Kalon Biological Ltd

HSV type 2 IgG

An enzyme immunoassay specific for IgG antibodies to type 2 Herpes simplex virus in human serum

INTENDED USE

The kit is supplied for research use only. Not for use in diagnostic procedures. The results obtained should be considered as provisional until confirmed by an alternative method and should not be used in isolation but together with all the information available to the researcher.

This is a brief protocol and assumes that the operator is familiar with performing enzyme linked immunosorbent assays. Contact Kalon Biological Ltd if more information is required.

INTRODUCTION

Infection with the two strains of herpes simplex viruses (HSV1 and HSV2) causes a primary infection in the non-immune person. A common characteristic of herpes viruses is the ability to infect nerve tissue and to enter into a latent phase of infection. This then leads to cycles of reactivation, causing cold sores and other clinical manifestations.

HSV type 1 is predominantly isolated from infections in the mouth and face, while HSV type 2 is most frequently the cause of genital infections. However, the symptoms of HSV1 and HSV2 overlap and either virus can cause genital and orofacial infection and these infections are clinically indistinguishable. However, the anatomical site and the type of virus has an influence on the frequency of future reactivation: recurrence of genital HSV2 infections are more common than genital HSV1 infection, and orofacial HSV1 infections recur more frequently than orofacial HSV2 infections.

Neonatal infection can range from mild disease to a fatal disseminated infection. Overall the mortality is 65%, with only 10% escaping neurological complications. In the United States the incidence is about 1 in 2500 to 1 in 10000. The spread of HSV2 from the maternal genital tract to the newborn during labour is therefore a serious risk. This can be reduced with the use of antiviral drugs but unfortunately most serological assays are severely limited by cross reactivity between type 1 and 2 viruses. Since the majority of adults have antibodies arising from oral infections, the predictive power of these tests is negligible.

The Kalon HSV type 2 specific IgG assay is based on the use of a recombinant type 2 antigen (gG2) that has been modified to eliminate reactivity arising from type 1 infections whilst retaining the natural antigenic characteristics of HSV2.

PRINCIPLE OF THE ASSAY

The assay is based on the indirect ELISA principle.

Polystyrene microtitre plate wells are supplied pre-coated with a recombinant modified gG2 antigen of HSV2. Serum samples are diluted and incubated in these wells during which reactive antibody is captured. After a wash step the surface is probed for IgG class antibodies by incubation with an enzyme conjugated anti-IgG tracer. Following a second wash step, enzyme substrate and a chromogenic reagent are added to the wells. The enzyme incubation is halted by the addition of acid, which also has the effect of both changing and enhancing the amount of colour produced. This is measured in a photometer. The optical density is proportional to the amount of anti-HSV2 IgG present in the original sample. This is compared with a cut-off calibrator which is run in each assay and has been designed to distinguish between non-specific binding and a true positive reaction.

KIT PRESENTATION

	KIT PRESENTATION	1 plate	10 plate
❑	**Antigen coated microtitre plate** 8 well strips coated with a recombinant modified HSV gG2	96 wells	960 wells
❑	**Positive Control** Human serum	100 µL	250 µL
❑	**Cut-off Calibrator** Human serum	200 µL	500 µL
❑	**Negative Control** Human serum	100 µL	250 µL
❑	**Tracer Concentrate (×41)** Anti-human monoclonal antibody labelled with peroxidase	500 µL	4 mL
❑	**Assay Diluent** Buffered saline with protein stabiliser, surfactant and 0.01% Thiomersal	50 mL	500 mL
❑	**Wash Concentrate (×40)** Buffered saline and surfactant	50 mL	250 mL
❑	**Substrate Solution** Buffered peroxide source	15 mL	3 × 60 mL
❑	**TMB Chromogen** in dimethylsulfoxide	500 µL	4 mL
❑	**Stop Solution** 0.5 mol/L sulfuric acid	15 mL	175 mL
❑	**Resealable plastic bags**	2	12

Store reagents refrigerated. Do not use beyond the expiry date printed on the label.

ADDITIONAL REQUIREMENTS

- ❑ Disposable tip micropipettes to deliver volumes from 10 µL to 1 mL and preferably multichannel pipettes for volumes of 10, 100 and 200 µL.
- ❑ 37°C incubator
- ❑ Wash bottle or automatic microtitre plate washer.
- ❑ Microtitre plate photometer fitted with a 450 nm filter.
- ❑ Clean volumetric laboratory plastic or glassware.
- ❑ Disposable paper towels.
- ❑ Purified water

SAFETY PRECAUTIONS

Treat all serum or plasma samples - and the materials they come into contact with - as potentially infectious.

