



DIAGNOSTIC AUTOMATION, INC.

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IVD



See external label



2°C-8°C



Σ=96 tests

REF

Cat # 9068-11

CHEMILUMINESCENCE

HSV 2 IgM

Cat # 9068-11

SUMMARY OF ASSAY PROCEDURE

| Step | (20-25°C Room temp.) | Volume | Incubation time |
|------|--|--------|-----------------|
| 1 | Sample dilution 1:40 =5 µl / 200 µl | | |
| 2 | Diluted samples, controls & calibrator | 100 µl | 30 minutes |
| 3 | Washing buffer (3 times) | 350 µl | |
| 4 | Enzyme conjugate | 100 µl | 30 minutes |
| 5 | Washing buffer (3 times) | 350 µl | |
| 6 | Substrate A and Substrate B mixture | 100µl | 5 minutes |
| 7 | Read with Luminometer in 5~30 minutes | | |

NAME AND INTENDED USE

HSV 2 IgM Chemiluminescence ELISA is intended for the detection of IgM antibodies to herpes simplex virus 2.

SUMMARY AND EXPLANATION OF THE TEST

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 or premature infants. HSV 2 IgM Chemiluminescence ELISA is an accurate serologic method to detect HSV 2 specific antibody IgM in serum sample.

PRINCIPLE OF THE TEST

Purified HSV antigen is coated on the surface of microwells. Diluted patient serum is added to wells, and the HSV 2 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 PROVIDED

- | | |
|--|-------------------|
| 1. Microwell Strips: purified HSV 2 antigen coated wells | (12 x 8 wells) |
| 2. Absorbent Solution: Black Cap. | 1 vial (22 ml) |
| 3. Washing Concentrate 10x: White Cap. | 1 bottle (100 ml) |
| 4. Enzyme Conjugate: Red Color Solution. | 1 vial (12 ml) |
| 5. Cut-off Calibrator: Yellow Cap. HSV 2 M Index = 1.0 | (150 µl/vial) |
| 6. Negative Control: Range stated on label. Natural Cap. | (150 µl/vial) |
| 7. Positive Control: Range stated on label. Red Cap. | (150 µl/vial) |
| 8. Substrate A: H ₂ O ₂ in buffer. Natural bottle. | 1 vial (7 ml) |
| 9. Substrate B: Luminol in buffer. Amber bottle. | 1 vial (7 ml) |

STORAGE AND STABILITY

1. Store the kit at 2 - 8 °C.
2. Always keep microwells tightly sealed in pouch with desiccants. We recommend you use up all wells within 4 weeks after initial opening of the pouch.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light during storage or usage.

WARNINGS AND PRECAUTIONS

1. Potential biohazardous materials:
The calibrator and controls contain human source components which have been tested and found nonreactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984
2. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.

- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
- This product contains components preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

SPECIMEN COLLECTION AND HANDLING

- Collect blood specimens and separate the serum.
- Specimens may be refrigerated at 2 - 8 °C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing of serum sample.

PREPARATION FOR ASSAY

- Prepare 1x washing buffer. Prepare washing buffer by adding distilled or deionized water to 10 x wash concentrate to a final volume of 1 liter.
- Bring all specimens and kit reagents to room temperature (20-25 °C) and gently mix.

ASSAY PROCEDURE

- Prepare 1:40 dilutions by adding 5 µl of the samples, negative control, positive control, and calibrators to 200 µl of absorbent solution. Mix well.
- Place the desired number of coated strips into the holder.
- Dispense 100 µl of diluted sera, calibrators, and controls into the appropriate wells. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 30 minutes at room temperature.
- Remove liquid from all wells and repeat washing three times with washing buffer.
- Dispense 100 µl of enzyme conjugate to each well and incubate for 30 minutes at room temperature.
- Remove enzyme conjugate from all wells. Repeat washing three times with washing buffer.
- Mix equal volume of Substrate A & Substrate B, then dispense 100 µl of this mixture to each well.
- Read RLU with a microwell luminometer within 5~30 minutes.

CALCULATION OF RESULTS

Determination of Index values

- Calculate the mean of duplicate RLU values (B).
- Calculate the HSV 2 M Index of each determination by dividing the mean values of each sample (B) by Cut-off calibrator mean value (C).

For example:

| Sample | Well No | RLU (A) | Mean RLU (B) | INDEX B/C |
|--------------------|---------|---------|--------------|-----------|
| Cut-off Calibrator | A1 | 280550 | 290048 (C) | 1 |
| | B1 | 299546 | | |
| Positive Control | C1 | 610675 | 614924 | 2.1 |
| | D1 | 619173 | | |
| Negative Control | E1 | 6515 | 6274 | 0.02 |
| | F1 | 6033 | | |
| Patient Sample 1 | G1 | 346012 | 340868 | 1.18 |
| | H1 | 335724 | | |
| Patient Sample 2 | A2 | 437667 | 456418 | 1.6 |
| | B2 | 475168 | | |

QUALITY CONTROL

1. In order for the assay results to be considered valid the controls should be within the ranges indicated on the labels.
2. The RLU values vary with the different luminometer used.
3. Each laboratory should assay controls at levels in low, normal and elevated ranges for monitoring assay performance. Quality control trends should be maintained to monitor batch to batch consistency.

INTERPRETATION

| HSV 2 IgM Index | Interpretation |
|-----------------|--------------------------------------|
| < 0.90 | Negative HSV 2 IgM |
| 0.91 ~ 0.99 | Equivocal, sample should be retested |
| 1 ~ 2 | Low positive |
| 2 ~ 2.5 | Moderate positive |
| > 2.5 | High positive |

PERFORMANCE CHARACTERISTICS

Specificity and Sensitivity

A total of 68 patient samples were used to evaluate specificity and sensitivity of the test. HSV 2 IgM test results were compared to a commercial ELISA kit results.

| | | Reference ELISA | | | Total |
|---|-------|-----------------|---|-------|-------|
| | | N | E | P | |
| HSV 2 IgM Chemiluminescence ELISA | N | 56 (D) | 0 | 3 (B) | 59 |
| | E | 0 | 2 | 0 | 2 |
| | P | 0(C) | 0 | 7(A) | 7 |
| | Total | 56 | 2 | 10 | 68 |

$$\text{Sensitivity} = A / (A+B) = 7 / 10 = 70\%$$

$$\text{Specificity} = D / (C+D) = 56 / 56 = 100\%$$

$$\begin{aligned} \text{Accuracy} &= (A+D) / (A+B+C+D) \\ &= (7+56) / (7+3+0+56) = 63 / 66 = 95\% \end{aligned}$$

Expected Values:

95 random samples were determined with HSV 2 IgM Chemiluminescence ELISA. The test results were computed as IgM Index using a chosen reference serum (cut off) as IgM index 1. 2 were found to be positive (2%), and 93 were found to be negative (98%).

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 | 10.2% | 8.5% | 7.5% |
| Inter-assay | 12.1% | 9.7% | 8.4% |

LIMITATION OF THE PROCEDURE

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.

REFERENCES

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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.

| Date Adopted | Reference No. |
|--------------|-------------------|
| 2007-10-18 | DA-HSV 2 IgM-2009 |



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