

ENGLISH

Cat. No.: V17HHV6
Format: 4 x 10 well slides
HHV-426-03



Human Herpesvirus-6 IgM Immunofluorescent Assay

An immunofluorescent assay for the detection of Human Herpesvirus-6 IgM
antibodies



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Intended Use

The Biotrin Human Herpesvirus-6 IgM IFA is intended for the qualitative and semi-quantitative detection of anti-Human Herpesvirus-6 (HHV-6) IgM antibodies in serum and plasma.

Introduction

Human Herpesvirus-6 (HHV-6), first described in 1986, was isolated from patients with lymphoproliferative disorders¹. Subsequently, HHV-6 has been confirmed as the aetiological agent responsible for the childhood disease exanthem subitum (Roseola infantum)², and has been associated with a number of other disease manifestations in children, including fulminant hepatitis³, encephalitis⁴, histiocytic necrotising lymphadenitis⁵ and fatal disseminated infection⁶.

In adults, primary infection with HHV-6 is less common, with documented evidence showing that HHV-6 may be involved in cases of hepatitis⁷, mononucleosis-like illness⁸, atypical polyclonal lymphoproliferation⁹, 'post-viral chronic fatigue syndrome'¹⁰, multiple sclerosis¹¹, oral carcinoma¹², cervical carcinoma¹³ and bone marrow suppression in bone marrow transplant patients¹⁴.

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 age¹⁵. However, although the prevalence of HHV-6 antibody is high, the level of antibody diminishes to low titres following infection. Detection of anti-HHV-6 IgM in humans can be used as an aid in the diagnosis of primary infection with this virus.

Assay Principle

The Biotrin HHV-6 IFA system utilises the indirect immunofluorescent method of antibody detection and titre determination. Patient serum or plasma samples are incubated with immobilised HHV-6 antigen, which has been stabilised on a glass slide. If HHV-6 IgM 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 IgM and this complex is visualised with the aid of a fluorescent microscope. A positive antibody reaction is denoted by bright green fluorescence.

Precautions

Safety

- For *in vitro* diagnostic use only.
- 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, HTLV-I/II 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

IFA Test Kit FOR RESEARCH USE ONLY IN THE USA.

- 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 + IgM

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

3. Negative Control **:

CONTROL - IgM

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

4. Fluorescein Conjugate**:

CONJ ENZ 1X

1 x 1.5ml fluorescein conjugated goat (inactivated) anti-human IgM with Evans Blue and Rhodamine counterstains. Contains 0.1% Sodium Azide. (Ready-To-Use) (Yellow Cap).

5. Mounting Media:

MM

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

6. Wash Buffer Concentrate (PBS):

BUF WASH CONC

1 x Sachet. The aluminium-sealed packet contains 10 PBS tablets. Each tablet makes up 100ml of 1x Wash Buffer.

7. Slide Blotters:

BLT

4 x Absorbent blotters with 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 5µl to 50µl and 50µl to 200µl.
- Timer.
- 35-39°C incubator.
- Paper towels or absorbent paper.
- Dilution tubes and minifuge tubes (0.5ml).
- Benchtop minifuge.
- Incubation tray containing moistened tissue paper.
- Wash bottles and wash tray.
- Coverslips: 22 X 50mm No.1 thickness.
- Wax pencil.
- IgG Adsorbent (Commercially available).
- Fluorescent microscope with appropriate filter combination for FITC (excitation filter 495nm, barrier filter 515nm), a halogen light source is recommended. The fluorescein label has an excitation peak of 490nm and an emission peak of 520nm. Differences in endpoint reactivates and fluorescence intensities may be due to the type and condition of the fluorescence equipment used in your laboratory.

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.
- Do not use excessively lipemic samples without delipidization.
- Do not use contaminated samples.

Reagent and Specimen Preparation

Reagent Preparation

Prepare wash buffer by adding 1 PBS tablet to 100ml freshly prepared distilled or deionized water. Mix to dissolve. 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: All samples are pretreated to remove IgG. Add 90µl of the adsorbent to 10µl of test sample and mix well. Incubate for 15 minutes at room temperature. Centrifuge at 10,000rpm for 2 minutes at room temperature. The supernatant is then added to the slide well.

