HSV 2 IgG

Enzyme immunoassay for the quantitative determination of IgG class antibodies to Herpes Simplex 2 Virus in serum or plasma

CE

REF **GD7605 00**

IVD

INDICATION

Herpes Simplex Virus types 1 (HSV 1) and 2 (HSV 2) are large complex DNA-containing viruses which have been shown to induce, during infection, the synthesis of several proteins possessing an high number of crossreactive determinants and just a few of type specific sequences.
The majority of primary genital herpes infections and recurrent genital infections are caused by HSV 2. Non genital herpes infections, such as common cold sores, are caused primary by HSV 1.
The detection of virus-specific antibodies is important in the diagnosis of acute/primary virus infections or reactivation of a latent one, in the absence of evident clinical symptoms. Asymptomatic infections may happen for HSV in apparently healthy individuals and during pregnancy. Severe herpes infections may happen in immunosuppressed or immunocompromised patients.
Serological HSV-specific assays provide the clinician with a helpful and reliable diagnostic test for the monitoring of "risk" population and for the follow up of primary infections.

PRINCIPLE OF THE ASSAY

Microplates are coated with synthetic HSV 2 antigen which, in the first incubation, captures anti-HSV 2 antibodies, if present in the sample.
After washing out the other components of the sample, IgG antibodies are detected by means of an anti human IgG antibody, conjugated with horseradish peroxidase (HRP). The intensity of the colour, generated by the enzyme on the substrate/chromogen mixture in the last incubation, is proportional to the content of anti-HSV 2 antibodies in the sample.
IgG concentrations in samples are quantified by means of a standard curve calibrated in arbitrary unit per milliliter (arbU/ml), as no international standard is available.

KIT CONTENT

1. **Reagent A – Microplate**
   12x8 strips.
   8 wells breakable strips, coated with HSV 2 specific antigen. The strips are assembled on a plastic frame and contained in a sealed bag with desiccant. Bring the strips to room temperature before use, to prevent any moisture formation inside the bag.

2. **Reagent B1 – Enzymatic Tracer 20x**
   1 vial of 0.8 ml.
   Stabilized proteic buffer solution containing human anti-IgG polyclonal antibody conjugated with Horseradish peroxidase (HRP), 20x concentrated.

3. **Reagent B2 – Tracer Diluent**
   1 vial of 16 ml.
   Proteic buffer solution for the dilution of the concentrated tracer; it contains 0.02% Gentamicin sulphate and 0.3% Kathon GC as preservatives.

4. **Reagent C – Washing Solution 20x**
   1 vial of 60 ml.
   Concentrated solution to be diluted 1:20 with distilled water. It contains PBS buffer pH 7.4 with detergents and 0.1% Kathon as preservative.

5. **Reagent D – Chromogen**
   1 vial of 8 ml.
   Ready to use solution containing Tetramethylbenzidine (TMB) with activators and stabilizers, diluted in phosphate/citrate buffer.
   Avoid light exposure.

6. **Reagent E – Substrate**
   1 vial of 8 ml.
   Ready to use solution containing Urea peroxide diluted in phosphate/citrate buffer.

7. **Reagent F – Stop Solution**
   1 vial of 16 ml.
   Ready to use solution, it contains a mixture of 1 M Hydrochloric and Phosphoric acid.
   The reagent is **irritant**: x_i R36/37/38; S(1/2)26-45 Handle with care.

8. **Reagent G – Sample Diluent**
   1 vial of 60 ml.
   Proteic solution for sample preparation; it contains a detergent, proteic stabilizers, 0.09% Sodium azide and 0.3% Kathon GC as preservatives.

9. **HSV 2 IgG Standards**
   6 vials of 2 ml each.
   Ready to use liquids containing HSV 2 IgG at the following concentrations:
   S_0: 0 arbU/ml, **S_1**: 2.5 arbU/ml, **S_2**: 10 arbU/ml,
   S_3: 25 arbU/ml, **S_4**: 50 arbU/ml, **S_5**: 100 arbU/ml.
   Standards are prediluted in the Sample Diluent.

10. **Cardboard sealers**
    2 cardboard sealers to be used to cover the plate during the incubations.

11. **Package insert:** instruction for use GD7605 00 it/ing.

