RUBELLA IgG ELISA

FOR IN VITRO DIAGNOSTIC USE

Catalog No.: 0777102

RUBELLA IgG ENZYME-LINKED IMMUNO-SORBENT ASSAY

INTENDED USE

The ICN Pharmaceuticals, Diagnostics Division (ICN) Rubella IgG EnzymeLinked Immuno-Sorbent Assay (ELISA) is intended for the in vitro diagnostic detection and determination of IgG antibody to rubella virus in human sera. Individual serum specimens may be used for the determination of Immune Status. Paired sera, acute and convalescent, may be used to demonstrate waneconversion or a significant rise in antibody level, as an aid in the diagnosis of a recent or current infection.

SUMMARY

Rubella, also known as German Measles or 3-day measles, is an encephalomyocarditis virus of children and young adults. Infected persons usually present a benign, self-limiting disease characterized by rash, fever, headache, and arthritis. Only rarely has rubella in children and young adults been associated with serious symptoms (1). The evidence indicates that clinically-unapparent or asymptomatic infections are common (1).

The Centers for Disease Control and Prevention and the World Health Organization recommend all women of childbearing age and susceptible individuals be vaccinated. (3,4).

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MATERIALS REQUIRED BUT NOT SUPPLIED

1. Serum Diluent: ready for use. Contains proclin (0.1%) as a preservative, with established range printed on vial label. (96T: one bottle, 15 mL; 480T: five bottles, 15 mL each)
2. Serum Diluent: ready for use. Contains proclin (0.1%) as a preservative, with established range printed on vial label. (96T: one bottle, 16 mL each; 480T: five bottles, 16 mL each)
3. Chromogen/Substrate Solution, Tetramethylbenzidine (TMB), ready to use. (1:1, one bottle, 15 mL; 5:1, five bottles, 15 mL each)
4. Wash Buffer (20X concentrated): dilute 1 part concentrate + 19 parts deionized or distilled water. Contains TBST, Tween and proclin (0.1%) as a preservative, pH 7.2 ± 0.2. (96T: one bottle, 40 mL; 480T: one bottle, 250 mL)
5. Stop Solution: 1N sulfuric acid (H2SO4), ready to use. (96T: one bottle, 15 mL)

PRECAUTIONS

1. The human serum components used in the preparation of the Controls and Calibrators in this kit have been tested by an FDA approved method for the presence of antibody by the fluorescent antibody test (8), as well as Hepatitis B surface antigen and found negative. Because no test method can offer complete assurance that Hepatitis B, rubella virus, or other infectious agents are absent, specimens and human-based reagents should be handled with extreme care if there is any chance of contaminating other agents.

Note:

MATERIALS SUPPLIED

1. Rubella virus antigen (inactivated) coated micro-assay plate: 96 wells, configured in twelve 8 well strips. (96T: one plate; 480T: five plates)
2. Serum Diluent: ready for use. Contains proclin (0.1%) as a preservative, pH 7.5 ± 0.2. (96T: one bottle, 30 mL; 480T: two bottles, 60 mL each)
3. Chromogen: human serum. Sodium Azide (0.1%) and protamine (0.01%) added as preservatives, with high specfic activity printed on vial label. (96T: one vial, 0.50 mL; 480T: one vial, 1.0 mL each)
4. Positive Control: human serum. Sodium Azide (0.1%) and protamine (0.01%) added as preservatives, with established range printed on vial label. (96T: one vial, 0.50 mL; 480T: one vial, 1.0 mL each)
5. Negative Control: human serum. Sodium Azide (0.1%) and protamine (0.01%) added as preservatives, with established range printed on vial label. (96T: one vial, 0.40 mL; 480T: one vial, 0.80 mL each)
6. Micropipettes capable of accurately delivering 10-200 FL volumes (less than 3%, CV).
7. Diluted or dehydrated water.
9. Latex or semi-automated or automated wash equipment.
10. Single or dual wavelength microplate reader with 450 nm filter. If dual wavelength is used, set the reference filter to 650-650 nm. Read the operator’s manual or contact the instrument manufacturer to establish twenty-four performance specifications of the reader.
11. Reagents for serum dilution.
12. Refrigerate.

