SYMBOL KEYS

Temperature Limitation	Consult Instructions for use	Date of Manufacture	Batch Number / Lot Number
Manufacturer	*In vitro* Diagnostic Medical Device	This side up	Contains sufficient for <n> tests
Use by	Catalogue Number	Do not reuse	

Manufactured by:

Zephyr Biomedicals

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0222/VER-01

Qualisa™

Microwell Enzyme Immunoassay | **HSV 1,2 IgG**

Enzyme linked Immunosorbent Assay (ELISA) for Qualitative Detection of HSV 1,2 IgG antibody in Human Serum

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2°C to 8°C

INTENDED USE

Qualisa™ HSV 1,2 IgG is intended for the Qualitative detection of IgG antibodies to herpes simplex virus (HSV) infection, or for evaluating paired sera for the presence of a significant increase in herpes specific IgG. For in Vitro Diagnostic Use only.

INTRODUCTION

Herpes Simplex Virus is a common pathogen and its primary infection is usually asymptomatic. There are two immunologically distinct types of HSV: Type 1 and Type 2. HSV 1 is generally associated with oral infection and lesions above the waist, and HSV 2 is associated with genital infections and lesions below the waist. Clinical cases primarily are 1) eczema herpeticum with eczematous skin changes with numerous lesions, 2) Gingivo-stomatitis and 3) Herpes sepsis, almost only found in newly born premature infants. HSV 1,2 IgG is an accurate serologic method to detect HSV specific antibody in serum sample.

PRINCIPLE

Purified HSV antigen is coated on the surface of microwells. Diluted patient serum is added to wells, and the HSV IgG specific antibody, if present, binds to the antigen. All unbound materials are washed away. After adding enzyme conjugate, it binds to the antibody-antigen complex. Excess enzyme conjugate is washed off and TMB substrate is added. The enzyme conjugate catalytic reaction is stopped at a specific time and absorbance is determined for each well at 450nm and 630nm with an ELISA reader. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample.

MATERIALS AND COMPONENTS

Materials provided with the test kit:

1. Coated Microwells: Purified HSV 1,2 antigen coated wells.
2. Sample Diluent: Ready to use
3. Negative Control: Range stated on the label
4. Positive Control: Range stated on the label
5. Calibrator.
6. Wash Buffer Concentrate (20X).
7. Enzyme Conjugate: Ready to use.
8. TMB Substrate: Ready to use
9. Stop Solution: Ready to use

Materials required but not provided

1) Precision pipettes: 10µl, 20-200µl, 100-1000µl
2) Disposable pipette tips
3) Distilled water
4) Disposable Gloves
5) ELISA reader
6) ELISA washer

STORAGE AND STABILITY

1. **Qualisa™ HSV 1,2 IgG** kit is stable at 2-8°C up to expiry date printed on the label.
2. Coated Microwells should be used within one month upon opening the pouch provided that once opened, the pouch must be resealed to protect from moisture. If the colour of the desiccant has changed from blue to white at the time of opening the pouch, another coated Microwells pouch should be used.
3. Diluted Wash Buffer is stable up to one week when stored at 2-8°C.

SPECIMEN COLLECTION & PREPARATION

1. Collect Blood specimen by venipuncture according to standard procedure.
2. Serum only should be used.
3. Avoid grossly hemolytic, lipemic or turbid samples.
4. Preferably use fresh samples. However specimens can be stored up to 48 hours at 2-8°C, for short duration.
5. For longer storage, specimens can be frozen at -20°C. Thawed samples must be mixed prior to testing.
6. Do not heat inactivate before use.
7. Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.
8. Specimen should be free from particulate matter and microbial contamination.

PRECAUTIONS

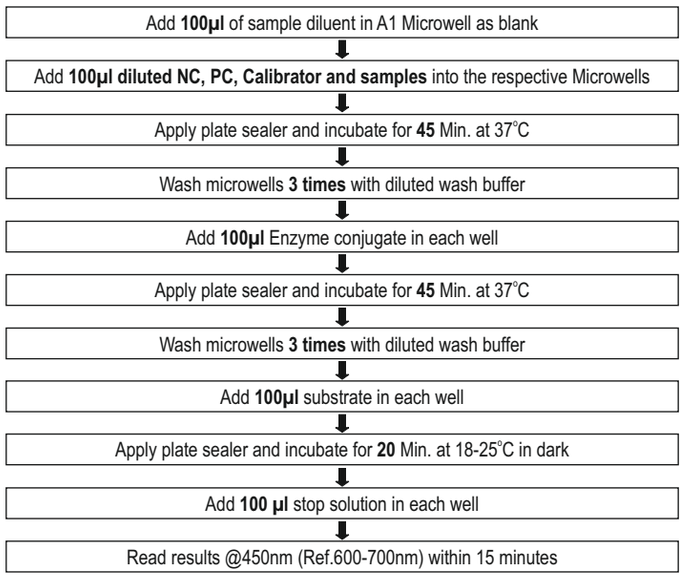
(1) Bring all reagents and specimen to room temperature before use.
(2) Do not pipette any material by mouth.
(3) Do not eat, drink or smoke in the area where testing is done.
(4) Use protective clothing and wear gloves when handling samples.
(5) Use absorbent sheet to cover the working area.
(6) Immediately clean up any spills with sodium hypochlorite.
(7) All specimens and controls should be considered potentially infectious and discarded appropriately.
(8) Neutralize acid containing waste before adding hypochlorite.
(9) Do not use kit after the expiry date.
(10) Do not mix components of one kit with another.
(11) Always use new tip for each specimen and reagent.
(12) Do not allow liquid from one well to mix with other wells.
(13) Do not let the strips dry in between the steps.

