ENGLISH

Catalogue No: V18HHV8
Format: 6x10 well slides
HHV-427-03

Human Herpesvirus-8 IgG Immunofluorescent Assay

An immunofluorescent assay for the detection of Human Herpesvirus-8 IgG Lytic antibodies.

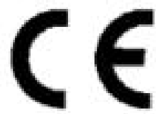

Table of Contents

Intended Use

Introduction

Assay Principle

Precautions
- Safety
- Procedural

Kit Components
- Materials Provided
- Additional Materials Required

Storage and Stability

Specimen Collection and Storage

Reagent and Specimen Preparation

Assay Procedure

Interpretation of Results

Quality Control Criteria

Expected Values

Limitations of Use

Performance Characteristics

Summary of HHV- 8 IgG IFA Procedure

References

Interpretation of Symbols

Additional Biotrin Products

Intended Use

The Indirect Immunofluorescent Assay (IFA) for Human Herpesvirus-8 (HHV-8) antibody is intended for the qualitative and semi-quantitative determination of IgG antibody to HHV-8 lytic antigens in human serum or plasma. Detection of HHV-8 IgG antibody in humans can be used as an aid in the diagnosis of primary infection or reactivation / reinfection with this virus.

Introduction

Human Herpesvirus-8 (HHV-8), also known as Kaposi's Sarcoma-associated Herpesvirus (KSHV), was discovered in 1994 when Chang *et al* identified two fragments of herpesvirus-like DNA in the lesions of a patient with AIDS-associated Kaposi's Sarcoma(1). The virus is classified as a gamma herpesvirus (genus rhadinovirus) and resembles EBV in its tropism for B cells and ability to exist in a latent state. There is now very strong epidemiological evidence of the causative role of HHV-8 in the pathogenesis of Kaposi's Sarcoma (KS). HHV-8 is detectable in all forms of the disease: Classic KS (a rare malignancy occurring in elderly Mediterranean men), African endemic KS, transplant-associated KS and AIDS associated KS.

Transmission through sexual contact plays an important part in the spread of HHV-8 among homosexual men(2). However HHV-8 can also be transmitted by saliva and transplanted organs(3).

The seroprevalence rate of HHV-8 in the general population has been reported in the scientific literature to vary from 5-35% depending on the type of immunologic assay employed and geographical area studied. In a number of reported studies, highly elevated IgG antibody titers were observed in KS patients but not in normal donors. Thus, the seroprevalance rate of HHV-8 is different from EBV, HHV-6, HHV-7, CMV, or HSV-1, where >80% of the population is antibody positive for these viruses.

The seroprevalence of HHV-8 among blood donors ranges from 5-10% in the United States and N. Europe(4), 10-35% in Italy and Mediterranean countries(5), to more than 50% in many African populations(6).

The prevalence of HHV-8 antibodies in the general population is thought to be correlated with the frequency of KS after transplantation(7). Transmission from the donor allograft to the recipient has also been documented. The association of HHV-8 in transplant recipients has led to the recommendation that organ donors and recipients should be screened for antibodies to HHV-8(8,9). In HIV positive patients, HHV-8 antibodies have been shown to precede and predict the development of KS(10). KS is the most common neoplasm in this patient group. HHV-8 has also been associated with body cavity lymphomas, multi-centric Castleman's disease, non-Hodgkin's lymphoma and multiple myeloma(11).

At present, the diagnosis for HHV-8 infection can be confirmed by PCR analysis and by immunological assays, e.g. IFA and ELISA. However, HHV-8 DNA can be detected in peripheral blood cells from only about half of infected persons with the use of standard PCR assays(12-14).

Since PCR detection systems appear to exhibit low sensitivity when DNA from peripheral blood cells is used as a template, serological assays have been proven more useful for epidemiology studies and diagnosis of HHV-8 infection, particularly for detecting previous exposure to the virus(11,12). Serological studies have detected specific antibodies to HHV-8 in 80-97% of HIV positive patients with KS, 54-100% of HIV positive patients who developed KS within 5 years of specimen collection and 16-56% of patients who did not develop KS(13). In contrast, PCR detected HHV-8 in only 40-50%, 30% and 5 -10% of the

same patient groups, respectfully.
Biotrin's HHV-8 kit is based on a cell line, which expresses lytic antigen, allowing detection of antibodies to lytic viral proteins.

