



Rubella IgG

(For Professional Use Only)

NAME AND INTENDED USE

ATLAS Rubella IgG is a solid phase enzyme linked immunosorbent assay. This test provides semi-quantitative measurement of Rubella IgG antibody in human serum.

SUMMARY AND EXPLANATION OF TEST

Rubella is a herpes virus. Infection with Rubella in children and adults is a self-limited, mild disease characterized by an erythematous rash, mild upper respiratory symptoms and suboccipital lymphadenopathy. After recovery, the individual is immune to subsequent infection with rubella.

Primary infection of a pregnant woman however, particular in the first trimester of pregnancy, may result in a high risk of fetal infection with severe complications. Congenital rubella is characterized by cataracts, deafness, congenital heart disease and other malformations that may occur singly or in combination. It is very important therefore, to identify those women who are not immune to Rubella and to immunize them well before they become pregnant.

This serological tests for detecting the presence of IgG antibodies to Rubella can provide valuable information regarding history of previous infection and diagnosis of active or recent infection¹. Following infection with rubella both IgG and IgM class antibodies to Rubella virus can be detected in serum. IgM class antibodies do not usually persist beyond 4 weeks while IgG antibodies may persist for life.

ATLAS Rubella IgG kit provides a reliable method for the diagnosis of acute Rubella infections, screening for Rubella immunity, diagnosis of congenital infections and vaccination follow-up³.

PRINCIPLE OF THE ASSAY

ATLAS Rubella -IgG semi-quantitative is a microwell sandwich ELISA. The wells are coated with purified Rubella antigens. The reference standards and test samples are incubated in the wells first. After incubation, the anti-Rubella antibodies will bind with coated antigens. The enzyme conjugate, goat anti-human IgG chemically conjugated with horseradish peroxidase, is then added to bind immunologically to antigen-antibody complex on the solid phase. Unbound enzyme conjugate is washed off. Upon addition of the substrate and chromogen, the intensity of color developed is proportional to the concentration of anti-Rubella IgG in reference standards and test samples and may be quantified by use of a photometric well reader at 450 nm wavelength.

WARNING AND PRECAUTION

1. ATLAS Rubella IgG Semi-quantitative is designed for in vitro use only.
2. The components in this kit are intended for use as an integral unit. The components from different lots should not be mixed and used.
3. References contains human serum should be treated as potentially infectious. All human bases products should be using appropriate precaution.

MATERIALS PROVIDED

1. Microwell Strips (96 wells): Purified Rubella antigens coated wells. 8 x 12 strips.
2. Washing Buffer Concentrate (20X) (50 mL): Prepare working solution by adding 950 ml purified water to 50 ml wash buffer.
3. Enzyme Conjugate (11 mL): Anti-human IgG conjugated to horseradish peroxidase.
4. Serum diluent (50 mL)
5. Solution A (11 mL): Buffer containing hydrogen peroxide
6. Solution B (11 mL): Tetramethylbenzidine.
7. Stop Solution: 2 N HCl.
8. Reference Standard Set (1.5 each vial): Negative, Positive, Calibrator (100 IU/mL). READY FOR USE. DO NOT DILUTE.
9. Well holder for securing individual wells.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Micro-well reader with wavelength at 450 nm.
2. Pipette with tips for measuring 5, 50 and 100 μ L.
3. Clean plastic washing bottle of 1000 mL capacity for use in washing micro-wells with working washing buffer during testing procedure.

REAGENT PREPARATION

Prepare the working washing buffer by adding the entire contents of the Wash Buffer Concentrate to 1000 mL distilled water in a clean plastic wash bottle. Mix gently to dissolve. Store at room temperature.

STORAGE AND STABILITY

1. Store the kits at 2-8°C and keep micro-wells in a dry bag with desiccants.
2. Unopened reagents are stable until expiration of the kit. Solution A and Solution B should be colorless; if the solution turns blue, it must be replaced. Do not expose these reagents to strong light during storage or usage.

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture and allow clotting. Separate the serum by centrifugation at room temperature. Do not heat and inactivate serum. If sera cannot be immediately assayed, they may be stored at -20°C for at least six months. Avoid repeated freezing and thawing of samples. Specimens obviously contaminated with bacteria should not be use. Specimens turbid with high lipid concentrations should be clarified prior to assay.

