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Varicella zoster IgG CLIA

REF

Catalog No.C30-914

IVD

FOR INVITRO DIAGNOSTIC USE ONLY

INTENDED USE

The Immunospec Varicella zoster IgG CLIA is an using chemiluminescence detection technology kit providing material for the detection of IgG-class antibodies to Varicella zoster virus in human serum or plasma.This assay is intended for *in vitro* use only.

SUMMARY AND EXPLANATION

Varicella and Herpes Zoster are two clinical manifestations resulting from infection by Varicella Zoster Virus (VZV). Varicella, or chicken pox, is a highly contagious disease that is generally a consequence of primary infection by VZV and typically affects children. Infection caused by VZV during pregnancy can cause disease or malformation of the fetus; if it occurs at the end of pregnancy, it can be fatal to the neonate.

Herpes Zoster is a disease that primarily affects adults and appears to be caused by reactivation of the virus, which can remain latent in the spinal sensorial ganglia following primary infection. The infection causes painful cutaneous eruptions along the path of the affected nerves.

Serological methods are usually adopted to determine the immune state of subjects at risk (chiefly immunodepressed patients) and in pre-natal and post-natal diagnosis of infected subjects .

PRINCIPLE OF THE TEST

Immunospec Varicella IgG kit is based on the chemiluminescence immunoassay technique. In the assay, Controls and Unknowns are incubated in microtitration wells coated with purified and inactivated VZV antigen. After incubation and washing, the wells are treated with the conjugate, composed of anti-human IgG monoclonal antibodies labeled with peroxidase. After a second incubation and washing step, substrate solution is added to the wells and read. The degree of enzymatic turnover of the substrate is determined by relative light unit (RLU) measurement using luminometer. The RLU measured is directly proportional to the concentration of Varcella zoster virus IgG antibodies presence

REAGENTS

The Immunospec *Varicella zoster IgG* CLIA kit contains sufficient reagent for 96 wells. Each kit contains the following reagents:

MATERIAL PROVIDED	QUANTITY
VZV- Antigen-Coated Microtitration Strip	One Plate
Wash Concentrate	One Bottle
Sample Diluent	One Bottle
Substrate A	One Bottle
Substrate B	One Bottle
Negative control	One Vial
Cut off control	One Vial
Positive control	One Vial
2nd Antibody Conjugate	One Bottle

MATERIAL NOT PROVIDED

- Microtitration plate reader capable of absorbance measurement at 450 nm
- Deionized/Distilled water
- Precision pipette to deliver 10 µL, 100 µL, and 1 mL
- Semi-automatic pipette to deliver 100 µL
- Automatic microtitration plate washer
- Absorbent materials for blotting the strips

VZV-Antigen-Coated Microtitration Strips:
One stripholder containing 12x8 (96) microtitration wells coated with Varicella zoster virus antigen. Store at 2-8°C until expiration date. Remove the support and strips to be used from the foil package, and place the unused strips in the polythene bag with the silica gel, expel the air and seal by pressing the closure. Once opened, the product is stable for 4 weeks at 2-8°C.

Wash Concentrate:
One bottle, 100 mL, containing a phosphate buffered saline, concentrated 10-fold containing 0.5% Brij weight by volume (w/v). Dilute with deionized/distilled water prior to use. Store at 2-8°C until expiration date.

Sample Diluent:
One bottle, 100 ml, containing a protein solution with 0.09% sodium azide as a preservative. Store at 2-8°C until expiration date.

***Varicella IgG Controls*:**
Three vials, negative, cut off and positive , each 2 mL of human serum in a 0.01 M phosphate buffer with 0.09% sodium azide as a preservative. Store at 2-8°C until expiration date.

2nd Antibody Conjugate:
One bottle, 12 mL, containing anti-human IgG monoclonal antibodies labeled with peroxidase, in a phosphate buffer solution with 0.02% Proclin. Store at 2-8°C until expiration date.

