Indirect Fluorescence Assay for Human Herpesvirus 6B (HHV-6B) IgG and IgM Antibodies
Product Number: I-H6B12

INTENDED USE

BIOCELL DIAGNOSTICS, INC.'s Indirect Immunofluorescence Assay (IFA) for Human Herpesvirus 6B (HHV-6B) IgG and IgM Antibodies is intended for the qualitative and semi-quantitative detection of IgG (Immunoglobulin G) and IgM antibodies to HHV-6B in human serum or plasma. Detection of HHV-6B IgG and IgM antibodies in humans can be used as an aid in the determinations of primary infection, reactivation and/or reinfection with this virus.

SUMMARY AND EXPLANATION OF TEST

Human herpesvirus 6 (HHV-6), known as one of the eight known herpesviruses that infect humans, is a double stranded DNA virus. HHV-6 is now classified into two viruses: HHV-6A and HHV-6B (1). HHV-6A, originally named human B-lymphotropic virus (HBLV), is the type species for Roseolovirus which was first isolated from immunosuppressed individuals and patients with lymphoproliferative disorders (1, 2-4).

HHV-6A is more neurovirulent, and as such is more frequently found in patients with neuroinflammatory diseases such as multiple sclerosis (4, 5). HHV-6B has been identified as the causative agent of roseola infantum, also known as exanthem subitum. The virus has been isolated from children with exanthem subitum and the association further supported by serologic studies (9-11). Roseola infantum is considered a benign non-seasonal disease of childhood with clinical indications of rash and fever. Infants are protected by maternal antibodies so that infection is most frequent between 6 and 18 months of age. Although IgM antibodies of HHV-6A and HHV-6B are cross-reactive against each other, serological information is critical in diagnosis since detection of IgM antibodies by the IFA technique is both sensitive and specific, without cross-reaction to other human herpesviruses (4, 7, 12-15).

HHV-6 is genetically and serologically distinct from the other known human herpesviruses (3, 4, 6). Serological studies, using the indirect fluorescence assay (IFA), have shown that antibody to the virus is often found in the general population. Primary infection usually occurs before the age of two. A high percentage of humans above the age of one are positive for anti-HHV-6 antibody (6-8). Primary infection in adults is rare because of the high rate of infection in childhood. While primary infection in healthy adults usually results in mild illness, more serious complications can arise in immunocompromised patients or patients undergoing organ or bone marrow transplantation (6).

PRINCIPLE OF THE TEST

BIOCELL DIAGNOSTICS, INC.'s immunofluorescence assays use the indirect method of antibody detection and titer determination. Patient serum or plasma samples are applied to the cultured cells containing inactivated HHV-6B virus provided on microscope slide wells. A 30 to 60 minute incubation allows any specific antibody in the sample to react and form an antigen-antibody complex with HHV-6B viruses in the infected cells. In a brief washing step, nonspecific antibody and other unreacted serum proteins are eliminated. Fluorescein-conjugated goat anti-human IgG or IgM is then applied. The anti-human IgG or IgM conjugate combines with human IgG or IgM, if present, during a 30 minute incubation. After a brief wash to remove unreacted conjugate, the slides are viewed by fluorescence microscopy. A positive antibody reaction is denoted by bright green fluorescence at the antigen sites. Semi-quantitative detection occurs by testing the serial dilutions of positive samples and determining the endpoint titers.

MATERIALS SUPPLIED AND STORAGE CONDITIONS

AG SLD — **HHV-6B Antigen Slides (REF: I-H6B12):** Slides containing human lymphocytes infected with HHV-6B on each glass well. The slides are ready for use after removal from the protective pouch. Slides are stable until the expiration date stated on the pouch label when stored at 2-8°C.

ADDITIONAL MATERIALS / EQUIPMENT REQUIRED AND STORAGE CONDITIONS

POS — HHV-6 IgG positive control (REF: P-HV6G), IgM positive control (REF: P-HV6M). Pre-diluted and ready to use. 0.5 mL dropper vial with red cap. Contains preservatives. Stable at 2-8°C until date stated on the label.

