PRINCIPLES OF THE PROCEDURE

Direct immunoassay identification is represented by the immunofluorescent antibody method introduced by Coons, et al and Coons and Kaplan.[7,8] It is a one-step procedure in which a specific conjugated antiserum is reacted with the antigen substrate. If the antigen is present, the conjugated antiserum will bind to it, forming a stable antigen-antibody complex having a bright apple-green fluorescence when viewed with a properly equipped fluorescence microscope.

The indirect immunoassay identification is represented by the immunofluorescent antibody method introduced by Weller and Coons.[9] It is a two-step procedure in which a specific (unconjugated) primary antiserum is reacted with the antigen substrate. If the antigen is present, the antiserum will bind to it, forming a stable antigen-antibody complex. This complex is visualized by adding a conjugated secondary antiserum which binds with the initial antigen-antibody complex resulting in a positive reaction of bright apple-green fluorescence when viewed with a properly equipped fluorescence microscope.

REAGENTS

Bion DIRECT IDENTIFICATION ANTIGEN CONTROL SLIDES are individually foil-wrapped slides with wells containing microorganisms alone or with tissue culture cells infected with a specific viral or chlamydial agent in addition to wells containing only the uninfected tissue culture cells. The infected tissue culture cells serve as a positive control, and the uninfected tissue culture cells serve as a negative control. The specific microbial antigen is identified on the product label.

STORAGE AND STABILITY

The Bion DIRECT IDENTIFICATION ANTIGEN CONTROL 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 diagnostics may perform this 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. These slides are a control reagent not to be used to test human serum.
6. The antigen control slides have been fixed and contain no detectable live viral or chlamydial agents. However, they should be handled and disposed of as any potentially biohazardous laboratory material.
7. Do not remove slides from pouches until ready for testing. Do not use if pouch has been punctured, as indicated by a flat pouch.
8. Antigen substrate slides should be brought to room temperature (20-25°C) prior to use.
9. Abnormal test results may be seen if the antigen control slides are allowed to dry during the staining procedure.
10. Refrigeration (2-8°C) of antigen control slides immediately upon arrival will insure stability until labeled expiration date.
12. Antigen control slides should not be used beyond stated expiration date.
13. Avoid microbial contamination of all reagents involved in the testing procedure or incorrect results may occur.
14. Incubation times or temperatures other than those specified may give erroneous results.
15. Reusable glassware must be washed and thoroughly rinsed free of detergents.
16. Care should be taken to avoid splashing or generation of aerosols.
17. 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.
18. 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.

PROCEDURE

The recommended procedure of the antiserum manufacturer should be observed. The following methods are general procedures. Incubation times and temperatures may vary between manufacturer. The recommended wash time will vary according to the type of antiserum being used (i.e., monoclonal or polyclonal). Detailed descriptions of immunofluorescence techniques may be found in the references listed in the bibliography.[2,3,4,10,11]

There are two general methods for fluorescence staining applicable to the Bion DIRECT IDENTIFICATION ANTIGEN CONTROL SLIDE:

A. Direct Immunofluorescence Staining Method, and
B. Indirect Immunofluorescence Staining Method.

The major procedural difference between the direct and indirect technique, is that only one antibody is required in the direct method; whereas, both a primary and secondary antibody are required in the indirect method.

MATERIALS PROVIDED

Lot Number provided on label. LOT
Bion ADENOVIRUS ANTIGEN CONTROL SLIDE; or
Bion CHLAMYDIA - INCLUSIONS ANTIGEN CONTROL SLIDE; or
Bion CHLAMYDIA - ELEMENTARY BODIES ANTIGEN CONTROL SLIDE; or
Bion CYTOMEGALOVIRUS ANTIGEN CONTROL SLIDE; or
Bion ENTEROVIRUS PANEL (COXSACKIE B5, ECHO 11, POLIO 3) ANTIGEN CONTROL SLIDE; or
Bion HERPES SIMPLEX VIRUS TYPES 1 & 2 ANTIGEN CONTROL SLIDE; or
Bion INFLUENZA A & INFLUENZA B VIRUS ANTIGEN CONTROL SLIDE; or
Bion MEASLES (RUBEOLA) VIRUS ANTIGEN CONTROL SLIDE; or
Bion MUMPS VIRUS ANTIGEN CONTROL SLIDE; or
Bion PARAINFLUENZA VIRUS TYPES 1, 2 & 3 ANTIGEN CONTROL SLIDE; or
Bion RESPIRATORY SYNCYTIAL VIRUS ANTIGEN CONTROL SLIDE; or
Bion VARICELLA ZOSTER VIRUS ANTIGEN CONTROL SLIDE.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Phosphate Buffered Saline (PBS)
2. Mounting medium
3. Pasteur pipettes and bulbs
4. Moist chamber
5. Plastic squeeze wash bottle
6. Coplin jars or staining dishes with slide rack
7. 24 x 60 mm #1 coverslips
8. Felt tip marking pen
9. Fluorescence microscope with a mercury or tungsten-halogen light source, a 390-490 nm excitation filter and 515-520 nm barrier filter, and optics to give an approximate total magnification of 200X or 250X. The excitation wavelength is 490 nm and the emission wavelength is 520 nm.

