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# Rubella IgM_CLIA

REF

Catalog No.C30-909

IVD

*For In Vitro Diagnostic Use Only*

## INTENDED USE

Immunospec Rubella IgM CLIA kit provides material for the detection of IgM-class antibodies to Rubella virus in human serum or plasma using chemiluminescence detection technology. This assay is intended for *in vitro* use only.

## SUMMARY AND EXPLANATION

The Immunospec Rubella virus, most commonly known as the German or 3-day measles is an RNA virus that belongs to the family Togaviridae and contains three major structural proteins, E1, E2 and C. It is spread through direct or droplet contact from nasopharyngeal secretions. The infection is highly contagious and affects children, 5-14 years as well as young adults. Rubella infection, however, is largely benign with symptoms ranging from subclinical to a disease characterized by an erythematous rash, low-grade fever, headache, lymphadenopathy, arthralgia, and conjunctivitis. Immunizations and natural infection both confer lifelong immunity and reinfection is extremely rare. Congenital Rubella infection, unlike acquired infection, may cause disastrous clinical effects to the unborn child. A fetus may be stillborn or have such abnormalities as bone and cardiovascular defects, mental retardation, encephalitis, hepatomegaly, splenomegaly, thrombocytopenic purpura, cataracts and microcephaly. Because of severity of the complication from infection, detection in pregnant women is paramount. Therefore, it is important that the level of immunity be determined in women of child-bearing age, pregnant women, neonates who were exposed in utero, and others who may have been in close contact. The clinical recognition of Rubella infection is highly unreliable, and subclinical cases are frequent. Serological testing has been shown to be an effective method of detecting infection.

## PRINCIPLE OF THE TEST

The test for the assay of Rubella IgM is based on the principle of the capture of these immunoglobulins and subsequent identification of those, which are specific, making use of their ability to bind an antigen conjugated to peroxidase. The μ-capture is performed using monoclonal antibodies bound to the solid phase (microtitration strips). The antigen is composed of purified and inactivated Rubella virus antigen..

## REAGENTS

*RVG IgM* CLIA kit contains sufficient reagents for 96 wells. Each kit contains the following reagents:

| MATERIAL PROVIDED | QUANTITY |
|---|---|
| *Antibody*-Coated Microtitration Strip | One Plate |
| Wash Concentrate | One Bottle |
| Sample Diluent | One Bottle |
| Substrate A | One Bottle |
| Substrate B | One Bottle |
| Calibrator 0 ( neg. cont ) | One Vial |
| Calibrator 1 ( cut off cont ) | One Vial |
| Calibrator 2 (pos cont) | One Vial |
| Rubella-HRP Conjugate | One Bottle |

## MATERIAL NOT PROVIDED

- Microtitration plate luminometer
- 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
- Incubator

### Antibody-Coated Microtitration Strips:

One stripholder containing 12x8 (96) microtitration wells coated with Anti-human IgM antibody. 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 BSA solution with 0.09% sodium azide as a preservative. Store at 2-8°C until expiration date.

### Rubella IgM Controls:

Three vials, positive, negative and cut off, 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.

### Rubella-HRP Conjugate:

One bottle, 12 ml, containing Rubella antigen 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 Substrate B. 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 all reagents and material 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 material. The material in this kit has been tested by CE recommended 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.. Avoid microbial contamination of reagents, especially of the conjugate, wash buffer and diluent. Avoid contamination with Substrate Solution and 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 Substrate 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 : At least 30 minutes prior to use mix 1 part of Substrate A 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 Calibrators 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 Sample Diluent.
3. Pipette 100 µL of each diluted serum sample and Calibrators to the appropriate wells. Leave one well for the blank.
4. Incubate for 15 minutes at 37°C.
5. 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 0.35 mL of the Wash Solution into each well, and (c) repeat step (a) and (b) four times.*
6. Add 100 µL of HRP-conjugate into each well.
7. Incubate for 15 minutes at 37°C.
8. 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.
9. Add 50 µL of substrate mixture to each well using a dispenser.
10. Shake the wells for 10 sec.
11. Read the wells within 4 minutes using a microplate luminometer.

## RESULTS

Calculate the mean RLU for each calibrator and unknown.

Qualitative results:

The Cut-off control corresponds to Calibrator 1.

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

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.

Quantitative results:

Arbitrary Units (AU): Positive results can be expressed in AU, interpolating the RLU values of the 3 calibrators and comparing the value of the sample with this curve.

## 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 Calibrator 1 must be at least >1000. Calibrator 2 must have a RLU at least 3 times that of Calibrator 1.

## PERFORMANCE CHARACTERISTICS

1. Sensitivity and Specificity

154 well selected human sera, obtained from a clinical laboratory , were analyzed by this Rubella IgM CLIA and reference Elisa method. Out of 154 samples, 17 were positive for the presence of IgM antibodies to Rubella virus by this assay and reference method also showed 17 of them as positive. The results are summarized below ( see Tab.1 ).

| | Positive | Negative | FN (false negative) | FP (false positive) |
|---|---|---|---|---|
| This assay | 17 | 137 | 0 | 0 |
| Test A | 17 | 137 | 0 | 0 |

## REFERENCES


1. G.B. Wisdom: Enzyme-Immunoassay. Clin. Chem. 22: 1243 (1976).
2. L. Schaefer, J. Dyke et al. Evaluation of microparticle enzyme immunoassays for immunoglobulins G and M to Rubella virus and Toxoplasma gondii on the Abbott IMx automated analyzer. J. Clin. Microbiol. 27: 2410 (1989).
3. Chaye, HH, et al: Cellular and humoral immune responses to Rubella virus structural proteins E1, E2, and C. J. Clin Microbiology, Washington, DC, 1992, pp. 596-599.
4. Mahony, JB, and Chernesky, MA: Rubella virus. In Rose, NR, et al (eds): Manual of Laboratory Immunology, ed. 4. ASM, Washington, DC, 1992, pp. 600-605.



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