NEG — Universal negative control (REF: N-UNIR). Pre-diluted and ready to use. 0.5 mL dropper vial with green cap. Contains preservatives. Stable at 2-8°C until date stated on the label.

PROS — IgG removal reagent for IgM test (REF: M-PROS). 2.0 mL vial. Contains preservatives. Stable at 2-8°C until date stated on the label.

FITC — FITC conjugate: Fluorescein labeled goat anti-human IgG (heavy and light chain specific) (REF: F-COG3) or goat anti-human IgM (μ) (REF: F-COM3). Ready to use. Contains Evan's Blue counterstain, protein stabilizer and preservatives. 1.5 mL dropper vial with yellow cap. Stable when stored in dark at 2-8°C until date stated on the label.

PBS — Phosphate buffered saline (PBS) (REF: M-PBS1). pH 7.4 ± 0.2. Reconstitute packet contents in 1 liter distilled or deionized water. Mix until all salts are thoroughly dissolved and store at 2-30°C until date stated on the label.

MM — Mounting medium (REF: M-GLY1). 2.0 mL dropper vial with white cap. Store at 2-30°C until date stated on the label.

BLT — 12 well 5 mm blotters (REF: M-BLT1). Store at room temperature.

1. Coverslips: 24 X 60 mm, No. 1 thickness glass
2. Distilled or deionized water
3. Test tubes, racks, pipettes, microtiter plates and safety pipetting devices for making sample dilutions

4. Graduated cylinder, 1L
5. 37°C incubator
6. Moist chamber for incubating slides
7. Slide staining dish with slide holder
8. Plastic wash bottle
9. Disposal basin and disinfectant (i.e., 10% bleach solution)

Fluorescence microscope: A fluorescence microscope equipped with the following was used to calibrate the controls and conjugate:

- 20X or 40X objectives
- Light source: 50W Mercury vapor
- Excitation filter: 480 / 40 (EX)
- Dichroic (Barrier) filter: 510 Long Pass (BS)
- Emission filter: 515 Long Pass (EM)

The fluorescein label has an excitation peak of approximately 480 nm and an emission peak of approximately 515 nm. Differences in endpoint reactivities and fluorescence intensities may be due to the type and condition of the fluorescence equipment used in your laboratory.

PRECAUTIONS

IFA Test: No US Standard of Potency. For Research Use Only.

- Follow normal precautions when handling laboratory reagents. Wear suitable protective clothing, gloves, and eye protection. Dispose of waste per all local, state, and federal laws.
- The slide wells do not contain viable organisms; however, consider the slides **potentially bio-hazardous material** and handle accordingly.
- The controls are **potentially bio-hazardous materials**. Source materials from which these products were derived were found negative for HBsAg and antibodies to HIV-1/2 and HCV by FDA-approved test methods.
- Evan's Blue is a carcinogen. Avoid contact with skin or eyes.
- Some components contain less than 0.1% sodium azide (w/v) which is toxic if ingested and forms potentially explosive copper and lead azide compounds in laboratory plumbing. To prevent, rinse sink thoroughly with water after disposing of solutions containing sodium azide.
- Cross-contamination of patient samples or reagents on a slide can cause erroneous results.
- Avoid microbial contamination of serum samples or reagents as erroneous results may occur.
- Collect the wash solution in a disposal basin. Treat the waste solution with disinfectant (i.e., 10% bleach solution).
- Store all components at their recommended or suggested temperatures. Do not freeze unless otherwise stated.
- Do not use the components beyond the stated expiration date of each component.
- Allow reagents to warm to room temperature before use.
- Do not remove the slides from their protective pouch until ready for use.

