

HHV-6 IgG EIA, Cat No: V15HHV6, HHV-317-07 07/09, EN



ENGLISH

**Cat No: V15HHV6
Kit Format: 12X8 well EIA
HHV-317-07**



Human Herpes Virus - 6 IgG Enzyme Immunoassay

An enzyme immunoassay for the detection of Human Herpesvirus 6 IgG antibodies



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Intended Use

The Biotrin Human Herpesvirus-6 (HHV-6) IgG EIA is intended for use for the qualitative detection of HHV-6 IgG antibodies in human serum.

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 more 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. 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 IgG Enzyme Immunoassay detects IgG class antibodies to HHV-6 in human serum. Specific IgG antibodies to HHV-6 IgG antigen when present in serum, combine with Human Herpesvirus-6 antigen attached to the polystyrene surface of the microwell test strips. Residual serum is removed by washing and peroxidase conjugated anti-human IgG is then added. The microwells are washed and a colourless substrate system, tetramethylbenzidine/hydrogen peroxide (TMB/H₂O₂) is added. The substrate is hydrolysed by the enzyme and the chromogen changes to a blue colour. After stopping the reaction with acid, the TMB becomes yellow.

Precautions

Safety

- For *in vitro* diagnostic use only
- This kit is intended for use by qualified laboratory staff only
- All human source material used in the preparation of controls and the calibrator have been tested and found negative for HbsAg and antibodies to HIV1/2 and HCV. However, since no test can offer complete assurance that infectious agents are absent, treat as potentially infectious material.
- 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.
- The concentration of sodium azide in the Controls and Calibrator is classified as harmful and subject to the following risk phrases (R22, R32): "Harmful if swallowed and contact with acids liberates very toxic gases."
- 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.
- The substrate contains TMB, which may irritate the skin and mucous membranes. Any substrate, which comes in contact with the skin, should be rinsed off with water.

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 Buffer Concentrate.
- 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
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12 x 8 wells streptavidin coated biotinylated HHV-6 peptides

2. Positive Control** (Red Cap Colour)

CONTROL	+	IgG
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1 x 200 uL of positive sera (contains 0.1% sodium azide and 0.005% gentamycin sulphate)

3. Negative Control** (Green Cap Colour)

CONTROL	-	IgG
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1 x 200 uL of negative sera (contains 0.1% sodium azide and 0.005% gentamycin sulphate)

4. Cut Off Calibrator** (Yellow cap)

CAL

1 x 400 uL of human sera (contains 0.1% sodium azide and 0.005% gentamycin sulphate)

5. Enzyme Conjugate (Green solution) (Ready to Use)

CONJ	ENZ	1X
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1 x 15 mL of sheep anti-human IgG HRP conjugate with Proclin™ (0.1%) and protein stabilisers.

6. Sample Diluent (Ready-to-use)

DIL	SPE	1X
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2 x 50 mL Tris buffered saline and Proclin™ (0.01%).

7. Wash Buffer Concentrate (Clear cap)

BUF	WASH	20X
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1 x 60 mL of concentrated (20X) phosphate buffered saline with Tween 20 (0.25%) and Proclin™ (0.1%).

8. TMB Substrate (Brown bottle) (Ready to Use)

SUBS	TMB
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1 x 15 mL of tetramethylbenzidine (TMB) solution in a citric-acid citrate buffer

9. Stop solution (Red cap) (Ready to Use)

SOLN	STP
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1 x 15 mL 1M Phosphoric acid

10. Instructions for Use



**** Potentially Biohazardous Material**

Proclin™ 300 is a registered trademark of Rohm and Haas Company.



(Xn - Harmful) Control and Calibrator Sera Safety Precaution:

Concentration of sodium azide in these components is classified as **Harmful** and subject to the following risk phrases (R22, R32) "Harmful if swallowed" and "contact with acids liberates very toxic gas."