A. DIRECT IMMUNOFLUORESCENCE ASSAY

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

Fluorochrome conjugated antiserum (specific for the corresponding antigen on the Antigen Control Slide).

DIRECT TEST PROCEDURE

1. **SLIDE PREPARATION**
   Remove whichever control slides 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.

2. **CONJUGATE APPLICATION**
   Using a Pasteur pipette or dropper bottle, as available, apply one drop (20-50 µl) of the corresponding fluorochrome conjugated antiserum under evaluation to both the positive and negative wells on the control slide. Do not touch the antigen surface with the pipette or dropper tip.

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

   NOTE: 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.

4. **RINSE**
   Remove slides from the moist chamber and rinse GENTLY with PBS using a squeeze wash bottle. Do not focus PBS stream directly onto the wells. Tilt the slide down and run the PBS stream across the slide above the wells, allowing the PBS to run off the bottom edge of the slide.

5. **WASH**
   Place slides in Coplin jars or staining dishes and wash in PBS for not less than one minute or more than two minutes, agitating gently at entry and prior to removal.

6. **COVERSLIP**
   Remove slides one at a time from the PBS wash, shake off excess PBS and immediately add a small amount of mounting medium to each well. Gently coverslip the slide 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.

7. **READ**
   Examine stained slide 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 re-coverslip slide.

B. INDIRECT IMMUNOFLUORESCENCE ASSAY

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

1. Primary antiserum (specific for the corresponding antigen in the Control Slide)
2. Fluorochrome conjugated secondary antiserum (corresponding with the primary antibody)

INDIRECT TEST PROCEDURE

1. **SLIDE PREPARATION**
   Remove whichever control slides 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.

2. **PRIMARY ANTISERA APPLICATION**
   Using a Pasteur pipette or dropper bottle, as available, apply one drop (20-50 µl) of the corresponding primary antiserum under evaluation to both the positive and negative wells on the control slide. Do not touch the antigen surface with the pipette or dropper tip.

3. **INCUBATION 1**
   Incubate in a moist chamber at room temperature (20-25°C) for 30 minutes.

   NOTE: 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.

4. **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. Tilt the slide down and run the PBS stream across the slide above the wells, allowing the PBS to run off the bottom edge of the slide.

5. **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.

6. **CONJUGATE APPLICATION (SECONDARY ANTISERA)**
   Remove slides from PBS wash one at a time, shake off excess PBS, dry around the outside edges if necessary and return each slide to the moist chamber. Apply one drop (20-50 µl) of the appropriate fluorochrome conjugated secondary antiserum to each well of the appropriate slide, making sure that each well is completely covered.

7. **INCUBATION 2**
   Incubate in a moist chamber at room temperature (20-25°C) for 30 minutes. Protect slides from excessive light.
8. **RINSE 2**
   Remove slides from moist chamber and rinse GENTLY with PBS using a squeeze wash bottle. As suggested in Step 4., do not focus the PBS stream directly onto the wells.
9. **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.
10. **COVERSLIP**
    Remove slides from the last PBS wash one at a time, shake off excess PBS and immediately add a small drop of mounting media to each well. Gently coverslip the slide 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.
11. **READ**
    Examine the slide 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.

READING OF TEST RESULTS

NEGATIVE

A negative reaction in the positive well is indicated by a lack of apple-green fluorescence when viewed with a fluorescence microscope with the cells appearing reddish-orange due to the counterstain. This reaction indicates that the antisera under evaluation does not react with the cells containing the specific antigen. This reaction, when observed in the negative, uninfected cell culture well, indicates that the antiserum under evaluation does not react nonspecifically with the cells containing the specific antigen.