PREPARATION FOR ASSAY

1. Bring all reagents and samples to room temperature (20-25°C) and mix gently before beginning the test.
2. Have all reagents and samples ready before the start of the assay. Once the test has begun it must be performed without any interruptions to get the most reliable and consistent results.
3. Use new disposable tips for each specimen.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Mark data sheet with sample identification.
2. Prepare 1:101 dilutions of test samples, by adding 5 μ L of samples to 0.5 mL sample diluent in the separate glass tubes. (Reference standards are ready for use and do not require dilution).
3. Dispense 100 μ L of Negative Control into well #2, and #3. The Positive Control into well #4 and #5, and Calibrator into #6 and #7 the Patient Samples into the remaining wells in duplicate.
4. Incubate for 30 minutes at room temperature.
5. Wash five times with washing buffer.
6. Dispense 100 μ L Enzyme Conjugate into each well except blank well.
7. Incubate for 30 minutes at room temperature.
8. Wash five times with the Washing buffer.
9. Dispense 100 μ L of Solution A and then 100 μ L of Solution B.
10. Incubate for 15 minutes at room temperature.
11. Stop reaction by adding 50 μ L of Stop Solution each well and read at 450 nm with micro-well reader against Blank well (Only Solution A and Solution B).

PROCEDURAL NOTE

1. Wash the microwells and remove water thoroughly to get the best results.
2. Pipette all reagents and samples into bottom of the well. Vortex mixing or shaking of wells after sample and reagent pipeting is not required.
3. The appropriate number of wells should be secured in a holder and all reagent and sample caps should be removed prior to the start of testing. This will permit pipetting at equally intervals without interruption. A maximum of 30 patients' samples should be assayed at one time in order to minimize error due to timing differences between specimens

CALCULATION OF RESULTS

Any microwell reader capable of determining absorbance at 450 nm may be used. The IgG value of anti-Rubella in each patient is obtained as follows:

$$\text{EU/mL of Sample} = \frac{\text{O.D. of Sample}}{\text{O.D. of Calibrator}} \times \text{IU/mL of Calibrator}$$

EXPECTED VALUES AND INTERPRETATION OF RESULT

1). Determination of Immune Status:

A positive result (equal to or greater than 15 IU/mL) reflects prior exposure. An antibody titer greater than 20 IU/mL is generally considered to be protective against reinfection.

A negative result (less than 15 IU/mL) indicates probably no protection exists against infection. However, the test result will usually be negative in infected persons during the latent period (2-3 weeks after infection).

2) Diagnostic of acute Rubella combined with specific IgM detection:

When paired samples, taken at an interval of 2 weeks, are tested in parallel, a rise in titer (IU/mL) by a factor of 2 or more is highly indicative for acute Rubella infection. Diagnosis, however, is preferably based on specific IgM detection by Atlas IgM Semi-quantitative.

3). Determination of seroconversion after vaccination:

The first sample should be taken before vaccination and the second at least 8 weeks after vaccination. The paired samples are tested in parallel. Negative results for the first sample with a positive result for the second indicates effective vaccination.

VALIDATION OF TEST

1. Negative Control: mean absorbance value should be <0.2 units.
2. Positive Control mean absorbance value should be greater than O.D. 1.00
3. A test may be validated if the above criteria are met.

LIMITATIONS OF THE PROCEDURE

For diagnostic purpose, the anti-Rubella IgG values should be used as an adjunct to other data available to physician.

QUALITY CONTROL

Each laboratory should utilize internal controls several levels to monitor assay performance. The controls should be treated as unknown. Results obtained should be in agreement with the assigned values of the control.

ANALYTICAL SPECIFICITY

Cross-reactivity has been shown by studies demonstrating that 4 sera positive for the following antibodies were negative by the UBI Rubella IgG EIA test: Varicella-zoster virus, Epstein-Barr Virus, herpes simplex Virus (HSV) type 1 and Rheumatoid factor.

CORRELATION STUDY

A total of 72 samples were tested. The results are summarized in the following:

Atlas	Commercial EIA	
	Pos (+)	Neg (-)
	(+)	43
(-)	3	22

Sensitivity Atlas relative to Commercial EIA = 95%

Specificity of Atlas relative to Commercial EIA = 91.7%

Two examples equivocal by Atlas were excluded from these calculations.

REFERENCES

1. Katz S.L. Rubella (German Measles). Zinssmer Microbiology, 18th Edition. Jolik, Willett, Amos (ed) Chapter 75:1067, 1985. 2
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3. Voller, A. and Bidwell, D.E. A Simple Method for Detecting Antibodies to Rubella. Br. J. Exp Pathol. 56:338, 1975.
4. Gravell, m., Dorcett, P.H., Gutenson, O. and Ley, A.C. Detection of Antibody to Rubella Virus by Enzyme-linked Immunosorbent Assay. J. Infect. Dis. 136:5300,1977.

ATLAS MEDICAL

William James House, Cowley Rd,

Cambridge, CB4 4WX, UK

Tel: ++44 (0) 1223 858 910

Fax: ++44 (0) 1223 858 524

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