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 10 uL, 100 uL, 1 mL and 5 mL 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

- 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. The serum should be separated as soon as possible and refrigerated (2-8°C) or stored frozen (-20°C) or colder if not tested within two days.
- It is recommended that haemolysed, icteric, lipaemic or microbially contaminated sera are not used for testing
- Self-defrosting freezers are not recommended for storage
- Test specimens should not be subjected to repeated freeze-thaw cycles

Reagent Preparation

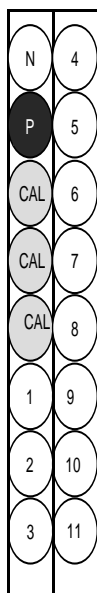
For each 8-well strip add 4 mL of Wash Buffer Concentrate to 76 mL of deionised water. Ensure that any crystals in the wash concentrate have been dissolved. To ensure that the crystals dissolve, the wash concentrate may be placed in a 35-39°C incubator for approximately 1 hour.

Specimen/Calibrator/Control Preparation

For each sample, the negative control, the positive control and the calibrator; dispense 1 mL of Sample Diluent into a labelled test tube or equivalent. Add 10 uL of sample/negative control/positive control/calibrator and mix.

Assay Procedure

NOTE: Ensure all reagents are equilibrated to room temperature (20-25°C) before commencing assay. 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.



1. Allow all components to equilibrate to room temperature (20-25°C) before use.
2. Determine the number of 8-well strips required. Establish an identification and distribution plan for controls and samples as indicated. The first strip is suitable for testing 3 patient samples. Each additional strip allows for testing of a further 8 patient samples.
3. Remove the required number of microwells from the foil sachet and insert into strip holder and cover with a plastic lid / sealant tape. Return the remaining strips to the pouch and reseal with desiccant.
4. Prepare the Wash Buffer, Controls, Calibrator and Patient Specimen.
5. Pipette 100 uL of prepared Patient Specimens (in singleton), Controls (in singleton) and Calibrator (in triplicate) into their respective microwells.
6. Cover plate and incubate for 30 minutes at 35-39°C.
7. Remove cover and wash six (6) times with Wash Buffer. After washing, firmly tap the plate against an absorbent paper towel.
8. Pipette 100 uL enzyme conjugate into each well.
9. Cover plate and incubate for 30 minutes at 35-39°C.
10. Wash six (6) times with diluted wash buffer. After washing, firmly tap the plate against an absorbent paper towel.
11. Pipette 100 uL TMB substrate into each well.
12. Incubate for exactly 10 minutes at room temperature (20-25°C).
13. Pipette 100 uL of Stop Solution into all wells in the same sequence and timing as the TMB addition. Mix well.
14. Read the absorbance of each well within 30 minutes.

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 HHV-6 IgG antibodies is determined in relation to the Cut-off Calibrator.

Important Note: The calibration factor is specific for each batch and is located inside the lid of your kit box. Obtain the calibration factor value before commencing calculations.

Calculation of Cut-Off Calibrator (COC) Value

- 1) Determine the COC by assaying the calibrator in each assay in triplicate.
- 2) Determine the mean OD value of the three calibrator results. Multiply this mean by the calibration factor, the resulting value is the Cut-Off Calibrator (COC) Value and it is the value to be used to determine index values.
- 3) An index value is calculated by dividing the sample/control absorbance by the COC value.

Table 1.

Index	Result
<0.9	Negative
0.9-1.1	Equivocal
>1.1	Positive

Table 2

RESULT	INTERPRETATION
Negative	No detectable IgG antibody. No evidence of recent or past exposure. If specific IgG antibodies are not detected and a recent infection is suspected, this can be confirmed by testing a further specimen 7-14 days later.
Equivocal	Equivocal samples should be retested. Samples that remain equivocal after repeat testing should be repeated by an alternative method or another sample should be collected.
Positive	Presence of detectable IgG antibody suggests recent or past exposure to HHV-6.

Quality Control Criteria

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

- (a) The Negative Control and Calibrator absorbances meet the specification given on the inside of the box lid.
- (b) The Positive Control Index meets the specification given on the inside of the box lid.

Expected Values

In a HHV-6 infection, a high level of IgG may be suggestive of a recent infection. A significant rise in specific IgG antibodies in paired samples is indicative of recent HHV-6 infection.

Limitations of use

- Results must be correlated with the patient’s clinical and epidemiological profile and other clinical results in making the diagnosis of HHV-6 infection. The results from this kit are not by themselves diagnostic and should not be used as the sole diagnostic tool.
- Test results of specimens from immunocompromised patients may be difficult to interpret.
- This test should be performed on serum only. The use of whole blood, plasma or other specimen matrix has not been established.

