

HHV-8 IgG EIA, Cat. No.: V19HHV8, HHV-428-03 07/09, EN



**ENGLISH**

Cat No: V19HHV8  
Kit Format: 96 well EIA  
HHV-428-03



## **Human Herpesvirus-8 IgG EIA**

An enzyme immunoassay for the qualitative detection of Human Herpesvirus-8 IgG antibodies in human serum and plasma.



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## **Intended Use**

The Human Herpesvirus-8 (HHV-8) IgG bi-peptide Enzyme Immunoassay is intended for the qualitative detection of IgG antibodies 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, or can provide evidence of previous HHV-8 infection.

## **Introduction**

Human Herpesvirus-8 (HHV-8) is also known as Kaposi's Sarcoma-associated Herpesvirus (KSHV). 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 for the causative role of HHV-8 in the pathogenesis of Kaposi's Sarcoma (KS)<sup>(1)</sup>. 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. In HIV positive patients, HHV-8 antibodies have been shown to precede and predict the development of KS<sup>(2)</sup>.

Transmission through sexual contact plays an important part in the spread of HHV-8 among homosexual men<sup>(3)</sup>. The seroprevalence of HHV-8 among blood donors ranges from 5 -10% in the United States and N. Europe<sup>(4)</sup>, 10 - 35% in Italy and Mediterranean countries<sup>(5)</sup>, to more than 50% in many African populations<sup>(6)</sup>.

HHV-8 has also been associated with body cavity lymphomas, also called primary effusion lymphomas (PEL), multi-centric Castleman's disease (MCD), non-Hodgkin's lymphoma and multiple myeloma<sup>(7)</sup>.

At present, HHV-8 infection can be diagnosed 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<sup>(9-12)</sup>. Since PCR detection systems appear to exhibit low sensitivity when DNA from peripheral blood cells is used as a template, serological assays have proved more useful for epidemiology studies and diagnosis of HHV-8 infection, particularly for detecting previous exposure to the virus<sup>(8,9)</sup>.

The Biotrin HHV-8 IgG EIA kit is based on a synthetic peptide mix which allows for the detection of antibodies to lytic HHV-8 viral proteins.

## **Assay Principle**

The BIOTRIN HHV-8 ELISA is a direct EIA based on the binding of HHV-8 specific antibodies to lytic peptide antigens coupled to microtitre test strips. Specifically bound antibodies are detected by an anti-human IgG peroxidase conjugate and a subsequent substrate reaction. The use of lytic peptide epitopes derived from different viral proteins ensures both a high sensitivity and specificity. There is no detectable cross-reactivity with HIV.

## Precautions

### Safety

- For *in vitro* diagnostic use only.
- This kit is intended for use by qualified laboratory staff only.
- Reagents marked with \*\* are considered POTENTIALLY BIOHAZARDOUS MATERIAL. Each donor unit used in the preparation of the positive control, cut-off calibrator and negative control was tested by an FDA-cleared method for HBsAg and antibodies to HIV and HCV and found to be negative. However, because no test method can offer complete assurance that infectious agents are absent, all these reagents and all patient specimens should be handled in accordance with established safety procedures.
- Some reagents contain Kathon™ CG, which may be corrosive. Stop Solution contains sulphuric acid, which is also corrosive. Avoid contact with the skin and eyes. If contact occurs rinse off immediately with water and seek medical advice.
- Some reagents contain Thiomersal, which may be toxic if ingested.
- The substrate contains TMB which may irritate the skin and mucous membranes. Any substrate that comes in contact with the skin should be rinsed off with water.
- Dispose of all clinical specimens, infected or potentially infected material in accordance with good laboratory practice. All such materials should be handled and disposed of 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 mouth and never eat or drink at the laboratory workbench.

### Procedural

- 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.
- Do not use kit or individual reagents past their expiry date.
- Do not use contaminated samples or reagents.
- Do not mix or substitute reagents from different kit lot numbers.
- Deviation from the protocol provided may cause erroneous results.
- Allow all reagents to come to room temperature (20 - 25°C) and mix well prior to use.
- Avoid leaving reagents in direct sunlight and/or above 2-8°C for extended periods.
- High Quality distilled or deionised water is required for the Wash Solution.
- 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.
- Remove only the volume of conjugate required for the assay. Do not pour unused reagent back into the bottle or pipette directly from the bottle. If so contamination may occur.

