en

Immunoassay

REF **CMK0401 / CMK0402 / CMK0403 / CMK0404 / CMK0405**

50 tests*1 / 100 tests*1 / 100 tests*2 / 100 tests*5 / 50 tests*2

HSV-1 IgM CLIA Microparticles

This assay is based on a chemiluminescent microparticle immunoassay (CLIA Microparticles) for the quantitative detection of HSV-1 IgM (IgM antibodies to herpes simplex virus type 1) in human serum or plasma.

Key to Graphical Symbols Used

LOT	batch code		use by
	manufacturer	Σ	contains sufficient for <n> tests
IVD	*in vitro* diagnostic medical device		temperature limitation
REF	catalogue number		consult instructions for use
EC REP	authorized representative in the European Community		

EC REP

OBELIS S.A.
Bd. Général Wahis, 53
1030 Brussels
Belgium

AUTOBIO DIAGNOSTICS CO., LTD.
No.87 Jingbei Yi Road
National Eco & Tech Development Area
Zhengzhou
China
450016

CE IVD

For any technical assistance please contact us in English at:
Email: customerservice@autobio.com.cn
Contact your local dealers for all product related questions in your local language

Introduction

HSV (Herpes simplex virus) is an enveloped, DNA-containing virus morphologically similar to the other members of the Herpetoviridae family. HSV-1 and HSV-2 (Herpes simplex virus 1 and 2), also known as HHV-1 and -2 (Human herpes virus 1 and 2), are two members of the herpes virus family, Herpesviridae, that infect humans.[1]

Active virus excretion in genital secretions of pregnant women may result in severe neonatal HSV infection contracted when the infant passes through an infected genital tract. When HSV lesions are present during delivery, 40% to 60% of the neonates can be affected. Transmission of HSV infection to neonates is associated with high morbidity and mortality rates if untreated.[2]

The first humoral immune response to infection is the synthesis of specific anti-HSV IgM antibody which becomes detectable one week after infection. Normally this is a proof of recent or recurrent infection. Detection of IgG allows assessment of the patient's immune status and provides serological evidence of prior exposure to HSV. This may aid in the diagnosis of recent (primary or recurrent) HSV infection in paired sera by the presence of seroconversion to HSV-1 or HSV-2 antibody.

Measurement Principle

This assay is based upon the two-step capture method. In the first step, sample and mouse monoclonal anti-human IgM coated microparticles are combined. During the incubation, the antibodies present in the sample bind to the anti-human IgM coated on the microparticles. After the washing, in the second step, Enzyme Conjugate is added to the reaction mixture. During the incubation, the HSV-1 antigen in the Enzyme Conjugate is allowed to react with the HSV-1 IgM already bound to the solid phase in the first step. After a second washing, a complex is generated among the solid phase, antibodies in the sample and enzyme-linked antigens by immunological reactions. Chemiluminescent Substrate is added and the complex catalyzes substrate, resulting in a chemiluminescent reaction. The resulting chemiluminescent reaction is measured as RLUs. The RLU is proportional to the amount of HSV-1 IgM in the sample.

Materials Provided

1. Calibrators

6 vials lyophilized calibrator A through F. The matrix is PBS (phosphate buffered saline) containing BSA (bovine serum albumin). Contains a selection of preservative.

Reconstitute each lyophilized calibrator with 1.0 mL of distilled water. Allow the reconstituted material to stand for at least 5 minutes. Then invert the calibrator to mix it completely.

2. Reagent pack

Reagent pack provided ready to use.

Σ	50*1	100*1	100*2	100*5	50*2
Microparticles Solution	1.2mL*1	2.3mL*1	2.3mL*2	2.3mL*5	1.2mL*2
Enzyme Conjugate	5.5mL*1	11.0mL*1	11.0mL*2	11.0mL*5	5.5mL*2
Sample Diluent	5.5mL*1	11.0mL*1	11.0mL*2	11.0mL*5	5.5mL*2

- Microparticles Solution

Mouse monoclonal anti-human IgM coated microparticles in PBS buffer containing casein. Contains a selection of preservative.

- Enzyme Conjugate

Horseradish peroxidase labeled HSV-1 antigens in a Tris-HCl buffer containing bovine serum and casein. Contains a selection of preservative.

- Sample Diluent

Tris-HCl buffer containing BSA. Contains a selection of preservative.

Assay Analyzers on which the kit can be used

- AutoLumo A2000 Plus
- AutoLumo A2000 Plus B
- AutoLumo A1000

The chemiluminescent microparticle immunoassay (CLIA Microparticles) is intended for use on Assay Analyzer which is AutoLumo A2000 Plus, AutoLumo A2000 Plus B or AutoLumo A1000.