Several anti-herpesvirus drugs, such as ganciclovir, have shown activity against HHV-8 *in vivo*, but only limited clinical data is available. Further clinical research is needed on several bleeds to define the rise and fall of antibody levels to HHV-8 in order to correlate that information with HHV-8 infection and disease states such as KS.

Assay Principle

Biotrin's indirect immunofluorescent assay system is a rapid, simple method for determination of antibody to Human Herpesvirus-8 lytic antigens. The fluorescent antibody assay utilises the indirect method of fluorescent antibody staining. The procedure is carried out in two basic reaction steps:

In step one, the human serum or plasma to be tested is brought into contact with fixed infected cells. Antibody, if present in the test sample, will complex with the antigen in the cellular substrate. If the sample being examined contains no antibody for this particular antigen, no complex is formed and all the serum components are washed away in the rinse cycle.

The second step involves adding fluorescein-labelled anti-human antibody. If antibody to HHV-8 is present (a positive reaction), a bright apple-green fluorescence can be seen with the aid of a fluorescence microscope.

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 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-8 Antigen Slides:

SLIDE

6 x 10 well slides of human lymphocytes expressing HHV-8 lytic antigens on each glass well. The slides are ready for use after removal from protective pouch.

2. HHV-8 IgG Positive Control*:

CONTROL	+	IgG

1 x 500µl HHV-8 IgG antibody positive human control. Contains sodium azide. Ready-To-Use.

3. Negative Control*:

CONTROL	-	IgG

1 x 500µl HHV-8 IgG antibody negative human control. Contains sodium azide. Ready-To-Use.

4. FITC anti-Human IgG Conjugate*:

CONJ	IgG

1 x 1.8ml fluorescein conjugated goat (inactivated) antihuman IgG (heavy and light chain) with Evans Blue and Rhodamine counterstains. Contains sodium azide. Ready-To-Use.

5. Mounting Media:

MM

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

6. Wash Buffer Concentrate (PBS):

BUF	WASH	CONC

3x Sachets. Each aluminium sealed packet of powdered buffer makes one litre 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

Additional Materials Required

- High quality distilled or deionised water
- Accurate 20µl,100µl and 200µl pipettes and disposable tips
- Serum collection equipment
- Timer
- Wash bottles and wash tray
- Test tubes, racks, pipettes, microtitre plates and safety pipetting devices for making sample dilutions
- 37ºC incubator
- Moist chamber for incubating slides
- Slide holder rack and staining dish for washing slides
- Coverslips: 22x50mm No. 1 thickness glass.
- Fluorescent microscope: A fluorescent microscope equipped with the following was used to calibrate the Controls and Conjugate:
- 10x eyepiece
- 16x or 40x objectives
- Epi-illuminator with 50W halogen lamp
- FITC-excitation filter KP490
- Yellow absorbing filter K530
- Red suppression filter BG38

The fluorescein label has an excitation peak of 490nm and an emission peak of 520nm. Difference in endpoint reactivities 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 2-25ºC.
- 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 or plasma samples collected over a period of time to demonstrate seroconversion or significant titre increase should be collected 7-14 days apart and stored at -20ºC. These samples should then be tested simultaneously.

Specimen and Reagent Preparation

Reagent Preparation

Wash Buffer: Add the entire contents of a PBS packet to 1L of freshly prepared distilled or deionized water. Store in a clean closed container at 2-8ºC for up to 4 weeks.
Note: Addition of salts while rapidly stirring the water will facilitate solubilisation.
All remaining reagents are supplied ready to use and at working dilution.

Specimen Preparation

Qualitative Test: Dilute the sample 1:64 in Wash Buffer. Prepare all dilutions in a minimum volume of 100µl Wash Buffer.

Quantitative Test: The sample 'titre' can be determined by preparing two-fold serial dilutions of the sample in Wash Buffer, starting with a 1:64 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 (20-25ºC) before use.

1. **Slide Preparation**
   Remove desired number of slides from protective pouch and mark between wells with a wax pencil to avoid contamination. Dispense 1 drop (approximately 20µl) of each diluted test sample and 1 drop (approximately 20µl) of the ready to use Positive and Negative Controls and 1 drop of Wash Buffer onto numbered wells.
   Note: Add sufficient volume to completely cover each well, but avoid cross mixing of contents between wells.