Performance Characteristics

Sensitivity and Specificity

A total of 212 single serum samples were tested at a public hospital laboratory on the Biotrin HHV6 IgG EIA. The sera, previously characterised by the public hospital laboratory’s in-house HHV6 IgG IFA, consisted of 155 IFA positive and 57 IFA negative samples. The data is summarised in Table 3.

Table 3
Human Herpesvirus-6 IgG Serological Sensitivity and Specificity of
BIOTRIN ELISA versus HHV-6 Status

HHV-6 Status	Positive	Equivocal*	Negative	Total
Positive	151	1	3	155
Negative	6	2	49	57
Total	157	3	52	212

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

$$= \frac{151}{(151 + 3 + 1)} \times 100 = 97.4\%$$

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

$$= \frac{49}{(49 + 6 + 2)} \times 100 = 86\%$$

$$\text{Agreement} = \frac{200}{212} = 94.3\%$$

*Retesting of equivocal samples was not conducted, as the samples were unavailable.

Note: Sensitivity and specificity refers to the comparison of the Biotrin assay results to that of other assays normally used to diagnose HHV-6. There was not an attempt to correlate the assay's results with disease presence or absence. No judgement can be made on the comparison's accuracy to predict disease. Since the above studies were performed on a pre-selected, retrospective, population, no calculations for the assay's positive and negative predictive value may be done or inferred.

Reproducibility

The reproducibility of the Biotrin Human Herpesvirus-6 IgG EIA kit was determined by testing 8 sera 3 times each on three kit batch numbers on three different days. Within-run, between day, between batch and total precision were calculated and are presented in Table 4.

**Table 4
Reproducibility Data
Biotrin Human Herpesvirus-6 IgG EIA
Precision Measures (Using Index Value*)**

Sample	n	*Mean	Within		Between Day		Between Batch		Total	
			*S.D	CV	*S.D	CV	*S.D	CV	*S.D	CV
Positive	24	2.56	0.21	8.4%	0.17	6.8%	0.36	14.2%	0.40	15.6%
Cut-off	24	1.00	0.10	10.2%	0.00	0.00	0.00	0.0%	0.09	9.4%
Negative	24	0.17	0.04	21.7%	0.02	8.9%	0.10	59.4%	0.09	54.5%
#1	24	3.72	0.35	9.5%	0.20	5.4%	0.51	13.8%	0.58	15.6%
#2	24	4.64	0.35	7.5%	0.00	0.0%	0.25	5.4%	0.39	8.5%
#3	24	2.57	0.29	11.2%	0.00	0.0%	0.21	8.0%	0.33	12.9%
#4	24	2.04	0.21	10.4%	0.00	0.0%	0.32	15.5%	0.33	16.4%
#5	24	2.21	0.20	9.0%	0.00	0.0%	0.00	0.0%	0.19	8.5%
#6	24	1.60	0.09	5.8%	0.00	0.0%	0.06	3.9%	0.10	6.5%
#7	27	0.63	0.12	19.7%	0.04	6.3%	0.00	0.0%	0.13	19.8%
#8	27	1.07	0.08	7.0%	0.04	3.6%	0.20	18.7%	0.19	17.3%

All values are calculated from Index Values
SD = Standard Deviation; CV = Coefficient of Variation

Summary of HHV 6 IgG EIA Procedure

Important Note:

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

Prepare Wash Buffer



Dilute Samples, Controls and Calibrator 1 in 101 in Sample Diluent



Pipette 100 uL of prepared samples, Controls and Calibrator into their respective wells



Incubate for 30 minutes @ 35-39°C



Wash 6 times with Wash Buffer



Add 100 uL of Enzyme Conjugate



Incubate for 30 minutes @ 35-39°C



Wash 6 times with Wash Buffer



Add 100 uL of TMB Substrate



Incubate for 10 minutes @ room temperature



Add 100 uL Stop Solution



Read results within 30 minutes at 450nm with reference filter of 600-650nm (if available)

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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



Additional Biotrin Products

Biotrin International offers a unique portfolio of Human Herpesvirus assays.

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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