POSITIVE

A positive reaction is indicated by the appearance of specific apple-green fluorescent antibody staining patterns in the infected cells when viewed with a fluorescence microscope. This reaction, when observed in the positive, infected cell culture well, indicates that the antiserum under evaluation has reacted specifically with its target antigen. In the negative well, the uninfected cell cultures should appear reddish-orange due to the counterstain indicating that the antisera under evaluation does not react nonspecifically with the cells containing the specific antigen.

NOTE: As each positive well contains 10-50% infected cells mixed with uninfected cells, only 10-50% of the cells should exhibit the specific fluorescent antibody staining pattern, with the uninfected cells appearing reddish-orange due to the counter-stain.

Each control must demonstrate the expected reaction in order to validate the test. Descriptions of fluorescent antibody staining patterns that are observed with some monoclonal antiserums can be found in TABLE 1 - FLUORESCENT ANTIBODY STAINING PATTERNS IN DIRECT IDENTIFICATION ANTIGEN CONTROL SLIDES. These patterns may vary with different manufacturers' antiserums. If the controls fail to react as described, 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.

TROUBLESHOOTING

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

LIMITATIONS OF THE PROCEDURE

1. Bion Direct Identification Control slides are prepared using defined viral or chlamydial strains. Monoclonal antibody reagents are also prepared using defined viral or chlamydial strains and may not detect, or detect with less sensitivity, all antigenic variants.
2. Intensity of reaction may vary between laboratories due to differences in type or condition of fluorescence microscope employed, assay procedure used, as well as the experience level of personnel performing the assay.
3. 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 or check the specific filters being used.
4. Control wells may exhibit nonspecific fluorescence due to contamination of the antiserum or PBS rinse-wash solutions with bacteria or fungi. It is very important that personnel reading the staining results have experience in fluorescence microscopy.
5. The longer wash times used with polyclonal antibodies may yield falsely negative results when used with monoclonal antibodies. The recommended procedure of the antiserum manufacturer must therefore be strictly adhered to.
6. Inherent in the manufacture of these products, an occasional mix of material may occur (i.e. a few positive cells in the negative well). As a reagent control, these slides will still indicate the specificity of monoclonal antibody reagents and the performance of fluorescence microscopes.

SPECIFIC PERFORMANCE CHARACTERISTICS

Bion DIRECT IDENTIFICATION ANTIGEN CONTROL SLIDES have been evaluated for the presence or absence of specific microbial antigens in positive and negative cell cultures using commercial monoclonal and/or polyclonal antisera. In each case, positive reactions were identified with the specific antigen when stained with its corresponding antibody. There was no cross-reactivity observed between the antigen infected or uninfected cell cultures when they were reacted with monoclonal or polyclonal antisera not specific for that particular antigen.[12]

BIBLIOGRAPHY

1. Hanson, C.V., Immunofluorescence and Related Procedures, in Laboratory Diagnosis of Viral Infections, Lennette, E.H. (ed), Marcel Dekker, Inc. New York, 1985.
2. Gardner, P.S., J. McQuillin, Rapid Virus Diagnosis; Application of Immuno-fluorescence, Ch. 4, Butterworths, London, 1980.
3. Gardner, P.S., Immunofluorescence, in Clinical Virology Manual. Specter, S., G. Lancz, (eds), Elsevier, New York, 1986.
4. Lyerla, H.C., F.T. Forrester, Immunofluorescence Methods in Virology. U.S. Dept. of Health and Human Services, Atlanta, Georgia, 1979.
5. Lennette, E.H., D.A. Lennette, Approaches to Diagnosis of Viral Diseases, in Immunologic Analysis; Recent Progress in Diagnostic Laboratory Immunology. Nakamura, R.M., W.R. Dito, E.S. Tucker, (eds), Masson Publishing, Inc., New York, 1982.
6. Koprowski, H., Monoclonal Hybridoma Antibodies in the Diagnosis of Rabies, in Immunologic Analysis; Recent Progress in Diagnostic Laboratory Immunology. Nakamura, R.M., W.R. Dito, E.S. Tucker, (eds), Masson Publishing, Inc., New York, 1982.
7. Coons, A.H., H.J. Creech, R.N. Jones, E. Berliner, The Demonstration of Pneumococcal Antigen in Tissues by the Use of Fluorescent Antibody, J. Immunol., 45:159, 1942.
8. Coons, A.H., M.H. Kaplan, Localization of Antigen in Tissue Cells, J. Exp. Med., 91:1, 1950.
9. Weller, T.H., A.H. Coons, Fluorescent Antibody Studies With Agents of Varicella and Herpes Zoster Propagated In Vitro, Proc. Soc. Exp. Biol. Med., 86:789, 1954.
10. Goldman, M., Fluorescent Antibody Methods. Academic Press, New York, 1968.
11. Minnich, L.L., T.F. Smith, G.C. Ray, Rapid Detection of Viruses by Immuno-fluorescence, Cumitech 24, Specter, S. (ed), ASM, Washington, D.C., 1988.
12. Data on file, Bion, Des Plaines, IL.