2. **Incubate the Samples**
   Incubate the slides in a moist chamber for 30 minutes at 35-39ºC.

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 (20-25ºC) with a change of Wash Buffer solution after 5 minutes with gentle shaking. Blot the paint mask surrounding the test wells with the blotters provided.

4. **Incubate with Conjugate**
   Apply 1 drop (approximately 20µl) of the ready to use Conjugate to each test well. Incubate the slides in a moist chamber for 30 minutes at 35-39 ºC.

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 coverslip.

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 2-8ºC. 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-8 IgG antibodies if fluorescent staining of the infected cells is absent.

Positive Reaction

- For lytic antigen, the whole cell, both cytoplasm and nucleus, will fluoresce.
- A sample can be considered HHV-8 IgG positive if green fluorescent staining of the infected cells is present at a dilution ≥ 1:64 and the staining pattern is similar to that of the Positive Control. Dull green cells that do not have an appearance similar to the Positive Control are graded negatives.

  +4 = Brilliant green fluorescence indicating very high titre HHV-8 IgG antibody response.
  +3 = Bright green fluorescence indicating high titre HHV-8 IgG antibody response.
  +2 = Green fluorescence indicating medium titre HHV-8 IgG antibody response.
  +1 = Dull green fluorescence indicating weak titre HHV-8 IgG antibody response. This also indicates the end-point dilution or 'titre' of the sample.

- Titration of HHV-8 IgG positive samples provides quantitative information. In a titration series, the highest serum dilution demonstrating a '+1' reaction is interpreted as the endpoint titre.
- To provide an internal control, each well on the microscope slide contains both HHV-8 infected and uninfected cells. Preparation of the slide in this manner is intentional. Uninfected cells, stained red by the counterstain, provide a contrasting background.

Significance of Interpretation

No discernible fluorescence of the infected cells found at the screening dilution.	Test sample is HHV-8 IgG antibody negative.
Random green cells displaying no discernible fluorescence of the infected cells.	Test sample is HHV-8 IgG antibody negative
Infected cells that are not graded at least 1+ reaction	Test sample is non-reactive and is deemed HHV-8 IgG antibody negative.
Specific positive fluorescence of the infected cells found at the screening dilution or at higher dilutions.	Test sample is HHV-8 IgG antibody positive, indicating previous HHV-8 infection. Seroconversion or a four-fold or greater rise in IgG antibody titre in paired serum samples indicates recent infection with HHV-8.
Fluorescence found in both infected and uninfected cells	Test sample is exhibiting a non-specific reaction.

Quality Control Criteria

Each assay must include the Positive Control, the Negative Control and a blank well containing only Wash Buffer. Results of an assay are considered valid if the following criteria are met:

1. The HHV-8 IgG Positive Control furnished with this kit yields a fluorescence intensity $\geq$ +2.
2. The HHV-8 IgG Negative Control furnished with this kit yields no visible fluorescence.
3. The well containing only Wash Buffer yields no visible fluorescence.

Note: The well containing only Wash Buffer acts as a Conjugate control to ensure the Conjugate is not reacting with the cell substrate.

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

Expected Values

Seroprevalence

Disease prevalence is usually determined after extensive testing for antibody levels, in any given population, according to age, sex, geographical location, and socio-economic status.
The seroprevalence of HHV-8 among blood donors ranges from 5-10% in the United States and N. Europe[(4)], 10-35% in Italy and Mediterranean countries[(5)], to more than 50% in many African populations[(6)].

Limitations of Use

- 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.

- A single positive result for HHV-8 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 plasma or serum taken 7-14 days apart should be done. A four-fold or greater rise in titre between the first and second sample is indicative of a current or a recent infection.

- In the Biotrin study non-specific positive reactions were found to occur in samples from patients with certain autoimmune diseases such as anti-nuclear antibody (ANA). Both infected and uninfected cells will fluoresce, thus obscuring a positive HHV-8 reaction. Therefore, observation of an autoimmune reaction cannot eliminate the possibility of HHV-8 infection. In the test for the lytic antibody, the possibility of a positive ANA reaction being read as positive for lytic antibody does exist. Comparison of the readings from a sample with ANA with the particulate reaction of the Positive Control could be helpful to eliminate false positives.