MICROBIOLOGICAL STATE AND CLEANING GRADE

1. All the materials of human origin resulted negative to HBsAg, HIV 1&2 and HCV FDA approved tests. Anyhow, as no test can guarantee the absolute absence of infective agents, handle reagents as potentially infected, especially standards, controls and samples. All objects come in direct contact with samples and all residuals of the assay should be treated or eliminated as potentially infected. Best procedures for inactivation are treatments with autoclave at 121 °C for 30 minutes or with sodium hypochlorite at a final concentration of 2.5% for 24 hours.
2. Avoid any contact with skin and mucous membrane, in particular for Stop Solution.
3. Use protective disposable talk-free gloves.
4. Avoid contaminating reagents when taking them from the vials. We recommend to use automatic pipettes with disposable tips. When dispensing reagents, do not touch with tips the wall of wells in order to avoid cross-contaminations.
5. For the washing step, use only the Washing Solution provided in the kit and follow carefully the indications reported in "WASHING INSTRUCTION".
6. Avoid the substrate/chromogen to come in contact with oxidizing agents or metallic surfaces; avoid intense light exposure during incubation or reagent preparation.

STORAGE AND STABILITY

1. The kit has to be stored at 2-8 °C and used before the expiry date stated on the label.
2. Unused strips have to be placed in the bag containing the desiccant and firmly sealed before re-store at 2-8 °C. After opening the strips are stable up to the expiry date stated on the label.
3. The diluted washing solution can be stored for one week at room temperature or 3 weeks at 2-8 °C.
4. Diluted tracer is stable one week at 2-8 °C, if stored in a disposable sterile container.
5. When preparing chromogen/substrate we recommend the use of plastic disposable containers. The chromogen/substrate solution is stable for 4 hours at room temperature, protected from light.
6. All other reagents can be repeatedly used up to exhaustion if stored at 2-8 °C, provided that they are handled carefully to avoid any environment contamination. Under these conditions the reagents are stable up to the expiry date stated on the labels.

AUXILIARY MATERIALS

- Semi automatic pipettes of 10, 200 and 1000 µl
- Vortex mixer and absorbent paper
- Chronometer
- Ultrapure Elisa grade water
- Photometric reader of microplates or microstrips, linear up to at least 2 OD and supplied with filters of 450 nm and 620-630 nm.
- Microplate incubator set at 37 (±1) °C.
- Automatic microplates washing device or manual apparatus capable of aspirating and dispensing volumes of 300 µl.

SAMPLES

Either serum or plasma can be used. If the assay is not immediately performed, the samples should be kept at 2-8 °C for one week; otherwise they should be stored at - 20 °C. Avoid repeated freeze-thaw cycles. Samples must not be turbid, lipemic, haemolyzed and microbiologically contaminated.

REAGENTS PREPARATION

- WASHING SOLUTION: dilute 1:20 with distilled or ELISA grade water (e.g. 60 ml of Reagent C + 1200 ml of distilled water) and mix carefully before use. It is recommended to store diluted washing solution at room temperature for immediate use.
- TRACER: dilute concentrated tracer (Reagent B1) 1:20 with Tracer Diluent (Reagent B2) and mix carefully on vortex.
- CHROMOGEN/SUBSTRATE: prepare in disposable plastic container, according to needs, the substrate/chromogen solution by mixing Reagent D with Reagent E in equal volumes.

WASHING INSTRUCTION

A good washing procedure is essential to obtain correct and precise analytical results.
We therefore recommend to use a good quality ELISA microplate washer, maintained at a good level of washing mechanical performances.
Generally, 3-5 automatic washing cycles of 0.3 ml/well are sufficient to avoid false positive reactions and remove high background. Anyhow we recommend to calibrate the washing system on the kit itself so to match the declared analytical performances.
In case of manual washing, we suggest to perform 5 washing cycles, dispensing and aspirating 0.3 ml/well per cycle.
In any case the liquid washed out from the plates must be inactivated with a sodium hypochlorite solution at a final concentration of 2.5%, before being thrown away or autoclaved, as it must be considered as potentially infected.

ASSAY PROCEDURE

1. At least one hour before use, bring all reagents, standards and samples to room temperature (18-30 °C), mixing them carefully on vortex.
2. Do not mix reagents from different lots.
3. We recommend to distribute standards and samples in duplicate.
4. Distribution and incubation times must be the same for all wells in the same analysis.
5. Avoid long interruptions between each step of the assay procedure.
6. It is suggested to eliminate the excess of washing solution from the microplate after washing by blotting it gently on an absorbent paper pad.
7. The colour developed in the last incubation is stable for a maximum of one hour. Otherwise, in case of reading after 10-15 min after dispensing stop solution, immediately place the strips **in the dark**.
8. We recommend to read the plate with an ELISA automatic reader able to subtract the background at 620-630 nm and to read the absorbance of samples and standards at 450 nm. The "blanking" of the instrument is to be carried out in the blank reagent well where only substrate-chromogen and stop solutions are added.