- Reagent delivery should be aimed at midpoint of the side of the wells, taking care not to scratch the side with the pipette tip.
- Do not allow the wells to dry up at any stage during the assay procedure.
- Always keep the upper surface of the wells free of droplets. Drops should be gently blotted dry on completion of the procedural step.
- Ensure that the bottom surface of the plate is clean and dry before reading.
- Before commencing the assay an identification and distribution plan should be established.
- Do not heat inactivate sera.
- Do not remove the plate from its protective pouch until ready to use.

## Kit Components

### Materials Provided

1. Coated ELISA plate  

PLA	IgG
-----	-----

12 x 8 wells streptavidin coated biotinylated HHV-8 lytic peptides
2. Positive Control\*\* (Red Cap Colour)  

CONTROL	+	IgG
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1 x 2ml of prediluted positive human serum or plasma in a stabilising buffer (containing 0.01% sodium azide, 0.01% Thiomersal)
3. Negative Control\*\* (Green Cap Colour)  

CONTROL	-	IgG
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1 x 2ml of prediluted negative human serum or plasma in a stabilising buffer (containing 0.01% sodium azide, 0.067% Kathon™ CG)
4. Cut-off-Calibrator\*\* (Brown Cap Colour)  

CAL
-----

1 x 2ml of prediluted weakly positive human serum or plasma in a stabilising buffer (containing 0.01% sodium azide, 0.067% Kathon™ CG)
5. Enzyme Conjugate Diluent  

CONJ	ENZ	DIL
------	-----	-----

1 x 17ml of IgG enzyme conjugate diluent (containing 0.01% gentamicin sulphate, 0.01% Thiomersal)
6. Enzyme Conjugate Concentrate  

CONJ	ENZ	10X
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1 x 1.7ml of IgG enzyme conjugate concentrate (containing 0.01% gentamicin sulphate, 0.01% Thiomersal).
7. Sample Diluent (Ready-to-use)  

DIL	SPE	1X
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1 x 110ml PBS buffer containing stabilisers and Kathon™ CG (0.067%).
8. Wash Concentrate  

BUF	WASH	25X
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1 x 55ml of concentrated (25X) Tris buffer with Tween 20 (2.75%) and Kathon™ CG (0.067%).

9. Substrate

<b>SUBS</b>	<b>TMB</b>
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1 x 17ml of tetramethylbenzidine (TMB) solution

10. Stop Solution

<b>SOLN</b>	<b>STP</b>
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1 x 17ml of 0.5M H<sub>2</sub>SO<sub>4</sub>

11. Instructions for Use



**\*\* Potentially Biohazardous Material**

**Kathon™ CG is a registered trademark of Rohm and Haas Company.**

***Additional Materials Required***

- Serum collection equipment
- 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 10µl, 100µl, 1ml and 5ml volumes
- Plastic lid or sealing tape for microwell plate
- Timer
- Manual or automatic washing device
- 35 - 39°C incubator
- Paper towels or absorbent paper
- ELISA plate reader with 450nm filter (additional 630 – 650nm filter is optional)

**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.
- 8-well strips should be stored in the resealable pouch along with the sachets of desiccant.
- All unused components should be returned to 2-8°C storage immediately after use.
- Reconstituted Wash Solution is stable for 1 month when stored at 2-8°C.

**Specimen Collection and Storage**

Either serum or plasma can be used in the Biotrin HHV-8 IgG EIA. Once collected by venipuncture, blood should be allowed to clot at room temperature (20-25°C) followed by centrifugation at 1500 x g for 10 minutes. If not for immediate testing within 8 hours, the serum or plasma can be placed at 2-8°C for up to 2-3 days or frozen at -20°C if extended storage or shipment is required (samples are stable at

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-20°C for at least 1 year). Citrated plasma is compatible with the test procedure. Microbially contaminated sera should not be used for testing. Finally, test specimens should not be subjected to repeated freeze-thaw cycles.

**Note:** It is recommended that samples are not heat inactivated.

## Reagent and Specimen Preparation

### Reagent Preparation

- Reagent volumes are based on singleton sample testing.
- Wash Solution  
For each 8-well Strip add 4ml of Wash Concentrate to 96ml of deionised water. Prepared reagent is stable for 1 month if stored at 2-8°C.
- Enzyme Conjugate preparation  
For each 8-well Strip add 100µl of Enzyme Conjugate to 900µl of enzyme conjugate diluent. Prepared solution should not be stored.

All remaining reagents are supplied ready - to - use and are at working dilution.

### Specimen Preparation

For each sample dispense 1ml of Sample Diluent into a labelled test tube or equivalent. Add 10µl of serum or plasma sample and mix.