Semi-Quantitative Test: The sample “titre” can be determined by preparing two-fold serial dilutions of the sample in Wash Buffer, starting with a 1:20 dilution of sample in Adsorbent, 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. 20µl) of each diluted test sample and 1 drop (approx. 20µl) 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 3 hours 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. 20µl) 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 fluorescent 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 IgM 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 primary HHV-6 infection.

+4 = Brilliant green fluorescence indicating very high titre HHV-6 IgM antibody response.

+3 = Bright green fluorescence indicating high titre HHV-6 IgM antibody response.

+2 = Green fluorescence indicating medium titre HHV-6 IgM antibody response.

+1 = Dull green fluorescence indicating weak titre HHV-6 IgM antibody response. This also indicates the end-point dilution or "titre" of the sample.

- Titration of HHV-6 IgM positive samples provides quantitative information. In a titration series, the highest serum dilution demonstrating a "+1" reaction is interpreted as the end-point titre.
- 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.
- The fluorescent staining pattern of HHV-6 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 whole cell fluorescence. Fluorescence can also range from granular to homogenous.

Significance of Interpretation

No discernible fluorescence of the infected cells found at the screening dilution.	Test sample is HHV-6 IgM antibody negative.
Specific positive fluorescence of the infected cells found at the screening dilution or at higher dilutions.	Test sample is HHV-6 IgM antibody positive, indicating current infection.
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 IgM Positive Control provided with this kit yields a fluorescent intensity $\geq +2$ of the infected cell.
- 2) The HHV-6 IgM Negative Control provided with this kit yields no specific fluorescence of the infected cell.

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 titre decreases to low levels following infection. Therefore, a high level of IgM may be suggestive of a primary infection.

Limitations of Use

For Research use only in U.S.A.

- A serological test such as the IFA serves as an aid to detect viral infection, but its use should not be the sole criteria. The test results should be compared with the patient's clinical and epidemiological profile and other clinical laboratory results.
- Non-specific 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 reaction. Therefore, observation of an autoimmune reaction cannot eliminate the possibility of HHV-6 infection.
- A sample obtained too early during infection may not contain detectable levels of IgM antibody. If a viral 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 titre of viral specific IgM and IgG. Either seroconversion or significant rise in titre is indicative of primary infection.

- Because of the possibility of contamination of cord blood with maternal IgM, it is prudent to confirm positive viral IgM antibody results on cord blood samples by testing a follow up specimen from the infant, preferably within the first 5 days of life.
- Specific IgM antibodies are usually detected in patients with recent primary infection. IgM antibodies may be found in patients with reactivated or secondary infections.
- Biotrin recommends pretreatment of test samples to remove IgG 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.

Performance Characteristics

Sensitivity and Specificity

Diagnostic Sensitivity

9 HHV-6 IgM positive samples were tested using Biotrin's HHV-6 IgM IFA. The 9 samples tested positive indicating a sensitivity of 100%.

$$\% \text{ Sensitivity} = \text{True positives} / (\text{True positives} + \text{False negatives} + \text{Equivocals}) \times 100$$

$$9 / (9 + 0) \times 100 = 100\%$$

Sensitivity = 100%

Diagnostic Specificity

77 normal donor serum samples were tested for HHV-6 IgM using Biotrin's HHV-6 IgM IFA. 76 were found to be negative for HHV-6 IgM antibodies. 99% specificity was obtained based on the following equation:

$$\% \text{ Specificity} = \text{True negatives} / (\text{True negatives} + \text{False positives} + \text{Equivocals}) \times 100$$

$$76 / (76 + 1) \times 100 = 99\%$$

% Specificity = 99%

Cross Reactivity

In order to establish the specificity of the Biotrin HHV-6 IgM IFA, 17 serum samples were tested. The following table summarises the results from the serum samples taken from patients with the following diseases.

Virus	Biotrin's HHV6 IgM IFA
CMV IgM	0/2
EBV IgM	0/5
HSV IgM	0/2
VZV IgM	0/2
ANA	0/2
MCTD	0/2
Lyme	0/2

Table 1: Cross Activity Results

Interferences (Analytical Specificity)

Interference studies include tests for haemolysis, lipemia, bilirubin and rheumatoid factor. A brick red counterstain was noted on all stained slides and the interpretation of the fluorescence intensity was not interfered with.

