Rubella IgG

ENZYME IMMUNOASSAY TEST KIT

Enzyme Linked Immunosorbent Assay (ELISA) for Quantitative Detection of Rubella IgG antibody in Human Serum

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2°C to 8°C

INTENDED USE

Rubella IgG is intended for the Quantitative detection of IgG antibodies to Rubella virus infection in human serum. For in Vitro Diagnostic Use only.

INTRODUCTION

Rubella is a herpes virus. Generally, rubella is considered a mild adolescence disease; however, a maternal infection could be transmitted through the placenta to the fetus, causing congenital rubella. Congenital rubella may result in chronic cardiac disease, growth retardation, hepatosplenomegaly, malformations and other severe abnormalities. Children born asymptomatic may develop these abnormalities later in life.

To reduce risk of such severe complications, accurate serological methods must be performed to determine the serologic status of childbearing-aged women. The presence of rubella specific IgG in the bloodstream attests immunity to rubella. An increase in rubella IgG denotes an acute infection and differentiates rubella from other exanthematous diseases.

PRINCIPLE OF THE ASSAY

Purified rubella antigen is coated on the surface of microwells. Diluted patient serum is added to wells, and the rubella IgG specific antibody, if present, binds to the antigen. All unbound materials are washed away. A subsequent incubation with Anti-human IgG agglutinating sera conjugated with horseradish peroxidase binds to the antigen-antibody complex. Excess enzyme conjugate is washed off, and TMB substrate is added. The enzyme conjugate catalytic reaction is stopped at a specific time and absorbance is determined for each well at 450nm and 630nm with an ELISA reader. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample.

MATERIALS AND COMPONENTS

Materials provided with the test kit:

1. Coated Microwells: Rubella antigen coated wells.
2. Sample Diluent. Ready to use.
3. Negative Calibrator: 0 IU/ml.
4. Positive Calibrator: 30 IU/ml.
5. Positive Calibrator: 100 IU/ml.
6. Negative Control: Range stated on the label.
7. Positive Control: Range stated on the label.
8. Wash Buffer Concentrate (20X).
9. Enzyme Conjugate. Ready to use.
10. Cut-off Calibrator: 15 IU/ml. Rubella G Index = 1.0.
11. TMB Substrate. Ready to use.
12. Stop Solution.
13. Pack Insert.
14. Protocol Sheet.
15. Plate Sealers.
16. Microwell Holder.

Materials required but not provided

1) Precision pipettes: 10-100µl, 20-200µl, 100-1000µl
2) Disposable pipette tips
3) Distilled water
4) Disposable Gloves
5) ELISA reader
6) ELISA washer
7) Avidity Buffer

STORAGE AND STABILITY

1. **Rubella IgG** kit is stable at 2-8°C up to expiry date printed on the label.
2. Coated Microwells should be used within one month upon opening the pouch provided that once opened, the pouch must be resealed to protect from moisture. If the colour of the desiccant has changed from blue to pink at the time of opening the pouch, another coated Microwells pouch should be used.
3. Diluted Wash Buffer is stable for up to one week when stored at 2-8°C.

SPECIMEN COLLECTION & PREPARATION

1. Collect Blood specimen by venipuncture according to standard procedure.
2. Only serum should be used.
3. Avoid grossly hemolytic, lipemic or turbid samples.
4. Preferably use fresh samples. However specimens can be stored up to 48 hours at 2-8°C, for short duration.
5. For longer storage, specimens can be frozen at -20°C. Thawed samples must be mixed prior to testing.
6. Do not heat inactivate before use.
7. Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.
8. Specimen should be free from particulate matter and microbial contamination.

PRECAUTIONS

(1) Bring all reagents and specimen to room temperature before use.
(2) Do not pipette any material by mouth.
(3) Do not eat, drink or smoke in the area where testing is done.
(4) Use protective clothing and wear gloves when handling samples.
(5) Use absorbent sheet to cover the working area.
(6) Immediately clean up any spills with sodium hypochlorite.
(7) All specimens and controls should be considered potentially infectious and discarded appropriately.
(8) Neutralize acid containing waste before adding hypochlorite.
(9) Do not use kit after the expiry date.
(10) Do not mix components of one kit with another.
(11) Always use new tip for each specimen and reagent.
(12) Do not allow liquid from one well to mix with other wells.
(13) Do not let the strips dry in between the steps.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25°C) and mixed by gently inverting or swirling prior to use. Do not induce foaming.
2. Dilute wash buffer 20 times (for example add 5ml concentrated buffer to 95ml of distilled or deionized water).

TEST PROCEDURE

1. Place the desired number of coated strips into the holder.
2. Prepare 1:40 dilutions by adding **5µl** of the test samples, negative control, positive control and calibrators to **200µl** of sample diluent. Mix well.
3. Dispense **100µl** of diluted serum samples, negative control, positive control and calibrator into the appropriate wells. For the reagent blank, dispense **100µl** of sample diluent in A1 well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for **45 minutes** at **37°C**.
4. Wash each well three times by filling approximately **350µl** diluted wash buffer & blot dry.
5. Dispense **100µl** of enzyme conjugate to each well and incubate for **45 minutes** at **37°C**.
6. Wash each well three times by filling approximately **350µl** diluted wash buffer & blot dry.
7. Dispense **100µl** of TMB Substrate to each well and incubate for **20 minutes** at room temperature, away from direct light.
8. Add **100µl** of Stop Solution to stop the reaction.
9. Read O.D. at 450 - 630 nm with an ELISA reader.