**Note:** Diluted samples should not be stored, if a repeat test is required a fresh preparation should be used.

### Assay Procedure

1. Allow all components to equilibrate to room temperature (20-25°C) before use.
2. Determine the number of wells required. Establish an identification and distribution plan for controls and samples as indicated in Figure 1 (below). The first strip is suitable for testing 2 patient specimens, each additional strip allows for testing of a further 8 patient specimens.

Figure 1 Strip 1

A		Negative Control
B		Negative Control
C		Cut-Off-Calibrator
D		Cut-Off-Calibrator
E		Positive Control
F		Positive Control
G		Patient No. 1
H		Patient No. 2

3. Remove the desired number of wells, place in a plastic frame and cover with a plastic lid/sealant tape. Return the remaining strips to the pouch and reseal along with desiccant.
4. Prepare Wash Solution (see "Reagent and Specimen Preparation").
5. Prepare patient specimen (see "Reagent and Specimen Preparation").
6. Remove cover from strips and pipette 100µl, in duplicate, of the ready-to-use Negative Control, Cut-Off-Calibrator, Positive Control and 100µl in singleton of the prepared patient specimens to the wells.
7. Cover the wells with a plastic lid/sealing tape and incubate for 30 minutes at 35 - 39°C.
8. Remove cover and wash each well 4 times with Wash Solution (250 - 300µl). After washing firmly tap the plate against an absorbent paper towel.
9. Prepare Enzyme Conjugate (see "Reagent and Specimen Preparation").
10. Pipette 100µl of the prepared IgG Enzyme Conjugate into all wells immediately after the wash step is completed.
11. Cover the wells with a plastic lid/sealing tape and incubate for 30 minutes at 35 - 39°C.
12. Remove cover and wash each well 4 times with Wash Solution (250 - 300µl). After washing firmly tap the plate against an absorbent paper towel.
13. Pipette 100µl of TMB Substrate into all wells immediately after the wash step is completed.
14. Cover the wells with a plastic lid/sealing tape and incubate for exactly 30 minutes at 35 - 39°C.
15. Pipette 100µl of Stop Solution into all wells and mix. Ensure that each addition is in the same sequence and time interval as the addition of Substrate.
16. Read immediately with an ELISA plate reader.

**Note:** Dual wavelength reading is recommended at 450nm with 630nm as the reference wavelength. If this function is not available on the ELISA plate reader use a single wavelength reading at 450nm.

### **Interpretation of Results**

The presence or absence of anti-HHV-8 IgG is determined in relation to the Cut – Off Calibrator (COC).

#### ***Cut – Off Calibrator Value***

- 1) Determine the COC value by assaying the Cut-Off Calibrator in each assay in duplicate.

- 2) Determine the mean OD value, this value is the COC value and is to be used to determine index values.
- 3) An index value is calculated by dividing the sample/control absorbance by the COC value.

**Interpretation (1): Absorbance**

Samples with an absorbance reading greater than the COC x 1.2 are considered reactive (positive) for anti-HHV-8 IgG.

Samples with an absorbance reading less than the COC x 0.8 are considered non-reactive (negative) for anti-HHV-8 IgG.

Samples with an absorbance reading greater than or equal to COC x 0.8 and less than or equal to COC x 1.2 are equivocal.

**Interpretation (2): Index Value**

Data comparison between different assay runs is facilitated by using an index value whereby sample absorbance is expressed relative to the assay cut-off calibrator. In this case, an index value <0.8 or >1.2 indicates sample negativity or positivity, respectively. Equivocality is indicated if the index value is in the range 0.8-1.2 inclusive.

$$\text{Index} = \frac{\text{Control/Sample absorbance}}{\text{Mean Cut-off Calibrator (COC) absorbance}}$$

Samples which are neither reactive (positive) or non-reactive (negative) are considered equivocal and should be re-tested. If the re-test result is equivocal then a second sample should be collected 7-14 days later. An equivocal result with the second sample may be considered unreactive (negative) for anti-HHV-8 IgG, however if recent infection is suspected, it may be confirmed by testing on an alternative method.

**Quality Control Criteria**

The Positive Control and Negative Control must always be included to determine the validity of test results. Results of an assay are considered valid if the following criteria are met:

1. The mean index of the Positive Control is greater than or equal to an index of 1.2
2. The mean index of the Negative Control is less than an index of 0.8.

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

**Limitations of Use**

For research use only in the U.S.A.

- Results must be correlated with the patient's clinical and epidemiological profile and other clinical laboratory results in making the diagnosis of HHV-8 infection.
- A non-reactive (negative) result does not exclude the possibility of HHV-8 infection. The development of a detectable antibody response may occur some days after

infection. In the case of suspected HHV-8 infection a negative result should be followed with a repeat test two weeks later.