Reproducibility

Intra Assay

One positive sample was tested 15 times on 2 separate batches. Refer to Table 2.

Controls	Batch 1	Batch 2
PC	2+	2+
NC	0	0
PBS	0	0
Positive sample	Batch 1	Batch 2
1	2+	2+
2	2+	2+
3	2+	2+
4	2+	2
5	1+/2+	2+
6	2+	2+
7	2+	2+
8	2+	2+
9	2+	1+/2+
10	2+	2+
11	2+	2+
12	2+	2+
13	2+	2+
14	2+	2+
15	2+	2+

Table 2: Intra Assay Reproducibility Results

Inter Assay

A panel of samples was run on ten different assays from two separate batches. Refer to Table 3.

Batch 1

Samples	Spec	Assay 1	Assay 2	Assay 3	Assay 4	Assay 5	Assay 6	Assay 7	Assay 8	Assay 9	Assay 10
PC	2+-4+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+
NC	0	0	0	0	0	0	0	0	0	0	0
PBS	0	0	0	0	0	0	0	0	0	0	0
Positive	≥1+	2+	1+	1+/2+	1+/2+	2+	2+	2+	2+	1+/2+	2+
Positive	≥1+	2+	1+	2+	2+	2+	2+	2+	2+	1+/2+	2+
Negative	0	0	0	0	0	0	0	0	0	0	0
Negative	0	0	0	0	0	0	0	0	0	0	0
Cut off	≥1+	1+	1+/2+	1+/2+	1+/2+	1+/2+	1+/2+	1+/2+	1+/2+	1+/2+	1+/2+

Batch 2

Samples	Spec	Assay 1	Assay 2	Assay 3	Assay 4	Assay 5	Assay 6	Assay 7	Assay 8	Assay 9	Assay 10
PC	2+-4+	2+	2+	2+	2+	2+	2+/3+	2+	2+	2+	2+
NC	0	0	0	0	0	0	0	0	0	0	0
PBS	0	0	0	0	0	0	0	0	0	0	0
Positive	≥1+	2+	2+	2+	1+2+	1+/2+	2+	2+	2+	2+	2+
Positive	≥1+	2+	1+/2+	2+	2+	2+	2+	2+	2+	2+	2+
Negative	0	0	0	0	0	0	0	0	0	0	0
Negative	0	0	0	0	0	0	0	0	0	0	0
Cut off	≥1+	1+/2+	1+	2+	2+	2+	1+/2+	1+/2+	1+/2+	2+	1+/2+

Table 3: Intra Assay Reproducibility Results

Summary of HHV-6 IgM 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 1:10 in IgG Adsorbent
Semi-Quantitative Determination: Start with a 1:20 dilution of sample in IgG Adsorbent, then add equal volumes of diluted sample and Wash Buffer for each consecutive dilution



Add 1 drop (~20µl) Positive Control to well #1 of slide
Add 1 drop (~20µl) Negative Control to well #2 of slide
Add 1 drop (~20µl) diluted sample to remaining wells (one sample per well)



Incubate slide @ 35-39°C for 3 hours



Wash slide with Wash Buffer



Add 1 drop (~20µl) Conjugate to each well



Incubate slide @ 35-39°C for 30 minutes



Wash slide with Wash Buffer



Place 1 small drop (~10µl) of Mounting Media in each well and add coverslip



Examine the slide under a fluorescent microscope

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Interpretation of Symbols

In-vitro diagnostic medical device

IVD

Batch code

LOT

Catalogue Number

REF

Temperature limitation



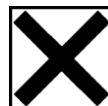
Use by end of



Manufacturer



Harmful if swallowed. Contact with acids liberates very toxic gases.



Instructions for Use



Additional Biotrin Products

Biotrin International offers a unique portfolio of Human Herpesvirus assays suitable for routine laboratory diagnosis

Cat #:	Description	Assay Format
V17HHV6	Human Herpesvirus-6 IgM IFA	4 x 10 well slides
V3HHV6	Human Herpesvirus-6 IgG 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

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