

**Cat No:** V3HHV6  
**Format:** 4 x 10 well slides



**Human Herpesvirus-6  
IgG Immunofluorescent Assay**

An Immunofluorescent assay for the detection of Human Herpesvirus-6 IgG antibodies

**FOR RESEARCH USE ONLY  
NOT FOR USE IN  
DIAGNOSTIC PROCEDURES**

## Table of Contents

Intended Use

Introduction

Assay Principle

Precautions

*Safety*

*Procedural*

Kit Components

*Materials Required*

*Additional Materials Required*

Storage and Stability

Specimen Collection and Storage

Reagent and Specimen Preparation

Assay Procedure

Interpretation of Results

Significance of Interpretation

Quality Control Criteria

Expected Values

Limitations of Use

Summary of HHV-6 IgG IFA Procedure

Bibliography

Interpretation of Symbols

## **Intended Use**

The Biotrin Human Herpesvirus-6 IgG IFA is intended for use as an *in vitro* Immunofluorescent assay for the detection of Human Herpesvirus-6 (HHV-6) IgG antibodies in serum.

## **Introduction**

Human herpesvirus-6 (HHV-6), first described in 1986, was isolated from patients with lymphoproliferative disorders<sup>1</sup>. Subsequently, HHV-6 has been confirmed as the aetiological agent responsible for the childhood disease exanthem subitum (Roseola infantum)<sup>2</sup>, and has been associated with a number of other disease manifestations in children, including fulminant hepatitis<sup>3</sup>, encephalitis<sup>4</sup>, histiocytic necrotising lymphadenitis<sup>5</sup> and fatal disseminated infection<sup>6</sup>.

In adults, primary infection with HHV-6 is less common, with documented evidence showing that HHV-6 may be involved in cases of hepatitis<sup>7</sup>, mononucleosis-like illness<sup>8</sup>, atypical polyclonal lymphoproliferation<sup>9</sup>, 'post-viral chronic fatigue syndrome'<sup>10</sup>, multiple sclerosis<sup>11</sup>, oral carcinoma<sup>12</sup>, cervical carcinoma<sup>13</sup> and bone marrow suppression in bone marrow transplant patients<sup>14</sup>.

Specific virological and serological tests found HHV-6 to be ubiquitous in the human population, with infection typically occurring during early infancy leaving few adults still susceptible to primary infection. The antibody prevalence is reported as greater than 80% in patients greater than 2 years of age<sup>15</sup>. However, although the prevalence of HHV-6 antibody is high, the level of antibody diminishes to low titers following infection. High levels of anti-HHV-6 IgG antibody in serum may act as an indicator of recent exposure to HHV-6.

## **Assay Principle**

The Biotrin HHV-6 IFA system utilises the indirect immunofluorescent method of antibody detection and titer determination. Patient serum samples are incubated with immobilised HHV-6 antigen, which has been stabilised on a glass slide. If HHV-6 IgG antibodies are present in the sample; a stable complex is formed with the antigen. Bound antibody is then reacted with a fluorescein conjugated goat anti-human IgG and this complex is visualised with the aid of a fluorescence microscope. A positive antibody reaction is denoted by bright green fluorescence.

## **Precautions**

### *Safety*

- FOR RESEARCH USE ONLY - NOT FOR USE IN DIAGNOSTIC PROCEDURES
- This kit is intended for use by qualified laboratory staff only.
- The kit contains materials of human origin, which are considered POTENTIALLY BIOHAZARDOUS MATERIAL. The Controls have been tested and found to be negative for HBsAg and antibodies to HIV 1/2 and HCV. However, because no test method can offer complete assurance of the absence of virus, treat all controls as potentially infectious.
- Some reagents contain Thiomersal, which may be toxic if ingested.
- Avoid contact with Evans Blue as it is a potential carcinogen. If skin contact occurs, flush with large volumes of water.
- Some reagents contain sodium azide, which may form potentially explosive metal azides with lead and copper plumbing. For disposal, reagents should be flushed with large volumes of water to prevent azide build up.
- Dispose of all clinical specimens, infected or potentially infected material in accordance with good laboratory practice. All such materials should be handled and disposed as though potentially infectious.
- Residues of chemicals, preparations and kit components are generally considered as hazardous waste. All such materials should be disposed of in accordance with established safety procedures.
- Wear protective clothing, disposable latex gloves and eye protection while handling specimens and performing the assay. Wash hands thoroughly when finished.
- Do not pipette materials by the mouth and never eat or drink at the laboratory workbench.