- Insufficient data is available to support the interpretation of results of tests performed on other body fluids or pools of sera/plasma.
- Test performance may be affected by deviation from the procedure, interpretation or recommended precautions.
- Assay performance has been validated based on testing controls in duplicate and samples in singleton.

### 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<sup>(4)</sup>, 10-35% in Italy and Mediterranean countries<sup>(5)</sup>, to more than 50% in many African populations<sup>(6)</sup>.

### Performance Characteristics

#### *Sensitivity and Specificity*

A panel of 166 samples was tested to determine the sensitivity of the HHV-8 IgG assay. The samples assayed were characterised by the clinical symptoms associated with HHV-8 infection and Kaposi Sarcoma. These samples were also tested by a HHV-8 IgG IFA and were determined positive. A panel of 114 samples was assayed to determine specificity for the HHV-8 IgG EIA. 114 of these samples were normal human sera. Samples were characterised as negative using different commercially available HHV-8 IgG assays.

HHV-8 Status	Positive	Negative	Equivocal	Total
Positive	150	2	14	166
Negative	3	106	5	114
Total	153	108	19	280

**Table 1:** Sensitivity and Specificity results were calculated.

Sensitivity:  $150/166 = 90.4\%$

Specificity:  $106/114 = 93\%$

Agreement:  $256/280 = 91.4\%$

Sensitivity = True Positives (TP) divided by (TP+False Negatives +Equivocals)x 100%

Sensitivity =  $(150/166)*100 = 90.4\%$

Specificity = True Negatives (TN) divided by (TN + False Positives + Equivocals) x 100%

Specificity = (106/114) \* 100 = 93%

***Intra-assay Reproducibility***

A series of serum specimens ranging in HHV-8 IgG levels from weak reactive to strongly reactive were each assayed a total of twenty times. Replicates were tested on 3 ELISA plates from 3 verification batches of product respectively. The resultant OD values were summated and the mean OD value, standard deviation (SD) and percentage coefficient of variation (%CV) were calculated, Table 2. These same results are presented in terms of assay index values in Table 3.

**The percentage CV expressed in terms of OD (indices) ranged from 7.28% in a strong reactive specimen (SR) to 8.022% in a weak reactive specimen (WR).**

Specimen	1			2			3		
	Mean OD	SD	% CV	Mean OD	SD	% CV	Mean OD	SD	% CV
SR	1.384	0.101	7.280	1.240	0.036	2.872	1.370	0.049	3.543
WR1	0.292	0.013	1.479	0.461	0.012	2.648	0.556	0.023	4.187
WR2	0.185	0.013	7.000	0.493	0.040	8.022	0.362	0.026	7.246

**Table 2:** Intra-assay reproducibility expressed in terms of optical density (OD) on 20 replicates of each of 3 different serum specimens ranging in HHV-8 IgG levels from weakly reactive to strongly reactive on 3 different batches. SR: strongly reactive and WR: weakly reactive.

Specimen	1			2			3		
	Mean Index	SD	% CV	Mean Index	SD	% CV	Mean Index	SD	% CV
SR	7.58	0.55	7.28	6.776	0.195	2.872	5.054	0.179	3.543
WR1	1.60	0.07	4.48	2.517	0.067	2.648	2.053	0.086	4.187
WR2	1.01	0.07	7.00	2.695	0.216	8.022	1.335	0.097	7.246

**Table 3:** Intra-assay reproducibility expressed in terms of Index values on 20 replicates of each of 3 different serum specimens ranging in HHV-8 IgG levels from weakly reactive to strongly reactive on three different batches. SR: strongly reactive and WR: weakly reactive.

### ***Interassay Reproducibility***

A series of serum specimens ranging in HHV-8 IgG levels from unreactive to strongly reactive were each assayed in 10 assays with 3 different operators. Samples were tested on 1 of product and results were combined to determine interassay reproducibility. Each sample was therefore assayed n=10 times. The resultant OD values were summated and the mean OD value, standard deviation (SD) and percentage coefficient of variation (%CV) were calculated, Table 4. These same results are presented in terms of assay index values in Table 5.