SPECIMEN COLLECTION AND STORAGE

Blood samples should be collected aseptically by qualified personnel. Allow blood samples to clot at room temperature prior to centrifugation. Hyperlipemic, hemolyzed or contaminated serum may cause erroneous results and should not be used. Store the collected serum or plasma samples in a tightly closed sterile container at 2-8°C, no longer than 5 days. If there's a delay in testing, store the samples at -20°C or colder. Avoid repeated freezing and thawing. Paired serum samples to demonstrate seroconversion or significant titer increase should be collected 7-14 days apart, stored and then tested simultaneously.

TEST PROCEDURE

1. Remove slides and reagents from refrigerated storage and allow to reach room temperature before opening. Keep conjugate stored in the dark at room temperature during testing.
2. For qualitative IgG antibody determination, prepare a 1:40 screening dilution of each test sample in PBS buffer (e.g.: 5 µL of serum sample + 195 µL PBS). Prepare all dilutions with a suggested minimum total volume of 100 µL with PBS buffer as the diluent.
3. For qualitative IgM antibody determination, prepare a 1:10 screening dilution of each test sample in IgG removal reagent (e.g.: 10 µL of serum sample + 90 µL IgG removal reagent) and mix well. Incubate mixture at room temperature for 5 - 10 minutes. Precipitation may occur but will not affect the IFA test results.
4. For semi-quantitative titration of sera, prepare further two-fold serial dilutions of the IgG and IgM sera samples with PBS as the diluent.
5. Positive and negative controls are ready to use and represent screening dilutions of 1:40 for IgG and 1:10 for IgM. The ready to use IgM positive control does not need treated with the IgG removal reagent.
6. Apply approximately 15- 20 µL of the diluted test sample(s) to each well. Add sufficient volume to completely cover each well, but cross-mixing of contents between wells should not occur.
7. Incubate the slides at 37°C in a moist chamber for 30 minutes for an IgG test and 60 minutes for an IgM test.
8. Rinse the slides gently in a light stream of PBS buffer. Avoid directing the stream at the wells.
9. Wash the slides for 10 minutes in PBS buffer with a change of buffer after 5 minutes. Agitate the slides by moving the rack up and down in the buffer.
10. Blot the mask surrounding the test wells using care not to touch the test well.
11. Apply one drop of conjugate to each test well. Incubate the slides at 37°C in a moist chamber for 30 minutes.
12. Repeat steps 8 (PBS buffer rinse), 9 (10-minute PBS buffer wash) and 10 (blot).
13. Apply a drop of mounting medium to each well and cover with a glass coverslip, avoiding air bubbles.
14. Observe the reactivity under fluorescence microscopy using 200 - 400X magnification. For best results, examine slides immediately after completion of the test. If this is not possible, store in the dark at 2-8°C and read within 24 hours. Allow refrigerated slides to warm before reading.

QUALITY CONTROL

1. To ensure the test is working properly, use a positive and negative control at least once for each day's testing.
2. Each day's test should include one PBS buffer well in place of a test sample. This is a conjugate control to ensure the conjugate is not reacting with the cell substrate.
3. The positive control must exhibit a 2+ to 4+ fluorescence at the screening dilution. The IgG positive control should give an endpoint titer of 1:640. The IgM positive control endpoint titer is expected to be 1:40-80.
4. The negative control must exhibit the absence of green fluorescence staining. Fluorescence that does not match the morphology and distribution of the positive control is considered non-specific.
5. If either of the controls does not react as specified, the test should be considered invalid, and the assay repeated.

INTERPRETATION OF RESULTS

Differences in endpoint reactivities and fluorescence intensities may be due to the type and condition of the fluorescence equipment used in your laboratory. Read control wells first during each run to ensure correct interpretation. It is best to test the positive control at the noted endpoint as well as the two-fold dilution immediately preceding and following the listed titer. It is normal for results obtained for an endpoint (1+) titer to differ between laboratories due to factors affecting the intensity of fluorescence.