Performance Characteristics

Sensitivity & Specificity

Diagnostic Sensitivity

For HHV-8 IgG antibodies, 33 samples from a known pool of positive samples were taken and tested with the Biotrin HHV-8 IgG IFA. All 33 tested positive indicating a sensitivity of 100%. The positive sample pool was verified with another commercially available assay.

% Sensitivity = True Positives / (True Positives + False Negatives + Equivocals) x 100
$\therefore$ 33 / (33 + 0 + 0) x 100 = 100%
Sensitivity = 100%

Diagnostic Specificity

The specificity of the Biotrin HHV-8 IgG IFA was assessed by testing 122 negative samples. These samples were confirmed negative on Biotrin's HHV-8 IgG IFA and two other commercially available assays using the two out of three rule. Out of these 122 samples, 114 were negative and 8 were positive. 94% specificity was obtained based on the following equation:

% Specificity = True negatives/(True Negatives + False Positives + Equivocals) $\times$ 100
$\therefore$ 114 / (114 + 8 + 0) x 100 = 94%
Specificity = 94%

Cross Reactivity

In order to establish the specificity of the Biotrin's HHV-8 IgG IFA, 42 serum samples were screened. The following table summarises the results from serum samples taken from patients with the following diseases:

Table 1

Virus	Biotrin's HHV- 8 IgG IFA
Lyme	0/3
Human T-Cell Lymphotrophic Virus (HTLV)	0/2
Antinuclear Antibody (ANA)[1]	3/3
HHV6 IgG	0/3
HIV-1	0/9
Hep C	0/2
Hep B	0/2
Cytomegalovirus (CMV IgG)	0/4
Epstein Barr Virus (EBV)	0/2
Herpes Simplex Virus (HSV)	0/3
Varicella Zoster Virus (VZV)	0/2

[1] Cross reacts (see Limitations of Use).

Interferences (Analytical Specificity)

Interference studies included tests for haemolysis, bilirubin and rheumatoid factor. Some non-specific fluorescence was noted. However, these did not interfere with the interpretation of the fluorescence intensity for a positive result. A brick red counterstain was noted on all stained slides.

Biotrin

Reproducibility

Intra -Assay Reproducibility

The following results were obtained when kit controls, a wash buffer control, medium and high titre IgG samples were screened 20 times on 3 separate production batches on the same day.

Table 2

Controls	**Batch 1**	**Batch 2**	**Batch 3**
PC	3+/4+	3+-4+	4+
NC	0	0	0
Wash Buffer	0	0	0
HIGH	**Batch 1**	**Batch 2**	**Batch 3**
1	3+/4+	3+/4+	3+/4+
2	3+	3+/4+	4+
3	3+/4+	3+	4+
4	3+	3+	4+
5	3+/4+	3+	4+
6	3+	3+	4+
7	3+	3+	4+
8	3+	3+/4+	4+
9	3+	3+	4+
10	3+	3+/4+	3+/4+
11	3+	3+	4+
12	3+	3+/4+	3+/4+
13	3+	3+	4+
14	3+	3+	4+
15	3+	3+	4+
16	3+	3+	4+
17	3+	3+	4+
18	3+	3+/4+	3+/4+
19	3+	3+/4+	4+
20	3+/4+	3+/4+	3+/4+
MEDIUM	**Batch 1**	**Batch 2**	**Batch 3**
1	2+	2+	2+/3+
2	1+/2+	2+	2+
3	2+	2+	2+/3+
4	2+	2+	2+
5	1+/2+	2+	2+
6	2+	2+/3+	2+
7	2+	2+	2+
8	2+	2+	2+/3+
9	1+/2+	2+	2+/3+
10	2+	2+	2+3+
11	2+	2+	2+
12	2+	2+	2+
13	1+/2+	2+	2+
14	1+/2+	2+	2+
15	1+	2+	2+
16	2+	2+	2+3+
17	2+	2+	2+
18	1+/2+	2+	2+/3+
19	2+	2+	2+/3+
20	2+	2+	2+

Inter-Assay Reproducibility

The following results were obtained when kit controls, a wash buffer control, low, medium and high titre IgG sera samples were screened on ten different assays in three production batches on different days.