**The percentage CV expressed in terms of OD (indices) ranged from 7.04% in a strongly reactive specimen to 15.84 % in a weakly reactive specimen.**

Specimen	Mean OD	SD	%CV	n
SR	1.429	0.101	7.04	10
MR	0.500	0.076	15.27	10
WR	0.319	0.051	15.84	10
UR1	0.058	0.008	13.13	10
UR2	0.061	0.011	18.19	10

**Table 4:** Inter-assay reproducibility expressed in terms of optical density (OD) for 10 assays of each of 5 different serum specimens ranging in HHV-8 IgG levels from non-reactive to strongly reactive. SR: strongly reactive, MR: medium reactive WR: weakly reactive and UN: unreactive.

Specimen	Mean Index	SD	%CV	n
SR	8.397	0.704	8.38	10
MR	2.934	0.436	14.87	10
WR	1.879	0.323	17.21	10
UR1	0.342	0.046	13.54	10
UR2	0.361	0.064	17.83	10

**Table 5:** Inter-assay reproducibility expressed in terms of index for 10 assays of each of 5 different serum specimens ranging in HHV-8 IgG levels from non-reactive to strongly reactive. SR: Strongly reactive, MR: medium reactive, WR: weakly reactive and UR: un-reactive.

**Crossreactivity:**

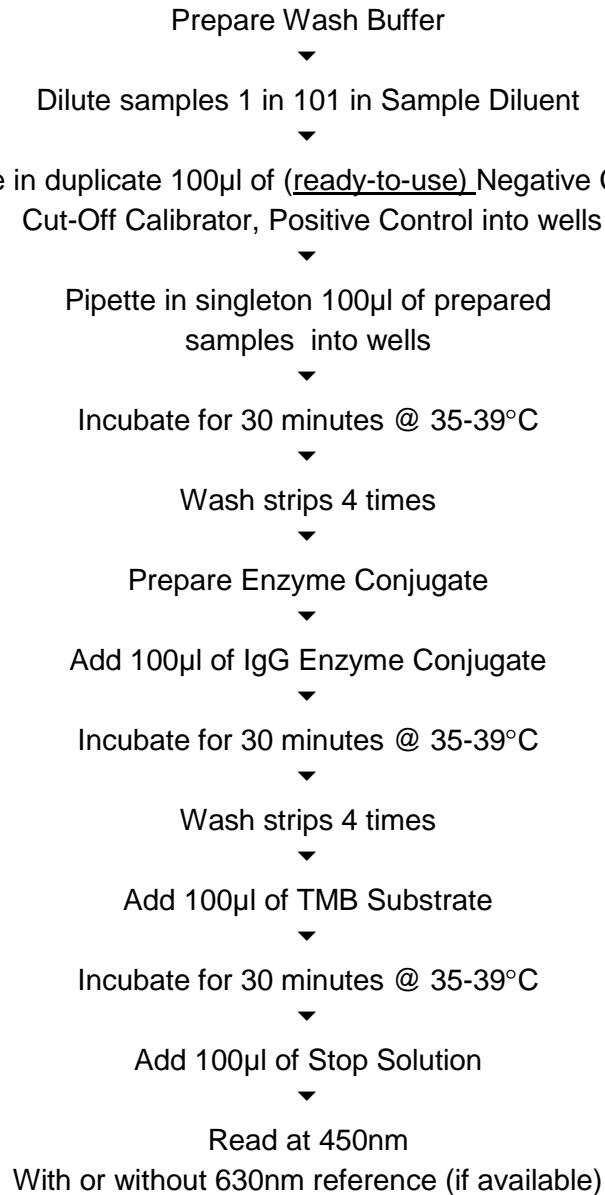
76 serum samples were screened in order to establish the specificity of the Biotrin HHV-8 IgG EIA. All samples were obtained from patients diagnosed with the following diseases:

Sample	Number of Positives
Lupus Erythematosus	0/5
Rhematoid Arthritis (RA)	0/3
Rhematoid Factor (RF)	0/5
Autoimmune	1/7
Lyme IgG	0/4
EBV IgG	2/24
Rubella IgG	0/5
CMV IgG	0/5
VZV IgG	2/5
Hep C	0/2
HIV	0/5
HTLV	0/2
HSV IgG	0/4

**Table 6:** n = 76 potential cross-reactive specimens assayed to determine non-specific binding. Results show the number of samples tested and the number of false positives

**Summary of Human Herpesvirus-8 IgG EIA Procedure**

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



## References

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12. Chang et al *Science* 265:1865-1869, 1994.

### Interpretation of Symbols

*In-vitro* diagnostic medical device



Batch code



Catalogue Number



Temperature limitation



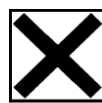
Use by end of



Manufacturer



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



Instructions for Use



### Additional Biotrin Products

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

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



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