ASSAY SCHEME

1. Dilute samples 1:50 with Sample Diluent (e.g.: 10 µl sample + 500 µl of Reagent G).
   Do not dilute standards. Carefully mix on vortex before dispensing.
2. Follow the scheme:

		HSV 2 antigens coated wells		
	REAGENTS	**Blank**	**Standard**	**Sample**
First incubation	Standard	-	100 µl	-
	Diluted sample	-	-	100 µl
	- Cover the strips with cardboard sealer - Incubate **60 minutes at 37 (± 1) °C**			
Wash	- Peel out the cardboard sealer and aspirate the reaction solution from all wells - Rinse 5 times with 300 µl of diluted washing solution, carefully aspirating off the remaining liquid			
Second incubation	Diluted tracer	100 µl	100 µl	100 µl
	- Cover the strips with cardboard sealer - Incubate **60 minutes at 37 (± 1) °C**			
Wash	- Peel out the cardboard sealer and aspirate the reaction solution from all wells - Rinse 5 times with 300 µl of diluted washing solution, carefully aspirating off the remaining liquid			
Colorimetric reaction	Chromogen/Substrate (Reagents D+E)	100 µl	100 µl	100 µl
	- Cover the strips with cardboard sealer - Incubate **20 minutes at room temperature** (20-25 °C), avoiding light exposure			
	Reagent F (Stop Solution)	100 µl	100 µl	100 µl
	Read the absorbance of each well against Blank at 450 and 620-630 nm			

VALIDITY OF THE ASSAY

The assay is to be considered valid if:

1. The OD 450 nm of the blanking well is lower than 0.100. Higher values indicate a chromogen/substrate contamination. In such a case, repeat the assay carefully checking the reagent.
2. After subtracting the blank, the mean OD 450 nm value for the Standard 0 arbU/ml is lower than 0.200. Higher values indicate an incorrect washing procedure. In such a case, check the efficiency of the washing device.
3. The mean OD 450 nm value of the Standard 2.5 arbU/ml is higher than the Standard 0 arbU/ml value.
4. The mean OD 450 nm value of the Standard 100 arbU/ml is higher than 1.000. Lower values indicate kit or calibrator decay. Before repeating the assay, check the expiry date of the kit.

CALCULATION OF RESULTS

We recommend to elaborate the standard curve with the 4 parameters curve fitting system and calculate the concentration of anti-HSV 2 antibodies in samples.
Values are expressed in arbitrary units for ml (arbU/ml). In case no automatic calculation is available, draw the standard curve on a lin-lin paper and calculate the IgG concentration in samples on a point-to-point fitting elaboration.

Example of calculation
Do not utilize for the calculation of results.

Standard	arbU/ml	OD 450 nm
S_0	0	0.033
S_1	2.5	0.247
S_2	10	0.468
S_3	25	0.893
S_4	50	1.727
S_5	100	2.487
Positive sample	48	1.663

RESULTS INTERPRETATION

From the study of a donors population, it has been observed that the value 10 arbU/ml can be used to discriminate negative from positive samples:

- Samples with **concentration < 10 arbU/ml** are considered **negative** to anti HSV 2 IgG antibodies.
- Samples with **concentration > 10 arbU/ml** are considered **positive** to anti HSV 2 IgG antibodies.

It is recommended each laboratory to establish its appropriate cut-off on the basis of the analyzed population and of other clinical and pathological data.

Particular attention is necessary in the results interpretation in case of samples from pregnant women, as HSV infections may cause severe neonatal malformations.

Note:

- As reported by literature, the HSV 2 test cross-reacts with HSV 1, due to the determinants which are common to both viral types.

ANALYTICAL PERFORMANCES

Reproducibility

a. Within Run

Within run precision has been determined on 20 replicates of three different samples in the same analytical run.
CV values ranging from 5.6 to 19.6% have been found, depending on OD 450 nm values.

b. Between Run

Between run precision has been determined on replicates of three different samples in 10 different analytical runs.
CV values ranging from 6.4 to 20.5% have been found, depending on OD 450 nm values.

Diagnostic sensitivity and specificity

Diagnostic sensitivity and specificity have been determined on a panel of positive and negative samples in comparison with a reference test.
The following results have been found:

Sensitivity: 100%
Specificity: ≥95%

PRECAUTIONS IN USE

Reagent F is **irritant (Xi)**. Refer to Safety Data Sheet.
The other reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes.
The use of laboratory reagents according to good laboratory practice is recommended.

Waste Management

Please refer to local legal requirements.

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