Substrate A:
One bottle, 6 mL containing substrate A. Store at 2-8°C until expiration date.

Substrate B :
One bottle, 6 mL, containing A. Store at 2-8°C until expiration date.

PRECAUTIONS

For *in vitro* use

The following universal Good Laboratory Practices should be observed:

Do not eat, drink, smoke or apply cosmetics where immunodiagnostic material is being handled. Do not pipet by mouth. Wear lab coats and disposable gloves when handling immunodiagnostic material. Wash hands thoroughly afterwards. Cover working area with disposable absorbent paper. Wipe up spills immediately and decontaminate affected surfaces. Avoid generation of aerosols. Provide adequate ventilation. Handle and dispose of all reagents and materials in compliance with applicable regulations.

WARNING: POTENTIAL BIOHAZARDOUS MATERIAL

This kit may contain some reagents made with human source material (e.g. serum or plasma) or used in conjunction with human source materials. The material in this kit has been tested by CE marked methods and found to be non-reactive for HIV-1/2 Antibodies, HCV and HBsAg. No available test method can offer complete assurance of eliminating potential biohazardous risk. Handle all reagents and patient samples at a Biosafety Level 2, as recommended for any potentially

infectious human material in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 4th Edition, April 1999.

WARNING AND PRECAUTION:

Some of the reagents in this kit contain sodium azide as a preservative at concentrations below the regulatory limit of < 0.1%. Although significantly diluted, concentrated sodium azide is an irritant to skin and mucous membranes, and may react with lead and copper plumbing to form explosive metal azides, especially if accumulated. Additionally, TMB and Sulfuric Acid, in concentrated amounts are also irritants to skin and mucous membranes. These substances are in diluted form and therefore may minimize exposure risks significantly but not completely. Provide adequate ventilation. Avoid contact with skin, eyes and clothing. In case of contact with any of these reagents, wash thoroughly with water and seek medical advice. Dispose all nonhazardous reagents by flushing with large volumes of water to prevent buildup of chemical hazards in the plumbing system.

For further information regarding hazardous substances in the kit, please refer to the component specific MSDS by request.

SPECIMEN COLLECTION AND HANDLING

Serum should be used, and the usual precautions for venipuncture should be observed. Specimens may be stored at 2-8°C for 2 days. For longer periods, store at –20°C. Do not use hemolyzed or lipemic specimens. Avoid repeated freezing and thawing of samples.

PREPARATION FOR ASSAY

A thorough understanding of this package insert is necessary for successful use of the product.Reliable results will only be obtained by using precise laboratory techniques and accurately following the package insert. Bring all kit reagents and specimens to room temperature (~25°C) before use. Thoroughly mix the reagents and samples before use by gentle inversion. Do not mix various lots of any kit component within an individual assay. Do not use any component beyond the expiration date shown on its label. Incomplete washing will adversely affected the outcome and assay precision. To minimize potential assay drift due to variation in the substrate incubation time, care should be taken to add the stopping solution into the wells in the same order and speed to add the TMB Chromogen Solution. Avoid microbial contamination of reagents, especially of the conjugate, wash buffer and diluent. Avoid contamination of the TMB Chromogen Solution with the Conjugate. Use a clean disposable pipette tip for each reagent. Avoid pipettes with metal parts. Containers and semi-automatic pipette tips used for the Conjugate and TMB can be reused provided they are thoroughly rinsed with deionized/distilled water and dried prior to and after each usage. The enzyme used as the label is inactivated by oxygen, and is highly sensitive to microbial contamination, sodium azide, hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies. Use high quality water. Avoid exposure of the reagents to excessive heat or sunlight during storage and incubation.

