SYMBOL KEYS

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

Manufactured by:

Zephyr Biomedicals

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0821/VER-01

Qualisa™

Microwell Enzyme Immunoassay | **Rubella IgM**

Enzyme linked Immunosorbent Assay (ELISA) for Qualitative Detection of Rubella IgM antibody in Human Serum

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2°C to 8°C

INTENDED USE

Qualisa™ Rubella IgM is intended for the Qualitative detection of IgM antibodies to Rubella virus infection in human serum based on capture principle. 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.

PRINCIPLE

The Rubella IgM assay is based on the principle of capture of these immunoglobulins by anti-human IgM monoclonal agglutinating sera coated on the solid phase. A subsequent incubation with Rubella antigen conjugated to horseradish peroxidase binds the IgM antibodies specific for the antigen and is revealed by the addition of the TMB substrate. When the enzymatic reaction is stopped by the addition of Stop Solution, a yellow colouring forms. The colour, which is proportional to the amount of specific antibodies present in the sample, can be read in an ELISA microplate reader. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

MATERIALS AND COMPONENTS

Materials provided with the test kit:

1. Coated Microwells: Purified anti-human IgM agglutinating sera coated wells.
2. Sample Diluent: Ready to use.
3. Negative Control: Ready to use.
4. Positive Control: Ready to use.
5. Wash Buffer Concentrate (20X).
6. Enzyme Conjugate: Ready to use.
7. TMB Substrate: Ready to use.
8. Stop Solution: Ready to use.

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

STORAGE AND STABILITY

1. **Qualisa™ Rubella IgM** 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 white at the time of opening the pouch, another coated Microwells pouch should be used.
3. Diluted Wash Buffer is stable up to one week when stored at 2-8°C.

SPECIMEN COLLECTION & PREPARATION

1. Collect Blood specimen by venipuncture according to standard procedure.
2. Serum only 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

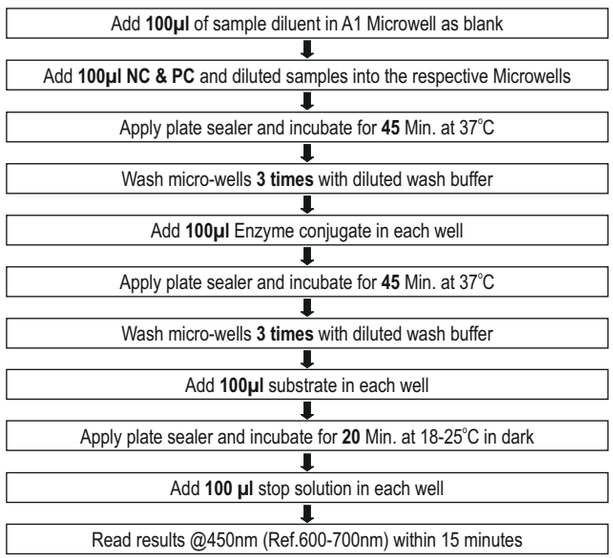
(1) Bring all reagents and specimen to room temperature before use.
(2) Do not pipette any material by mouth.
(3) Do not eat, drink or smoke in the area where testing is done.
(4) Use protective clothing and wear gloves when handling samples.
(5) Use absorbent sheet to cover the working area.
(6) Immediately 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 to 200 µl of sample diluent. **(Please do not dilute Positive Control and Negative Control, they are ready for use).** Mix well.
3. Dispense 100µl of diluted serum samples, negative control and positive control 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 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.



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 0.2.
2. The Rubella IgM Index for Negative and Positive Control should show negative and positive results.

CALCULATION OF RESULTS

1. Calculate the average value of the absorbance of the negative control.

2. Calculate the cutoff value using the following formula:
   Cut Off OD = 2 x Mean OD of Negative Control.

3. Calculate the Rubella IgM Index using the following formula:
   Rubella IgM Index= Sample OD/ Cut Off OD.

INTERPRETATION OF THE RESULT

IgM Index Value	Result
IgM Index value <0.90	Negative
IgM Index value 0.91-1.3	Grey zone
IgM Index value >1.3	Positive

PERFORMANCE CHARACTERISTICS

The precision of the assay was evaluated by testing three different sera of eight replicates over 3 days. The intra-assay and inter-assay C.V. are summarized below:

	Negative	Low positive	Positive
Intra-assay	6.3%	4.9%	3.5%
Inter-assay	8.9%	7.2%	5.5%

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 multiple channel pipettes to avoid time effect. A full plate of 96 wells may be used if automated pipetting is available.
4. Duplication NC, PC & samples is not mandatory but may provide information on reproducibility & application errors.

LIMITATIONS OF THE ASSAY

1. To prevent false negative and false positive IgM test results, caused by the presence of specific IgG and rheumatoid factor (RF) in some specimens, reagents provided in this kit has been formulated to resolve these interferences. However, in specimens with extremely high RF and high autoimmune antibodies, the possibility of these interferences cannot be ruled out entirely.
2. 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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