STORAGE AND SHELF LIFE OF REAGENTS

1. Store unopened/ sealed/under refrigeration at (22oC-25oC). The test may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. Unopened micro-assay plates must be stored between 2-8oC and 60% Relative Humidity. Unused strips must be immediately resealed in a sealable bag with desiccant and heat-sealed, and then returned to the refrigerator. (96T: one bottle, 16 mL; 480T: five bottles, 16 mL each)
3. Store HRP Conjugate between 2-8oC and 60% Relative Humidity.
4. Store the Calibrator, Positive and Negative Controls between 2-8oC and 60% Relative Humidity.
5. Store the Chromogen/Substrate Solution between 2-8oC and 60% Relative Humidity.
6. Store Wash Buffer at room temperature (21° to 25°C) for up to 5 days, or 1 week between 2° and 8° C.
7. Store Stop Solution as directed on label.

INTERPRETATION OF RESULTS

1. Handle all blood and serum as if capable of transmitting infectious agents.
2. Optimal performance of the ICN ELISAs kits depends upon the use of fresh sera should be used whenever possible. Serum specimens should be utilized within one week of collection to ensure maximum antibody recovery. If sera will be stored for more than 7 days, the specimens should be stored at -20°C or colder. Do not freeze-thaw cycles and degrade antibody. Samples that are improperly stored should not be used.
3. Reagent blank (when read against air blank) must be <0.150 Absorbance (A) reading. If reagent blank is 0.150 the run should not be completed. The reader on the blank well will continue to read the entire plate. Dispose of used plates after readings have been obtained.

QUALITY CONTROL

For the assay to be considered valid the following conditions must be met:

1. Calibrator and Controls must be run with each run test.
2. Reagent blank (when read against air blank) must be < 0.150 Absorbance (A) reading.
3. Negative Control must be ≥0.200 A at 450 nm (when read against blank).
4. Calibrator must be ≥0.250 A at 450 nm (when read against blank).
5. Positive Control must be ≥ 0.550 A at 450 nm (when read against blank).

This recommends that a Positive Control of known reactivity be included in each assay, run as part of the user’s quality control program. If above criteria are not met on repeat, contact ICN Technical Service.

1. Multiply the mean absorbance of the Calibrators by the factor assigned. This is the calibrator value. The factor is the Master Lot specific and is recorded on the Master Lot label.
2. The TPR value for each patient sample is calculated by dividing the sample absorbance by the calibrator value (obtained in Step 1).

Note:

Caution: Liquid waste at pH 4 must be neutralized prior to adding to the Sodium Hydroxide Solution (bases) to avoid formation of poison gas.

For in vitro diagnostic use only.
3. Samples that remain equivocal after repeat testing should be retested on an alternate method, e.g., immunofluorescence assay (IFA). If results remain equivocal upon further testing, an additional sample should be taken.

4. The results of ELISA performed on serum from immuno-compromised patients must be interpreted with caution. The presence of IgG antibodies against a particular virus or organ may not assure protection from that disease. For example, cases of individuals with a history of rubella vaccination experiencing infection after exposure to wild virus have been reported. These rubella infections are not known to be a risk to the fetus of a pregnant woman (2.3). Alternatively, certain immune individuals have been shown to have such low circulating IgG levels that they may appear negative or equivocal for that antibody when tested and then show a significant rise in antibody level when retested after exposure to the rubella virus (2.3).

5. The results of a single specimen antibody determination should not be used to aid in the diagnosis of recent infection. Paired samples (acute and convalescent) should be collected and tested concurrently to look for seroconversion or a significant rise in antibody level.

**PERFORMANCE CHARACTERISTICS**

Sensitivity and Specificity

A total of 462 specimens from two separate random populations were tested by the ICN Rubella IgG ELISA kit. The first population was evaluated against hemagglutination inhibition (HAI). The second population was evaluated against a commercially available Rubella IgG ELISA kit.

In the first population, there was agreement in 250 of 258 samples. One hundred and fifteen (113) were positive and 17 were negative. The remaining 8 samples were negative by HAI and positive on the ICN ELISA. The data showed 100% sensitivity and 94.8% specificity. The discrepant samples were evaluated by a reference method, a commercially available Rubella IgG IFA, 2 of 8 were confirmed as positive (1.4, 1.8) for a corrected specificity of 95.5%.

**REFERENCES**