Table 3a

Batch 1

Samples	Assay 1	Assay 2	Assay 3	Assay 4	Assay 5	Assay 6	Assay 7	Assay 8	Assay 9	Assay 10
PC	3+/4+	3+	3+/4+	3+	3+	3+	3+/4+	3+/4+	4+	3+4+
NC	0	0	0	0	0	0	0	0	0	0
Wash Buffer	0	0	0	0	0	0	0	0	0	0
High	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
High	3+/4+	3+/4+	3+/4+	3+/4+	3+/4+	3+/4+	3+/4+	3+	4+	3+/4+
Medium	1+/2+	2+	2+	1+/2+	1+/2+	1+/2+	2+	1+	1+/2+	1+
Medium	1+	1+	1+/2+	1+	1+	1+/2+	2+	1+	2+	1+
PC 1:256*	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+
Negative	0	0	0	0	0	0	0	0	0	0
Negative	0	0	0	0	0	0	0	0	0	0

Table 3b

Batch 2

Samples	Assay 1	Assay 2	Assay 3	Assay 4	Assay 5	Assay 6	Assay7	Assay 8	Assay 9	Assay 10
PC	4+	3+	3+/4+	3+/4+	3+/4+	3+/4+	3+	3+	3+	3+/4+
NC	0	0	0	0	0	0	0	0	0	0
Wash Buffer	0	0	0	0	0	0	0	0	0	0
High	3+/4+	3+	3+	3+	3+	3+	3+	3+	3+	3+
High	4+	3+/4+	3+/4+	3+/4+	3+/4+	3+/4+	3+/4+	3+/4+	3+/4+	3+/4+
Medium	2+	2+	1+/2+	2+	2+	1+/2+	1+/2+	1+/2+	1+/2+	2+
Medium	1+	1+	1+	1+/2+	1+	1+/2+	1+	1+	1+/2+	1+/2+
PC 1:256*	1+/2+	1+	1+	1+	1+	1+	1+	1+	1+	1+/2+
Negative	0	0	0	0	0	0	0	0	0	0
Negative	0	0	0	0	0	0	0	0	0	0

Table 3c

Batch 3

Samples	Assay 1	Assay 2	Assay 3	Assay 4	Assay 5	Assay 6	Assay 7	Assay 8	Assay 9	Assay 10
PC	3+	3+	3+/4+	4+	3+/4+	4+	3+/4+	3+/4+	4+	3+
NC	0	0	0	0	0	0	0	0	0	0
Wash Buffer	0	0	0	0	0	0	0	0	0	0
High	3+	3+	3+	4+	2+/3+	3+	2+/3+	3+	3+	2+/3+
High	4+	3+/4+	3+/4+	4+	3+/4+	3+/4+	3+/4+	3+/4+	3+/4+	3+
Medium	1+	1+	1+/2+	2+/3+	1+/2+	1+/2+	2+	1+/2+	2+	1+
Medium	1+	1+	1+	1+	1+	1+	1+	1+/2+	1+	1+
PC 1:256*	2+	1+/2+	1+/2+	2+	2+	1+/2+	1+	2+	2+	1+
Negative	0	0	0	0	0	0	0	0	0	0
Negative	0	0	0	0	0	0	0	0	0	0

* A titre of the Positive Control at a 1:256 dilution in Wash Buffer was included

Summary of HHV-8 IgG IFA Procedure

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

Qualitative determination: Dilute patient sample 1:64 in Wash Buffer
Quantitative titration: Start with a 1:64 dilution of sample in Wash Buffer, then add equal volumes of diluted sample and Wash Buffer for each consecutive dilution

Add ~20µl Positive Control to well #1 of slide
Add ~20µl Negative Control to well #2 of slide
Add ~20µl Wash Buffer to well #3 of slide
Add ~20µl diluted sample to remaining wells (one sample per well)

Incubate slide @ 35-39°C for 30 minutes

Wash Slide with Wash Buffer

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

Incubate slide @ 35-39°C for 30 minutes

Wash slides with Wash Buffer

Place 10µl of Mounting Media in each well and add cover slip

Examine the slide under a fluorescence microscope

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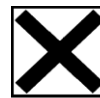
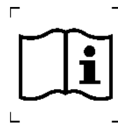
Interpretation of Symbols

In-vitro diagnostic medical device	
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

Additional Biotrin Products

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

Cat #:	Description	Assay Format
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

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www.biotrin.com