Materials Required but not Provided

1. Assay Analyzer
2. Reaction vessel(s) for sample and reagent reaction
3. Sample tubes(s) or cup(s) for sample containing
4. Diluent Universal
5. Chemiluminescent Substrate
6. System Wash for washing the pipetting needles
7. Wash Buffer used in the washing procedure
8. Distilled or deionized water

Metrological Traceability of Calibrators

The measurand or analyte in the HSV-1 IgM calibrators is traceable to the manufacturer's working calibrators.

Warnings and Precautions

1. For professional use only.
2. Follow the instruction for use carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this instruction for use.
3. Refer to the material safety data sheet and product labeling for any chemical hazards that may be present in this assay.
4. Handle the potentially contaminated materials and wastes safely according to local requirement.
5. CAUTION: This assay contains materials of animal origin. Bovine components originate from countries where BSE has not been reported.
6. Do not smoke, drink, eat or use cosmetics in the working area.
7. Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.
8. Use caution when handling patient samples to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
9. Conduct the assay away from bad ambient conditions. e. g. ambient air containing high concentration corrosive gas such as sodium hypochlorite acid, alkaline, acetaldehyde and so on, or containing dust.
10. Do not mix or use components from kits with different batch codes.
11. When storing the calibrators, be certain the vials are securely sealed.
12. Ensure the microparticles are resuspended before loading it on the analyzer.
13. Avoid foam formation in all reagents and sample types (samples, calibrators and controls).
14. Do not substitute any reagent in this kit from other manufacturers or other lots.
15. When any damage to the protective packaging or any change of analytical performance is observed, do not use the kit.
16. Do not use reagents beyond the labeled expiry date.

Storage

1. Store the kit at 2-8 ℃. Do not freeze. Avoid strong light. When stored as directed, all reagents are stable until the expiration date.
2. Refrigerate the reagent pack at 2-10 ℃ for a minimum of 2 hours prior to use.
3. Store the unsealed reagents pack upright on the analyzer or 2-10℃ for a maximum of 28 days. After 28 days, the reagent pack must be discarded. Once they are removed from the analyzer, store them at 2-10 ℃ in an upright position.
4. Seal and return the remaining reconstituted calibrators at 2-8 ℃ immediately after the experiment, under which conditions the stability will be retained for 1 week, for longer use, store reconstituted calibrators in aliquots and freeze at -20℃, which can be stored up to 2 months. Avoid multiple freeze-thaw cycles, do not freeze-thaw more than 3 cycles.

Sample

1. Collect samples in accordance with correct medical practices.
2. Plasma samples collected in tubes containing EDTA, heparin or sodium citrate have been tested and may be used with this assay.
3. Do not use heat-inactivated samples and samples with obvious microbial contamination. Do not use sodium azide preservative in samples.
4. Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the sample is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results. Be sure that the samples are not decayed prior to use.
5. Prior to shipment, it is recommended that samples be removed from the clot, serum separator or red blood cells.
6. Insufficient processing of the sample or disruption of the sample during transportation may cause depressed results.
7. Avoid grossly hemolytic, lipemic or turbid samples.
8. Cap and store the samples at 18-25 ℃ for no more than 8 hours, for longer use samples should be capped and stored at 2-8 ℃ up to 48 hours. Or freeze the samples that need to be stored or transported for more than 48 hours at -20 ℃. Avoid multiple freeze-thaw cycles. Mix thawed samples thoroughly by low speed vortex or by inverting 10 times. Visually inspect the samples, if layering or stratification is observed, continue mixing until samples are visibly homogeneous. After thawing, bring to room temperature and mix well by gently shaking.
9. Centrifuge the thawed samples containing red blood cells or particulate matter, or which are hazy or cloudy in appearance prior to use to ensure consistency in the results.
10. Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
11. For optimal results, inspect all samples for bubbles. Remove bubbles with a tip prior to analysis. Use a new tip for each sample to prevent cross contamination.

Measurement Procedure

1. **Check the consumable materials**

- Verify adequate volume of consumable materials is present prior to running the test.
- Refer to the Assay Analyzer's operation manual.

2. Load the kit

- Mix contents of new (un-punctured) reagent packs by gently inverting pack several times before loading on the analyzer. Avoid foam formation in all reagents. Don't invert the open (punctured) packs. If necessary, shake gently to mix horizontally after the first loading.
- Read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Refer to the Assay Analyzer's operation manual.

3. Order tests

- Place the sample tubes or cups on the sample rack, 10 μL of serum or plasma samples for each test. But consider the sample container and 150 μL of system dead volumes, which can be refer to the appropriate Assay Analyzer manuals for the minimum sample volume required.
- Load the sample rack and input the sample information on the system software interface.
- Select "run" to start the test, the analyzer automatically operates tests. It performs the following functions:
  - Moves the sample to the set point
  - Loads a reaction vessel into the process path
  - Aspirates and transfers Diluent Universal and sample into the reaction vessel and mixed well
  - Aspirates and transfers the diluted sample to the reaction vessel
  - Adds Microparticles Solution and Sample Diluent to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Enzyme Conjugate to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Chemiluminescent Substrate
  - Measures chemiluminescent emission to determine the quantity of HSV-1 IgM in the sample
  - Discards the used reaction vessel
  - Calculates the result
- Refer to the Assay Analyzer's operation manual.