### *Procedural*

- Do not use kit or individual reagents past their expiry date.
- Do not mix or substitute reagents from different kit lot numbers.
- Do not use contaminated samples or reagents.
- Deviation from the protocol provided may cause erroneous results.
- Performing the assay outside the time and temperature ranges provided may produce invalid results. Assays not falling within the established time and temperature ranges must be repeated.

- High quality distilled or deionised water is required for the Wash Buffer Concentrate. The use of poor quality or contaminated water may lead to background. Ensure Wash Buffer Concentrate is mixed thoroughly.
- Allow all reagents to come to room temperature (20-25°C) and mix well prior to use.
- Do not remove the slides from their protective pouch until ready to use. Allowing the slides to equilibrate to room temperature prior to opening the protective pouch will protect the contents from condensation.
- Avoid leaving reagents in direct sunlight and/or above 2-8°C for extended periods.
- When staining multiple samples on a slide avoid cross contamination between samples by marking between wells with a wax pencil.
- Application of excess Mounting Media may cause blurred fluorescence.
- Always use clean, preferably disposable, glassware for all reagent preparation.
- Care must be taken not to contaminate components and always use fresh pipette tips for each sample and component.
- Do not scratch the well with the pipette tip or dropper.
- Before commencing the assay, an identification and distribution plan should be established.

## Kit Components

### *Materials Provided*

1. HHV-6 Antigen Slides:

**SLIDE**

4 x 10 well slides to which human lymphocytes infected with HHV-6 have been stabilised. The slides are ready for use after removal from protective pouch.

2. Positive Control \*\*:

**CONTROL + IgG**

1 x 0.5 mL HHV-6 IgG antibody positive human control. Contains 0.1% Sodium Azide.(Ready-To-Use) (Blue Cap)

3. Negative Control \*\*:

**CONTROL - IgG**

1 x 0.5 mL HHV-6 IgG antibody negative human control. Contains 0.1% Sodium Azide. (Ready-To-Use) (Red Cap)

4. Fluorescein Conjugate:\*\*

**CONJ ENZ 1X**

1 x 1.5 mL fluorescein conjugated goat (inactivated) antihuman IgG (heavy and light chain specific) with Evans Blue and Rhodamine counterstains. Contains 0.1% Sodium Azide.(Ready-To-Use) (Yellow Cap)

5. Mounting Media:

**MM**

1 x 2 mL Tris buffered glycerol. Contains Thiomersal (0.01%). (Ready-To-Use) (Orange Cap)

6. Wash Buffer Concentrate (PBS):

**BUF WASH CONC**

1x Sachets. Each aluminium-sealed packet contains 10 PBS tablets. Each tablet makes up 100 mL of 1x Wash Buffer.

7. Slide Blotters:

**BLT**

Absorbent blotters have pre-cut holes for use in drying the slide mask.

8. Instructions for Use:



\*\* Potentially Biohazardous Material

### **Additional Materials required**

- Serum collection equipment
- Slide holder rack and staining dish for washing slides
- High quality distilled or deionised water
- Clean volumetric labware
- Test tubes or equivalent for sample preparation
- Graduated cylinders
- Accurate pipettes, micropipettes and disposable tips to deliver 5 uL to 50 uL and 50 uL to 200 uL
- Timer
- 35-39°C incubator
- Paper towels or absorbent paper
- Dilution tubes and minifuge tubes (0.5 mL)
- Benchtop minifuge
- Incubation tray containing moistened tissue paper
- Wash bottles and wash tray
- Coverslips: 22 X 50mm No. 1 thickness
- Fluorescence microscope with appropriate filter combination for FITC (excitation filter 495nm, barrier filter 515nm), a halogen light source is recommended
- Wax pencil

### **Storage and Stability**

- The kit is stable until the expiry date indicated on the outer box label, provided it is stored between 2-8°C. Note: The blotters may be stored between 2-25°C.
- All unused components should be returned to 2-8°C storage immediately after use.
- Reconstituted Wash Buffer is stable for up to 4 weeks when stored at 2-8°C.