Add **100µl** of sample diluent in A1 Microwell as blank

⬇

Add **100µl** diluted NC, PC, Calibrators and Samples into the respective Microwells

⬇

Apply plate sealer and incubate for **45 minutes** at **37°C**

⬇

Wash micro-wells **3 times** with diluted wash buffer

⬇

Add **100µl** Enzyme conjugate in each well

⬇

Apply plate sealer and incubate for **45 minutes** at **37°C**

⬇

Wash micro-wells **3 times** with diluted wash buffer

⬇

Add **100µl** substrate in each well

⬇

Apply plate sealer and incubate for **20 minutes** at **18-25°C in dark**

⬇

Add **100 µl** stop solution in each well

⬇

Read results @450nm (Ref.600-700nm) within **15 minutes**

RUN CRITERIA

The test run may be considered valid provided the following criteria are met:

1. The O.D. value of the reagent blank against air from a microwell reader should be less than 0.150.
2. If the O.D. value of the Cut Off Calibrator is lower than 0.250, the test is not valid and must be repeated.
3. The Rubella IgG Index for Negative and Positive Control should be in the range stated on the labels.

AVIDITY TESTING

Avidity is a measure of antigen to antibody binding. Avidity Test helps in discriminating primary infection from secondary infection. Sometimes it is not sufficient to test for IgM antibodies, as the presence of this class may be due to the persistence of IgM antibodies due to past infection or asymptomatic re-infection without risk for the fetus. For this reason it is useful to assay the avidity of IgG antibodies. The presence of low avidity is therefore an indication of recent or current infection. The avidity of IgG antibodies can be assayed with this same kit using an additional Buffer called Avidity Buffer (Cat No. 532010096) which is available on request.

For Procedure and Interpretation of results, kindly refer Pack Insert of Avidity Buffer.

CALCULATION OF RESULTS

Qualitative Determination of Rubella IgG

1. Rubella IgG index value can be calculated by dividing the mean absorbance of NC/PC/Sample by mean absorbance of Cut-Off calibrator (15 IU/ml)

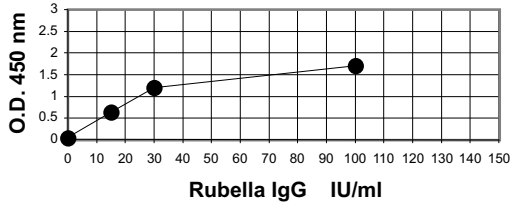
$$\text{Rubella IgG Index of NC} = \frac{\text{Absorbance of NC}}{\text{Absorbance of Cut-Off calibrator}}$$

$$\text{Rubella IgG Index of PC} = \frac{\text{Absorbance of PC}}{\text{Absorbance of Cut-Off calibrator}}$$

$$\text{Rubella IgG Index of sample} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Cut-Off calibrator}}$$

Quantitative Determination of Rubella IgG

For a quantitative estimate of anti-Rubella IgG levels of positive specimens in IU/ml, OD of cut-off and positive calibrators are plotted on Y-axis in graph versus their corresponding anti-Rubella IgG concentration of 0, 15, 30, and 100 IU/ml on X-axis. The estimates of levels in patient sera are read off the graph using their individual OD values. For example:



INTERPRETATION OF THE RESULT

IgG Index Value	Result	Interpretation
IgG Index value <0.90	Negative	Indicates absence of prior exposure to Rubella virus (< 13 IU/ml)
IgG Index value 0.91-0.99	Grey zone	Sample should be retested (13-15 IU/ml)
IgG Index value >1.0	Positive	Indicates prior exposure to Rubella virus (> 15 IU/ml)

Significant change in antibody level:

The ratio between the Rubella G Index of convalescent sample and that of pre-vaccination sample should be greater than 1.5 to be suggestive of a significant rise in antibody level.

PERFORMANCE CHARACTERISTICS

Sensitivity, specificity and accuracy were evaluated using a commercial available ELISA kit on 117 specimens. The correlation results are summarized in the following table:

		Reference ELISA			
		N	E	P	Total
Rubella IgG ELISA	N	71 (D)	0	0 (B)	71
	E	0	3	0	4
	P	0 (C)	1	41 (A)	42
	Total	71	4	41	117

Sensitivity = 100%
Specificity = 100%
Accuracy = 100%

IMPORTANT NOTE

1. This assay is a temperature sensitive assay. The best temperature condition for this assay is 37°C.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. It is recommended to use the multi channel pipettes to avoid time effect. A full plate of 96 wells may be used if automated pipetting is available.
4. Duplication of Calibrators & samples is not mandatory but may provide information on reproducibility & application errors.

LIMITATIONS OF THE ASSAY

1. A single serum sample cannot be used to determine recent infection.
2. A serum specimen taken in an early stage during acute phase of infection may contain low levels of IgG antibody and render a Rubella G Index result negative.
3. As with other serological assays, the results of these assays should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.

BIBLIOGRAPHY

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3. Katz, S.L. Rubella (German measles). Zinssmer Microbiology, 18th Edition. Jolik, Willett, Amos (ed). Chapt. 75:1067, 1985.
4. Data on file: Orchid Biomedical Systems (P) Ltd.

SYMBOL KEYS

	Temperature Limitation		Consult Instructions for use
	Manufacturer	IVD	*In vitro* Diagnostic Medical Device
	Use by	REF	Catalogue Number
	Date of Manufacture	LOT	Batch Number / Lot Number
	This side up	Σ	Contains sufficient for <n> tests
	Do not reuse		

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