PERFORMANCE CHARACTERISTICS

Precision:

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	8.2%	7.2%	6.3%
Inter-assay	9.1%	8.2%	6.5%

LIMITATIONS OF THE ASSAY

1. To prevent false negative and false positive IgM test results caused by the presence of specific IgM and rheumatoid factor (RF) in some specimens, reagents provided in this kit has been formulated to resolve these interferences. However, specimens with extremely high RF and high autoimmune antibodies, the possibility of these interferences cannot be ruled out entirely.
2. 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.
3. A negative serological test does not exclude the possibility of past infection. Following primary HSV infection, antibody may fall to undetectable levels and then be boosted by later clinical infection with the same or heterologous type. Such a phenomenon may lead to incorrect interpretations of seroconversion and primary infection, or negative antibody status. In addition, samples obtained too early during primary infection may not contain detectable antibody. Some persons may fail to develop detectable antibody after Herpes infection.

BIBLIOGRAPHY

1. Nahmias, A.J., J. Dannenbarger, C. Wickliffe and M. Muther. Clinical aspects of infection with herpes simplex viruses 1 and 2 in the human herpes viruses. An interdisciplinary Perspective (Nahmias, A.J., W.R. Dawdle and R.F. Schinazi eds) New York, Elsevier, pp 3-9, 1981.
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.

SYMBOL KEYS

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

Manufactured by:
Zephyr Biomedicals
A Division of Tulip Diagnostics (P) Ltd.
M 46-47, Phase III B, Verna Industrial Estate, Verna, Goa- 403 722, INDIA.

An ISO 13485 Certified Company

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0322/VER-01

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electra™
:::::::::::: HSV 1,2 IgM

Chemiluminescence Assay for Qualitative Detection of HSV 1,2 IgM antibody in Human Serum
FOR IN VITRO DIAGNOSTIC USE ONLY
Store at 2°C to 8°C

INTENDED USE

ELECTRA™ HSV 1,2 IgM is intended for the Qualitative detection of IgM antibodies to herpes simplex virus (HSV) infection or, for evaluating paired sera for the presence of a significant increase in herpes specific IgM. 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. CLIA HSV 1,2 IgM is an accurate serologic method to detect HSV specific antibody IgM in serum sample.

PRINCIPLE

ELECTRA™ HSV 1,2 IgM Qualitative CLIA assay is for use on **ELECTRA™** analyzers. **ELECTRA™ HSV 1,2 IgM** CLIA works on the principle of chemiluminescence wherein light is produced by a chemical reaction from a substance as it returns from an electronically excited state to the ground state. When catalysed by HRP, the oxidation of luminol by hydrogen peroxide produces an electronically excited form of 3-aminophthalate which on relaxation emits light with maximum intensity at λ=425nm.

Purified HSV antigen is coated on the surface of microwells. Diluted patient serum is added to wells, and the HSV IgM 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 substrate A & substrate B mixture is added. The light generated (RLU) is proportional to the amount of IgM specific antibody in the sample. The results are read by a microwell luminometer compared in a parallel manner with calibrator and controls.

MATERIALS AND COMPONENTS

Materials provided with the test kit:
1. Coated Microwells: purified HSV 1,2 antigen coated wells.
2. Sample Diluent
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. Substrate A: Chemiluminescent substrate containing enhanced luminol solution.
9. Substrate B: Chemiluminescent substrate containing stabilized peroxide solution.

Materials required but not provided:
1. Precision pipettes: 10-100µl, 20-200µl, 100-1000µl
2. Disposable pipette tips
3. Distilled water
4. Disposable Gloves
5. ELECTRA™ Analyzer

STORAGE AND STABILITY

1. **ELECTRA™ HSV 1,2 IgM** kit is stable at 2-8°C up to the 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.
4. Working Substrate (A+B) must be used immediately.