### **Specimen Collection and Storage**

- Samples should be obtained using aseptic laboratory techniques. Samples can be stored for up to 1 week at 2-8°C and at -20°C for longer periods. Repeated freezing and thawing should be avoided.
- Paired serum samples collected over a period of time to demonstrate seroconversion or significant titer increase should be collected 7-14 days apart and stored at -20°C. These samples should then be tested simultaneously.

### **Reagent and Specimen Preparation**

#### ***Reagent Preparation***

Prepare PBS by adding 1 tablet to 100 mL freshly prepared distilled or deionized water. Store in a clean, closed container at 2-8°C for up to 4 weeks. All remaining reagents are supplied ready to use and at working dilution.

#### ***Specimen Preparation***

**Qualitative Test:** Dilute each serum sample 1:20 in PBS. All dilutions should be at a minimum volume of 0.1 mL.

**Quantitative Test:** The sample "titer" can be determined by preparing two-fold serial dilutions of the sample in Wash Buffer, starting with a 1:20 dilution, and adding equal volumes of diluted sample and Wash Buffer for each consecutive dilution, until a "+1" grade of fluorescence is achieved (See "Interpretation of Results").

## Assay Procedure

**Allow all components to equilibrate to room temperature (20-25°C) before use.**

### 1. Slide Preparation

Remove desired number of slides from the protective pouch and mark between the wells with a wax pencil to avoid contamination. Dispense 1 drop (approx. 20 uL) of each diluted test sample and 1 drop (approx. 20 uL) of the ready to use Positive and Negative Controls onto numbered wells.

Note: Add sufficient volume to cover each well, but avoid cross mixing of contents between the wells.

### 2. Incubate the Samples

Incubate slide in moist chamber for 30 minutes at 35-39°C.

### 3. Wash the Slide

Rinse slides along the edge in a light stream of Wash Buffer using a wash bottle. Avoid directing the stream at the wells. Place slides in a wash tray containing Wash Buffer for 10 minutes at room temperature (20-25°C) with a change of Wash Buffer after 5 minutes. Blot the paint mask surrounding the test wells with the blotters provided.

### 4. Incubate with Conjugate

Apply 1 drop (approx. 40 uL) of the ready-to-use Conjugate to each test well. Incubate the slides in a moist chamber for 30 minutes at 35-39°C.

### 5. Wash the Slide

Repeat Step 3.

### 6. Apply Mounting Media

Apply 1 small drop of the Mounting Media to the centre of each well and apply a cover slip.

### 7. Examine the Slide

Examine under a fluorescence microscope using 200-500x magnification. For best results, examine slides immediately after completion of the test. (To obtain equivalent results, seal slides or keep humidified to minimise dehydration of Mounting Media. Store in dark at 2-8°C. Read within 3 days.)

### 8. Grading

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 (weak).

## Interpretation of Results

### *Negative Reaction:*

A sample is considered negative for HHV-6 IgG antibodies if fluorescent staining of the infected cells is absent.

### *Positive Reaction:*

A HHV-6 antibody positive reaction is denoted only when bright green fluorescence is observed in the infected cells at a dilution  $\geq 1:20$ . A positive reaction indicates previous HHV-6 infection. Seroconversion to IgG antibodies or a four-fold increase or greater rise in IgG antibody titer in paired serum samples indicates recent infection with HHV-6.

+4 = Brilliant green fluorescence indicating very high titer HHV-6 IgG antibody response.

+3 = Bright green fluorescence indicating high titer HHV-6 IgG antibody response.

+2 = Green fluorescence indicating medium titer HHV-6 IgG antibody response.

+1 = Dull green fluorescence indicating weak titer HHV-6 IgG antibody response. This also indicates the end-point dilution or “titer” of the sample.

- Titration of HHV-6 IgG positive samples provides quantitative information. In a titration series, the highest serum dilution demonstrating a “+1” reaction is interpreted as the end-point titer.

- To provide an internal control, each well on the microscope slide contains both HHV-6 infected and uninfected cells. Preparation of the slide in this manner is intentional. Uninfected cells, stained red by the counterstain, provide a contrasting background. These are added as controls for anti-nuclear and/or anticytoplasmic antibody reactions, which may be seen in patients with auto-immune diseases.

### **Significance of Interpretation**

No discernible fluorescence of the infected cells found at the screening dilution.	Test sample is HHV-6 IgG antibody negative.
Random green cells displaying no discernible fluorescence of the infected cells.	Test sample is HHV-6 IgG antibody negative
Specific positive fluorescence of the infected cells found at the screening dilution or at higher dilutions.	Test sample is HHV-6 IgG antibody positive, indicating previous HHV-6 infection. Seroconversion or a four-fold or greater rise in IgG antibody titer in paired serum samples indicates recent infection with HHV-6.
Fluorescence found in both infected and uninfected cells	Test sample is exhibiting a nonspecific reaction.

