



## Rubella IgM

(For Professional Use Only)

### NAME AND INTENDED USE

Atlas Rubella IgM is a solid phase enzyme linked immunosorbent assay. This test provides qualitative measurement of Rubella IgM antibody in human serum and aids in the diagnosis of recent infection with Rubella.

### 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 sub occipital 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 infection<sup>1,2</sup>. 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 IgM kit provides a reliable method for the diagnosis of acute Rubella infections, screening for Rubella immunity, diagnosis of congenital infections and vaccination follow-up<sup>3,4</sup>

### PRINCIPLE OF THE ASSAY

Atlas Rubella -IgM qualitative 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 IgM 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 IgM 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 IgM qualitative 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 purified water to 1 liter.
3. Enzyme Conjugate (11 mL): Anti-human IgM conjugated to horseradish peroxidase.
4. Serum diluent (11 mL)
5. Solution A (11 mL): Buffer containing hydrogen peroxide
6. Solution B (11 mL): Tetra methylbenzidine.
7. Stop Solution: 2 N HCl.
8. Reference Standard Set (0.3 each vial): Negative and Calibrator.
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 µL
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 1:20 dilutions by adding 5 µL of references or test samples to 100 µL sample diluent (blue color) in the dilution plate provided. Mix well.
3. Dispense 100 µL of diluted References and test samples into each assigned wells in duplicate.
4. Incubate for 30 minutes at room temperature.
5. Wash five times with washing buffer.
6. Dispense 100 µL 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 µL of Solution A and then 100 µL of Solution B.
10. Incubate for 15 minutes at room temperature.
11. Stop reaction by adding 50 µL of 2 N HCl solutions (Stop Solution) into 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 pipetting 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 IgM value of anti-Rubella in each patient is obtained as follows:

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

### EXPECTED VALUES AND INTERPRETATION OF RESULT

1) A **positive** result in Rubella IgM (equal or greater than 100 IU/mL) is indicative of acute Rubella infection in a time of zero to three months before the blood samples was obtained.

A **negative** result (less than 80 IU/mL) indicates probably excludes a recent Rubella infection.

**Equivocal:** Between 80-100 IU/mL for IgM

2) Diagnostic of acute Rubella combined with specific IgG detection: When paired samples, taken at an interval of 2 weeks, are tested in parallel, a rise in titer IgG by a factor of 2 or more is highly indicative for acute Rubella infection.

### VALIDATION OF TEST

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

### LIMITATIONS OF THE PROCEDURE

For diagnostic purpose, the anti-Rubella IgM 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.

## CORRELATION STUDY

A total of 95 samples were tested. The results are summarized in the following:

Atlas	Commercial EIA	
	Pos (+)	Neg (-)
	(+)	37
(-)	2	50

Sensitivity of Atlas relative to Commercial EIA = 94.9%

Specificity of Atlas relative to Commercial EIA = 96.2%

Four examples equivocal by Atlas were excluded from these calculations.

## ANALYTICAL SPECIFICITY

Cross-reactivity has been shown by studies demonstrating that 4 sera positive for the following antibodies were negative by the Atlas Toxo IgM, CMV IgM, HSV Type I IgM and Rheumatoid factor.

## REFERENCES

1. Katz S.L. Rubella (German Measles). Zinsser Microbiology, 18<sup>th</sup> Edition. Jolik, Willett, Amos (ed) Chapter 75:1067, 1985. 2
2. Herrmann K.L. Rubella virus. Manual of Clinical Microbiology, 3<sup>rd</sup> edition. Lennette, Balows, Hausler, Truant, (ed.) Chapter 86:862, 1980.
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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