ASSAY PROTOCOL

Preparation

1) Select the number of microwell strips required (four wells in total for the controls and calibrator and one for each test specimen).

2) To simplify the in-well dilution steps (6) and (7), test sera can be dispensed into an empty microtitre plate or microtubes (not the HSV type 2 plate!): 40 or 50 µL is sufficient. The 10 µL required for the assay in step (7) can then be dispensed, quickly and accurately, with a multichannel pipette.

3) Prepare the required volume of working strength tracer by mixing Tracer Concentrate and Assay Diluent 1 to 40, e.g. add 250 µL Tracer Concentrate to 10 mL Assay Diluent.

4) Prepare the required volume of working strength wash solution by diluting Wash Concentrate 1 in 40, e.g. make 25 mL Wash Concentrate to 1 L with purified water.

5) Prepare the required volume of working substrate solution by mixing TMB Chromogen and Substrate Solution 1 to 40; e.g. add 250 µL TMB Chromogen to 10 mL Substrate Solution. The substrate solution made in this way is colour coded pink as an aid to dispensing. A *blue* colour prior to use indicates that the solution is contaminated. In this circumstance a fresh dilution into clean glassware should be made.

Primary Incubation

6) Dispense 200 µL Assay Diluent into each well

7) Dispense 10 µL of the kit calibrator and the two kit controls into their designated microwells; the Cut-off Calibrator should be run in duplicate. Mix each well as you go by pumping the diluted serum in and out of the micropipette five times.

8) Dispense 10 µL of each test specimen into their designated microwells, mixing thoroughly as for the kit sera. Steps (6) and (7) should be completed within a combined time of 5 minutes or less - see step (2) for advice.

9) Seal the plate in the plastic bag and incubate at 37°C for 30 minutes.

10) Wash the microwells -

Alternately fill and aspirate the microtitre wells with 350 µL of fresh working strength wash solution a total of four times. Tap out residual wash solution on paper towelling ready for the next step.

Secondary Incubation

11) Dispense 100 µL working strength Tracer

12) Seal the plate in the plastic bag and incubate at 37°C for 30 minutes

13) Wash the microwells as before.

Enzyme Incubation

14) Dispense 100 µL working substrate solution

15) Incubate the plate uncovered at 18° to 25°C for 30 minutes

16) Dispense 100 µL Stop Solution into each microwell.

Assay Completion

17) Read the microtitre well optical densities at 450 nm within 30 minutes of adding Stop Solution. The microtitre plate reader should be blanked on air or using the 620 nm reading.

18) Verify the assay.

The assay can be considered valid if the protocol has been followed correctly, the Positive Control optical density is greater that 0.8 and the ratio of the Cut-off Calibrator to the Negative Control is greater than 2.0.

19) Interpret the results.

Score results with an optical density greater than Cut-off $\times$ 1.1 as positive.

Score results with an optical density less than Cut-off $\times$ 0.9 as negative.

Results between these values, that is $0.9 \leq$ Cut-off ≤ 1.1, are equivocal and should be repeated to confirm the status.

EXPECTED RESULTS

The HSV type 2 IgG assay was tested blind against 299 specimens collected from a UK genito-urinary medicine clinic and previously characterised for HSV1 and HSV2 antibodies by a monoclonal antibody blocking[3]. The incidence of HSV1 antibodies was 181/299 (61%). The HSV2 antibody results are shown below.

Reference Assay	Kalon HSV type 2 IgG	
	positive	negative
HSV2 positive	80	3
HSV2 negative	2	214

Relative Sensitivity = 80/83 = 96.4% (89.8% - 99.3%)*
Relative Specificity = 214/216 = 99.1% (96.8% - 99.5%)*
* 95% confidence intervals

The incidence of antibodies to HSV2 is expected to vary between populations, age and socio-economic group. For example, in a survey of 80 volunteer blood donors in the UK, two were positive (3%).

Children are less likely to have antibodies while babies born to HSV2 positive mothers are likely to have antibodies that are maternally derived, whether or not there is a congenital infection.

REFERENCES

1. Peto TEA & Juel-Jensen. In: Oxford Textbook of Medicine, 3rd edition. DJ Weatherall, JGG Ledingham & DA Warrell (eds) 1996.
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3. R.Gopal, T.Gibbs, MJ. Slomka, J.Whitworth, LM. Carpenter, A.Vyse and DWG Brown. Journal of Virological Methods 2000;**87:**71-80.

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Instructions for use K30-191