REAGENT PREPARATION

1. Dilute wash buffer 20 times (for example add 5ml concentrated buffer to 95ml of distilled or deionized water).
2. All reagents should be brought to room temperature (18-25°C) and mixed by gently inverting or swirling prior to use. Do not induce foaming.

TEST PROCEDURE

1. Place the desired number of coated strips into the holder.
2. Prepare 1:40 dilutions by adding 5µl of the test samples, negative control, positive control and calibrators to 200µl of sample diluent. Mix well.
3. Dispense 100µl of diluted serum samples, negative control, positive control and calibrator into the appropriate wells. For the reagent blank, dispense 100µl of sample diluent in A1 well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 45 minutes at 37°C.
4. Wash each well three times by filling approximately 350µl diluted wash buffer & blot dry.
5. Dispense 100µl of enzyme conjugate to each well and incubate for 45 minutes 37°C.
6. Wash each well three times by filling approximately 350µl diluted wash buffer & blot dry.
7. Dispense 100µl of TMB Substrate to each well and incubate for 20 minutes at room temperature, away from direct light.
8. Add 100µl of Stop Solution to stop the reaction.
9. Read O.D. at 450 - 630 nm with an ELISA reader.



RUN CRITERIA

The test run may be considered valid provided the following criteria are met:

1. The O.D. value of the reagent blank against air from a microwell reader should be less than 0.150.
2. If the O.D. value of the Calibrator is lower than 0.250, the test is not valid and must be repeated.
3. The HSV 1,2 IgG Index for Negative and Positive Control should be in the range stated on the labels.

CALCULATION OF RESULTS

1. To obtain Cut off Value (COV): Multiply the O.D of Calibrator by Factor (f) (which is lot specific & will be printed on the label of the calibrator vial).
2. Calculate HSV 1,2 IgG Index of each determination by dividing the OD values of each sample by obtained OD value of Cut off.

For example:
If the Factor (f) value on the label = 0.5
Calibrator O.D. = 2.224
COV = 2.224 x 0.5 = 1.11
Patient sample O.D. = 2.009
HSV 1 IgG Index = 2.009/1.11 = 1.887 (Positive result)
Patient sample O.D. = 0.088
HSV 1 IgG Index = 0.088/1.11 = 0.079 (Negative result)

INTERPRETATION OF THE RESULT

HSV 1,2 IgG Index Value	Result
HSV 1,2 IgG Index value <0.90	Negative for IgG antibody to HSV 1,2
HSV1, 2 IgG Index value 0.91-1.19	Equivocal, sample should be retested
HSV 1,2 IgG Index value >1.2	Positive for IgG antibody to HSV 1,2

PERFORMANCE CHARACTERISTICS

The precision of the assay was evaluated by testing three different sera of eight replicates over a period of one week. The intra-assay and inter-assay C.V. are summarized below:

	Negative	Low positive	Positive
Intra-assay	8.9 %	7.9 %	6.8%
Inter-assay	10.3 %	8.3 %	7.5%

LIMITATIONS OF THE ASSAY

1. As with other serological assays, the results of these assays should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.
2. Samples obtained too early during primary infection may not contain detectable antibody.
3. A single serum sample should not be used to aid in the diagnosis of recent infection. Paired samples should be collected and tested simultaneously to look for seroconversion.

BIBLIOGRAPHY

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2. Vestergaard, B.F., P.C. Grauballe and H. Spanggaard. Titration of herpes simplex virus antibodies in human sera by the enzyme-link immunosorbent assay (ELISA). Acta Pathol. Microbiol. Scand. Sect. B 85:446-448, 1977.
3. Coleman, R.M., L. Pereira, P.D. Bailey, D. Dondero, C. Wickliffe, and A.J. Nahmias. Determination of herpes simplex virus type-specific antibodies by enzyme-linked immunosorbent assay. J. Clin. Microbiol. 18(1983) 287.