4. Calibrate the curve

- Analyzer can read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Transfer the calibrators into the sample tubes or cups and place them on the sample rack. Conduct duplicate detection on the system.
- Load the sample rack and input calibrators' information on the system software interface.
- Select "run" to start the test and generate the calibration curve, calibration is required every 28 days.
- Once a calibration curve is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - Controls are out of range after repeated measurements
  - A reagent kit and Chemiluminescent Substrate with new batch code is used
  - Beyond the expiration date of a calibration curve
  - Important parts of the analyzer are replaced or repaired
- Refer to the Assay Analyzer's operation manual.

5. Dilute the sample

Samples with a HSV-1 IgM value exceeding 240 AU/mL may be diluted manually. Negative serum is used to dilute the samples. After dilution, multiply the result by the dilution factor. Antibodies to HSV-1 are heterogenous. A non-linear dilution behavior is frequently observed.

Measurement Results

The sample test results are determined automatically by the system software. The amount of HSV-1 IgM in the samples is determined from the measured light production by means of the stored calibration data. Refer to the Assay Analyzer's operation manual on reviewing the stored data.

Interpretation of Results

Results obtained with the HSV-1 IgM CLIA Microparticles can be interpreted as follows:

Nonreactive:< 6 AU/mL

Equivocal: 6-10 AU/mL.

Reactive: ≥ 10 AU/mL

A nonreactive result cannot always rule out acute HSV-1 infection, because the infection maybe be in its very early stage and the patient is still unable to synthesize HSV-1 specific IgM.

An equivocal result may be indicative either of recent infection or of past infection with long-lasting HSV-1 IgM. A second sample should be collected within a reasonable period of time (e.g., within one week). Serological data from detection of additional HSV-1 markers may provide useful information for clinical interpretation of results.

Diagnosis of infectious diseases should not be established on the basis of a single test result, but should be determined in conjunction with clinical findings and other diagnostic procedures as well as in association with medical judgment.

Control Procedure

Controls for the various concentration ranges should be run individually when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Limitations of the Procedure

1. This assay is intended as an aid for the clinical diagnosis. Conduct this assay in conjunction with clinical examination, patient's medical history and other test results.
2. If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
3. Samples from neonates, cord blood, pretransplant patients or body fluids other than serum and plasma, such as urine, saliva or amniotic fluid have not been tested.
4. For the samples who have received blood transfusions or other blood products in recent months, the positive result should be given careful analysis.
5. If the patient is immune-compromised or is receiving immunosuppressive therapy (for example, transplant recipients, AIDS patients), the reference value of their IgM antibodies serological detection is limited, and wrong medical explanation may be obtained.
6. This test measures concentrations within the range of 6-240 AU/mL. If HSV-1 IgM concentrations above the measuring range to be expected, it is recommended to dilute samples with Diluent Universal, the recommended dilution is 1:9 of this test, under this condition, allowing samples to be up to approximately 2400 AU/mL.

Performance Characteristics

1. Measurement Precision

3 samples were assayed in replicates of 2, twice per day across 20 testing days. Data from this study are summarized in the following table.

Sample	n	Mean (AU/mL)	Within-run	Total
			%CV	%CV
1	80	23.49	2.45	8.27
2	80	48.76	2.90	7.05
3	80	106.67	2.56	5.16

*Representative data; results in individual laboratories may vary from these data

2. Analytical Sensitivity

LOD is ≤ 1 AU/mL.

3. Analytical Specificity

Corss reaction: This assay is tested to have no cross reactivity with the HSV-2 IgM, Rubella IgM, Toxo IgM, CMV IgM, MP, CP, HBV, HCV, TP, HIV HEV and B19 IgM.

Interference: Controlled studies of potentially interfering substances or conditions showed that the assay performance was not affected by anticoagulants (sodium citrate, EDTA, heparin), bilirubin (up to 20 mg/dL), hemoglobin (up to 1000 mg/dL), triglyceride (up to 3000 mg/dL).

4. Relative Agreement

A comparison study was performed where samples were tested using this assay and a HSV IgM reference assay. The relative agreement is 96.4% (482/500).

Literature References

1. Ryan KJ, Ray CG, Sherris JC. Sherris medical microbiology: an introduction to infectious diseases. New York: McGraw-Hill; 2004.

2. Kimberlin DW. Herpes simplex virus infections of the newborn. Semin. Perinatol. 2007;31(1):19–25.