TABLE 1 - Fluorescent Antibody Staining Patterns in Direct Identification Antigen Control Slides

NOTE: Fluorescent staining patterns obtained from the use of specific monoclonal antisera may vary somewhat from manufacturer to manufacturer and, therefore, may not correspond exactly to the description given below.

ANTIGEN	FLUORESCENT PATTERNS IN INFECTED CELLS
Adenovirus	Individual cells with intensely stained nuclear speckles and somewhat weaker cytoplasmic staining.
Chlamydia, LGV-1 (Inclusions)	Staining of intracytoplasmic inclusion bodies near nucleus and pinpoint Elementary Bodies on and between cells.
Chlamydia (Elementary Bodies)	Staining of pinpoint Elementary Bodies on and between cells.
Cytomegalovirus	Cells with staining of intranuclear inclusions (Early Antigen) surrounded by cells with diffuse homogeneous nuclear staining of varying intensity (Immediate Early Antigen).
Herpes Simplex Type 1	Groups of cells with large intranuclear inclusion staining; or, groups of cells with web-like membranous cytoplasmic staining; or with both membranous cytoplasmic and nuclear staining.
Herpes Simplex Type 2	Groups of cells with large intranuclear inclusion staining; or, groups of cells with web-like membranous cytoplasmic staining; or with both membranous cytoplasmic and nuclear staining.
Influenza A	Speckled staining in the nucleus alone or in both the nucleus and cytoplasm.
Influenza B	Speckled staining in the nucleus alone or in both the nucleus and cytoplasm.
Measles Virus (Rubeola)	Cytoplasmic granules in small cell patches or multinucleated giant cells.
Mumps Virus	Fine and coarse staining of cytoplasmic particles in small patches of individual cells.
Parainfluenza Type 1	Fine and coarse staining of cytoplasmic speckles scattered throughout the cell sheet without appearing to alter the cell morphology.
Parainfluenza Type 2	Staining of coarse speckles in cytoplasm contained in cellular plaques.
Parainfluenza Type 3	Fine and coarse staining speckles in cytoplasm appearing like dust across the cells without disturbing the cell morphology.
Poliovirus Type 3	Solid staining of the entire cell, to speckly staining of some cells, to just the rim of the cell staining.
Respiratory Syncytial Virus	Particulate cytoplasmic staining of infected cells in small syncytial masses.
Varicella Zoster Virus	Cellular patches of fine, webby membranous cytoplasmic staining.

PRODUCT AVAILABILITY

The following DIRECT IDENTIFICATION ANTIGEN CONTROL SLIDES are available individually from Bion Enterprises, Ltd. (Bion):

Antigen Control Slides	Code No. REF
Adenovirus	QAD-3102
* Chlamydia - Inclusions	QCH-4102
* Chlamydia - Elementary Bodies	QCHE-4502
* Cytomegalovirus	QCM-2002
Enterovirus Panel including Coxsackie B5, Echovirus 11 and Poliovirus Type 3	QEP-6604
Herpes Simplex Virus Types 1 and 2	QHS-3504
Influenza A and Influenza B Virus	QAB-1504
Measles (Rubeola) Virus	QME-6002
Mumps Virus	QMU-8002
Parainfluenza Virus Types 1, 2 and 3	QP-8506
Respiratory Syncytial Virus	QRS-9002
Varicella Zoster Virus	QVZ-7002

* Not Marked

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