Positive reactivity may range in fluorescence intensity from brilliant to weak. Grade the fluorescence reaction according to the following intensity scale: 4+ (brilliant), 3+ (bright), 2+ (moderate), 1+ (dim) or - (absence of specific fluorescence).

Bright green fluorescent staining of the infected cells denotes an HHV-6B antibody positive reaction. The fluorescent staining pattern of HHV-6B infected cells is variable. Depending on the cell's stage of infection, the fluorescent pattern can vary from a small portion of the infected cell fluorescing to the whole cell fluorescing. Fluorescence can also range from granular to homogeneous. To provide an internal control, each well on the microscope slide contains HHV-6B infected and uninfected cells. Preparation of the slide in this manner is intentional. Uninfected cells, stained red by the counterstain, provide a contrasting background. Infectivity of the cells ranges from 20% - 50% for IgG and 10 - 30% for IgM. Titration of positive IgG or IgM samples will provide semi-quantitative information. In a titration series, the highest serum dilution demonstrating a 1+ reaction is interpreted as the endpoint. Absence of specific fluorescent staining of the infected cells denotes an antibody negative reaction.

1. No discernible fluorescence of the infected cells found at the screening dilution.	1. Test sample is HHV-6B IgG and/or IgM antibody negative.
2. Specific positive fluorescence of the infected cells found at the screening dilution or at higher dilutions.	2. Test sample is HHV-6B IgG and/or IgM antibody positive. IgM antibody positive and a four-fold or greater rise in IgG antibody titer in paired serum samples indicate recent infection with HHV-6B. IgG antibody positive only indicates previous infection or seroconversion.
3. Fluorescence found in both infected and uninfected cells.	3. Test sample is exhibiting a nonspecific reaction.

LIMITATIONS OF THE PROCEDURE

1. A serological test such as the IFA serves as an aid to detect HHV-6Binfection, but its use should not be the sole criterion. The test results should be used in conjunction with information available from the patient, clinical evaluation and other available diagnostic procedures.
2. A single positive result for HHV-6B IgG antibody is significant only in that it indicates previous contact or infection with HHV-6B. For epidemiological purposes a single result is useful. It should not be used, however, as an indication of current or recent infection with HHV-6B. To determine current or recent infection, simultaneous testing of paired specimens of plasma or serum taken 7-14 days apart should be done. A four-fold or greater rise in titer between the first and second sample is indicative of a current or recent infection.
3. In some cases, a maximal IgG antibody titer may have been reached during the time of initial sample collection, and an increase in titer will not be observed.
4. A sample obtained too early during infection may not contain detectable levels of IgM antibody. If an active infection is suspected, a second sample should be obtained 7-14 days later. The second sample should be tested concurrently with the first specimen to look for seroconversion or a significant rise in titer of viral-specific IgM or IgG. Either seroconversion or significant rise in titer is indicative of primary infection.
5. BIOCELL DIAGNOSTICS, INC. recommends pretreatment of test samples to remove IgG antibody when testing for IgM antibody. This additional step helps eliminate false negative and false positive results. When IgG antibody competes with IgM antibody for specific binding sites, IgG antibody can cause a false negative result. When IgG antibody forms immune complexes with the antigenic substrate that may then bind rheumatoid factor (IgM class), IgG antibody can cause a false positive result.
6. A negative result does not exclude HHV-6A infection. An additional sample should be tested in 4-6 weeks if early infection is suspected.
7. Nonspecific positive reactions such as antinuclear antibody and/or anticytoplasmic antibody reactions can occur in samples from patients with certain autoimmune diseases. Both infected and uninfected cells will fluoresce, and this may obscure a positive HHV-6A reaction. Therefore, observation of an autoimmune reaction cannot eliminate the possibility of HHV-6B infection.

EXPECTED VALUES

A high percentage of humans above the age of two are seropositive for anti-HHV-6B antibody (6-8). Studies using the IFA method indicate prevalence rates of 80-95% in older children and adults in the general population. No significant differences in prevalence rates due to geographical location have been reported.

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