### **Quality Control Criteria**

Each assay must contain the Positive Control and the Negative Control. Results of an assay are considered valid if the following criteria are met:

- 1) The HHV-6 IgG Positive Control provided with this kit yields a fluorescent intensity  $\geq +2$ .
- 2) The HHV-6 IgG Negative Control provided with this kit yields no visible fluorescence.

If the above criteria are not met, the assay is considered invalid and must be repeated.

### **Expected Values**

HHV-6 antibody prevalence is greater than 80% in-patients more than 2 years old. Although the prevalence is high, HHV-6 antibody titer decreases to low levels following infection. Therefore, a high level of IgG may be suggestive of a recent infection. A significant rise in IgG antibodies in paired samples is indicative of recent HHV-6 infection.

### **Limitations of Use**

- **FOR RESEARCH USE ONLY - NOT FOR USE IN DIAGNOSTIC PROCEDURES**
- A serological test such as the IFA serves as an aid to detect viral infection, but its use should not be the sole criterion. The test results should be compared with the patient's clinical and epidemiological profile and other clinical laboratory results.
- A single positive result for HHV-6 IgG antibody is significant only in that it indicates previous contact or infection with the virus. For epidemiological purposes, a single result is useful. It should not be used, however, as an indication of current or recent infection with the virus. To determine current or recent infection, simultaneous testing of paired specimens of 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 a recent infection.

**Summary of HHV-6 IgG IFA Procedure**

**Important Note: Please read the entire instruction leaflet before starting this assay. This summary is for quick reference only.**

Qualitative Determination: Dilute patient sample 1:20 in Wash Buffer

Quantitative Determination: Start with a 1:20 dilution of sample in Wash Buffer, then add equal volumes of diluted sample and Wash Buffer for each consecutive dilution



Add ~20 uL Positive Control to well #1 of slide

Add ~20 uL Negative Control to well #2 of slide

Add ~20 uL diluted sample to remaining wells (one sample per well)