PREPARATION OF REAGENTS:

Wash Solution:

Dilute 1:10 with deionized/distilled water prior to use. If crystals are present, they should be dissolved at 37°C before dilution. Pour 100 mL of the Wash Concentrate into a clean container and dilute by adding 900 mL of deionized/distilled water. Mix thoroughly by inversion. The wash solution is stable for 5 days at room temperature and 2 weeks at 2-8°C when stored in a tightly sealed bottle.

Microtitration Strips:

Select the number of coated strips required for the assay. The remaining unused wells should be placed in the resealable pouch with a desiccant pack. The pouch must be resealed to protect from moisture.

Substrate : Atleast 30 minutes prior to use mix 1 part of Substrate in 1 part of Substrate B (Example : 1ml of Substrate A and 1 ml Substrate B).The two components should be mixed thoroughly by inversion and store the substrate mixture at RT, avoid exposure to light.

Assay Procedure:

All specimens and reagents to reach room temperature (~25°C) before use. Serum Samples and Controls should be assayed in duplicate.

1. Mark the microtitration strips to be used.
2. Dilute serum samples 1:101 distributing 10 µL of serum into 1 mL of Assay Buffer.
3. Pipette 100 µL of each diluted serum sample and ready to use controls to the appropriate wells. Leave one well for the blank, performed using 100 µL of the TMB-substrate at the substrate incubation step
4. Incubate for 20 minutes at 37°C.
   Aspirate and wash each well four (4) times for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.
   NOTE: Use of an automatic microplate washer is strongly recommended. Incomplete washing will adversely affect assay precision. If a microplate washer is not available, (a) completely aspirate the liquid from each well, (b) dispense 300 uL of the Wash Solution into each well, and (c) repeat step (a) and (b) four times.
5. 5 Add 100 µL of Enzyme-Labeled 2nd Antibody into each well.
6. Incubate for 20 minutes at 37°C.
7. Aspirate and wash each well four times for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.
8. Add 100 µL of substrate mixture to each well using a dispenser.
9. Shake the wells for 10 sec.
10. Read the wells within 3 minutes using a microplate luminometer.

RESULTS

Calculate the mean RLU for each control and unknown.

Qualitative results:

If the RLU of the sample is higher than that of the Cut-Off, the sample is positive for the presence of specific IgM.

Calculate the ratio between the average RLU value of the sample and that of the Cut-Off. The sample is considered:

Positive: if the ratio is > 1.1.
Doubtful: if +/- 10% of the Cut-Off.
Negative: if the ratio is < 0.9.

If the result is doubtful, repeat the test. If it remains doubtful, collect a new serum sample.

LIMITATIONS OF THE PROCEDURE

- A serum sample obtained during the early phase of infection, when only IgM antibodies are present, may be negative by this procedure.
- The test result should be used in conjunction with information available from the evaluation of other clinical and diagnostic procedures.
- Avoid repeated freezing and thawing of reagents and specimens.
- Grossly hemolyzed, icteric or lipemic specimens should be avoided.
- Heat inactivated sera should be avoided.

QUALITY CONTROL

Subtract the value of the blank from all the other readings. The RLU values of cut off control must be at least >10. Positive control must have a RLU at least 1.5 times that of cut off.

PERFORMANCE CHARACTERISTICS

1. Sensitivity and Specificity

103 human sera were analyzed by this Varicella IgG CLIA and a commercial ELISA (Test A) as reference method. Out of 103 samples, 80 were positive for the presence of IgG antibodies to Varicella zoster virus by Immunospec CLIA and commercial Elisa showed 79 of them as positive. The results are summarized below.

2. Precision

	Positive	Negative	FN (false negative)	FP (false positive)
Immunospec	80	23		1
Test A	79	24		

REFERENCE

1. E.H. Wasmuth and w. Miller. J. Med. Virology 32:189 (1990)
2. M.L.Landry, S.D. Cohen, D. Mayo, C. Fong, W. Andiman: J Clin. Microbiology 25:832 (1987)
3. P.Larussa, S.Steinberg,,et.al. J. Clin. Microbiology 25:2059 (1987)

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