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.
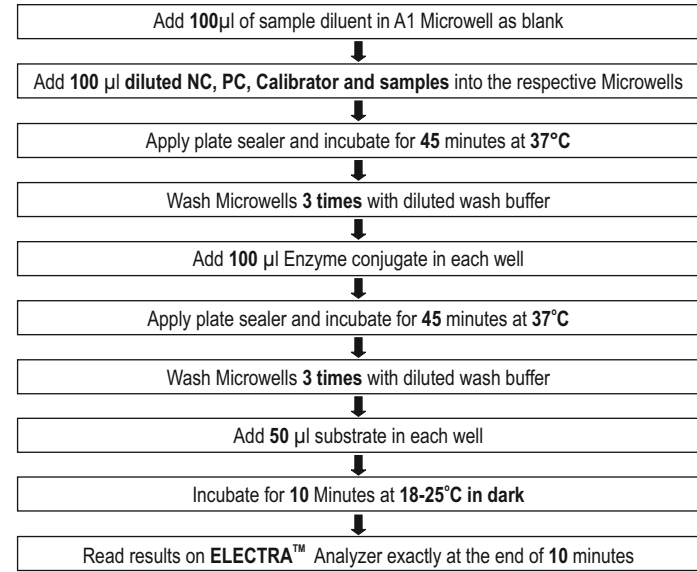(13) Do not let the strips dry in between the steps.

REAGENT PREPARATION
- 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.
- Dilute wash buffer 20 times (for example add 5ml concentrated buffer to 95 ml distilled or deionized water). Mix well before use.
- Prepare a Working Substrate by Mixing Substrate A and Substrate B in equal volume (1:1 ratio) before addition to the micro-wells.

No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
Substrate- A µl	250	450	650	850	1050	1250	1450	1650	1850	2050	2250	2450
Substrate- B µl	250	450	650	850	1050	1250	1450	1650	1850	2050	2250	2450

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, positive Control, negative Control and Calibrator to 200 µl 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 into each well and incubate for 45 minutes at 37°C.
6. Wash each well three times by filling approximately 350µl diluted wash buffer & blot dry.
7. Add 50µl of working Substrate (A+B) in all the micro-wells. Keep away from direct light while adding the substrate.
8. Cover the ELECTRA™ microplate and incubate for 10 minutes at room temperature (18-25°C) in dark.
9. Read the ELECTRA™ micro-plate exactly at 10 minutes in ELECTRA™ Analyzer.

Add **100**µl of sample diluent in A1 Microwell as blank
↓
Add **100** µl **diluted NC, PC, Calibrator and samples** into the respective Microwells
↓
Apply plate sealer and incubate for **45** minutes at **37°C**
↓
Wash Microwells **3 times** with diluted wash buffer
↓
Add **100** µl Enzyme conjugate in each well
↓
Apply plate sealer and incubate for **45** minutes at **37°C**
↓
Wash Microwells **3 times** with diluted wash buffer
↓
Add **50** µl substrate in each well
↓
Incubate for **10** Minutes at **18-25°C in dark**
↓
Read results on **ELECTRA**™ Analyzer exactly at the end of **10** minutes

RUN CRITERIA
The test run may be considered valid provided the following criteria are met:
1. If the RLU of the Calibrator is lower than 10,00,000 the test is not valid and must be repeated.
2. The HSV 1,2 IgM Index for Negative and Positive Control should be in the range stated on the labels.

CALCULATION OF RESULTS
1. To obtain Cut off RLU value: Multiply the RLU of Calibrator by Factor (f) printed on label of Calibrator.
2. Calculate the IgM Index of each determination by dividing the RLU values of each sample by RLU value of cut-off.

For example:
If Factor (f) value on label = 0.9
This factor (f) is a variable. It is specific for a lot manufactured and printed on label of Calibrator.

Obtained Calibrator RLU = 6598535
Cut-off RLU = 6012644 x 0.9 = 5411379
Patient sample RLU = 9665867
HSV 1,2 IgM Index = 9665867/5411379 = 1.78 (Positive result)
Patient sample RLU = 1270550
HSV 1,2 IgM Index = 1270550/5411379 = 0.23 (Negative result)

INTERPRETATION OF THE RESULT

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