Incubate slide @ 35-39°C for 30 minutes



Wash slide with Wash Buffer



Add 40 uL Conjugate to each well



Incubate slide @ 35-39°C for 30 minutes



Wash slide with Wash Buffer



Place 10 uL of Mounting Media in each well and add coverslip



Examine the slide under a fluorescence microscope

## Bibliography

1. Salahuddin, S.Z., Albashi, D.V., Markham, P.D., Joseph, S.F., Sturzenegger, S., Kaplan, M., Halligan, G., Biberfeld, P., Wong-Stall, F., Kramarsky, B. And Gallo, R.C. (1986) Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders. *Science* 234: 596-601.
2. Yamanishi, K., Okuno, T., Shiraki, K., Takahashi, M., Kondo, T., Asano, Y. And Kurata, T. (1988) Identification of human herpesvirus-6 as a causal agent for exanthem subitum. *Lancet* 1:1065-1067.
3. Asano, Y., Yoshikawa, T., Suga, S., Yazaki, T., Knodo, K. and Yamanishi, K. (1990) Fatal fulminant hepatitis in an infant with human herpesvirus-6 infection. *Lancet* 335: 862-863.
4. Asano, Y., Yoshikawa, T., Kajita, Y., Ogura, R., Suga, S., Yazaki, T., Nakashima, T., Yamada, A. and Kurata, T. (1992) Fatal encephalitis/encephalopathy in primary human herpesvirus-6 infection. *Arch, Dis Child.* 67:1484-1485.
5. Eizuru, Y., Minematsu, T., Minamishima, Y., Kikuchi, M., Yamanishi, K., Takahashi, M. and Kurata, T. (1989) Human herpesvirus-6 in lymph nodes. *Lancet* 1:40.
6. Prezioso, P.J., Cangiarella, J., Lee, M., Nuova, G.J., Borkowsky, W., Orlow, S.J. and Greca, M.A. (1992) Fatal disseminated infection with human herpesvirus- 6. *J. Pediatrics* 120:921-923.
7. Dubedat, S. and Kappagoda, N. (1989) Hepatitis due to human herpesvirus-6. *Lancet* 2:1463-1464.
8. Steeper, T. A., Horwitz, C.A., Ablashi, D.V., Salahuddin, S.Z., Saxinger, C., Saltzman, R and Schwartz, B. (1990) The spectrum of clinical and laboratory findings from Human Herpesvirus-6 (HHV-6) in patients with mononucleosislike illnesses not resulting from Epstein-Barr virus or cytomegalovirus. *Am. J. Clin. Pathol.* 93: 776-783.
9. Kruegar, G. R. F, Koch, B., Ramon, A., Ablashi, D.V., Salahuddin, S.Z., Josephs, S.F., Streicher, H.Z., Gallo, R.C. and Habermann, U. (1988) Antibody prevalence to HBLV (human herpesvirus-6, HHV-6) and suggestive pathogenicity in the general population and in patients with immune deficiency syndromes. *J. Virol. Methods* 21:125-131.
10. Buchwald, D., Cheney, P.R., Peterson, D.L., Henry, B., Wormsley, S.D., Geiger, A., Albashi, D.V., Salahuddin, S.Z., Saxinger, C., Biddle, R., Kikinis, B., Jolesz, F.A., Folks, T., Balachandran, N., Peter, J.B., Gallo, R.C. and Komaroff, A.L. (1992) A chronic illness characterized by fatigue, neurologic and immunologic disorders, and active human herpesvirus type 6 infection. *Ann. Intern. Med.* 116:103-113.
11. Soldan, S.S., Berti, R., Salem, N., Secchiero, P., Flamand, L., Calabresi, P.A., Brennan, M.B., Maloni, H.W., McFarland, H.F., Lin, H.-C., Patnaik, M. and Jacobson, S. (1997) Association of human herpes virus 6 (HHV-6) with multiple sclerosis: Increased IgM response to HHV-6 early antigen and detection of serum HHV-6 DNA. *Nature Medicine* 3:1394-1397.
12. Yadav, M., Chandrashekrana, A. Vasudevan, D.M. and Ablashi, D.V. (1994) Frequent Detection of Human Herpesvirus 6 in Oral Carcinoma. *J. Natl. Cancer Inst.* 86:1792-1794.

13. Chen, M., Popescu, N., Woodworth, C., Berneman, Z., Corbellino, M., Lusso, P., Albashi, D.V. and DiPaolo, J.A. (1994) Human Herpesvirus-6 infects cervical epithelial cells and transactivates human papilloma virus gene expression. *J. Virol.* 68:1173-1178.
14. Drobyski, W.R., Dunne, W.M., Burd, E.M, Knox, K.K., Ash, R.C. Horowitz, M.M., Flomberg, N. and Carrigan, D.R. (1993) Human Herpesvirus-6 (HHV-6) infection in allogeneic bone marrow transplant recipients: Evidence of a marrow-suppressive role for HHV-6 in vivo. *J. Inf. Dis.* 167:735-739.
15. Briggs, M., Fox. J. and Tedder, R.S. (1988) Age prevalence of antibody to human herpesvirus-6. *Lancet* i:1058-1059.

### Interpretation of Symbols

Batch code



Catalogue Number



Temperature limitation



Use by end of



Manufacturer



Harmful if swallowed.  
Contact with acids  
liberates very toxic gases



Instructions for Use



**Biotrin HHV Product Range**

Biotrin International offers a unique portfolio of Human Herpesvirus assays:

<b>Cat #:</b>	<b>Description</b>	<b>Assay Format</b>
V3HHV6	Human Herpesvirus-6 IgG IFA	4 x 10 well slides
V17HHV6	Human Herpesvirus-6 IgM IFA	4 x 10 well slides
V15HHV6	Human Herpesvirus-6 IgG EIA	96 well EIA
V18HHV8	Human Herpesvirus-8 IgG IFA	6 x 10 well slides
V19HHV8	Human Herpesvirus-8 IgG EIA	96 well EIA

Biotrin International Ltd.  
93 The Rise, Mount Merrion  
Co. Dublin  
Ireland  
Tel: +353 (01) 2831166  
Fax: +353 (01) 2831232  
e-mail: [info@biotrin.ie](mailto:info@biotrin.ie)  
[www.biotrin.com](http://www.biotrin.com)

**FOR RESEARCH USE ONLY  
NOT FOR USE IN DIAGNOSTIC  
PROCEDURES**