Rubella IgM capture EIA
An Enzyme Immunoassay for the detection of Rubella virus specific IgM in human oral fluid and serum / plasma samples

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For Research Use Only

Distributed by

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INTENDED USE AND APPLICATION
Enzyme Immunoassay (EIA) for the detection of human IgM antibodies to Rubella virus in serum, plasma and oral fluid specimens. This product is for research use only and for use by trained laboratory personnel.

SUMMARY AND EXPLANATION

The aetiological agent of rubella or German measles is the rubella virus, a small positive-sense ssRNA viurs belonging to the *Togaviridae* family and the only member of the genus *Rubivirus.* Rubella is a childhood disease, endemic throughout the world, although the use of vaccines in the developed world has reduced the incidence to low levels in these areas. Rubella can present as a mild disease associated with low grade fever, lymphadenopathy and a short-lived morbilliform rash(1). Postnatally acquired rubella is seldom associated with complications, apart from arthritis and arthralgia in up to 60% of post pubertal females and low incidences of encephalopathy, purpuric rash and transient thrombocytopenia (2). However infection with rubella during the first trimester of pregnancy can affect all organs and cause a variety of congenital defects including cataracts, congenital cardiac abnormalities and deafness (3).

Clinical diagnosis of rubella is unreliable and laboratory confirmation is essential since infection with a number of other viruses such as parvovirus B19, human herpes virus 6, measles and dengue result in rash and fever that are difficult to differentiate clinically (4). Laboratory diagnosis of current or recently acquired infection is usually performed by detection of rubella specific IgM and by demonstration of a rise in IgG antibodies within 4-7 days of symptoms. Rubella specific IgG and IgM synthesised by the foetus are detectable at birth in congenital rubella syndrome (CRS). However, since maternally derived IgG antibodies are also present in sera, the diagnosis of CRS is almost invariably made by detection or rubella-specific IgM antibodies.

TEST PRINCIPLE

In the Microimmune Rubella IgM capture EIA, undiluted oral fluid extracted from a collection device (e.g. "Oracol" or "OraSure®") or diluted serum / plasma is added to anti-human IgM coated microtitre wells in duplicate. IgM in the specimen binds to the wells and after washing, recombinant Rubella Antigen (rRA) is added to one of the duplicate sample wells and diluent to the other well. Rubella specific IgM in the sample, if present, binds the rRA. After washing the wells a monoclonal antibody to the rRA conjugated to horseradish peroxidase is added. After washing, tetramethylbenzidine (TMB) substrate is added to reveal the presence of specific IgM. The presence of Rubella specific IgM results in a colour change in the TMB from colourless to blue which then changes to yellow on stopping the reaction with 0.5M HCl. The colour change and intensity are monitored using a spectrophotometric plate reader set at 450nm with a correction filter between 620 and 650nm. The presence of Rubella specific IgM is indicated by an increase in optical density above the cut-off value in the antigen well compared to the control well.

MATERIALS PROVIDED

Each kit contains sufficient materials for 48 tests.

1. ANTI-HUMAN IGM PLATE: 8x 12 microwell strips coated with anti-human IgM antibody in a re-sealable pouch with desiccant. Open the pouch by cutting along the notched edges and separating the re-sealable joint. Return unused strips to the pouch with desiccant and store at 2..8°C. Strips must be used within 3 months of initial opening.

2. SERUM DILUENT (100 mL): One bottle containing phosphate buffered saline, protein stabiliser, detergent and red dye.

3. WASH BUFFER, 10x (100 mL): One bottle containing 10x phosphate buffered saline, detergent and preservative. Dilute 1 in 10 with good quality deionised or distilled water.

4. POSITIVE CONTROL (1.2 mL): One vial containing pre-diluted serum positive for Rubella IgM antibody in phosphate buffered saline containing detergent, protein stabiliser and antimicrobial agent. **Ready to use**. DO NOT DILUTE.

5.   NEGATIVE CONTROL (1.2 mL): One vial containing pre-diluted serum negative for Rubella IgM antibody in phosphate buffered saline containing detergent, protein stabiliser and antimicrobial agent. **Ready to use.** DO NOT DILUTE.

6. rRA, 10 x (0.6 mL): One vial containing 10 x concentrated recombinant Rubella Antigen in buffer containing stabilisers and antimicrobial agent (blue in colour). DILUTE IN ANTIGEN DILUENT BEFORE USE.

7. CONJUGATE (10 mL): One vial containing anti-Rubella E1 antibody conjugated to horseradish peroxidase (HRP) in a buffered solution containing protein stabilisers, detergent, antimicrobial agent and blue dye. **Ready to Use**.

8. TMB SUBSTRATE (10mL): One vial containing 3,3',5,5' tetramethylbenzidine. **Ready to Use**.

9. STOP SOLUTION (10mL): One vial containing 0.5M hydrochloric acid. **Ready to Use**.

10. ANTIGEN DILUENT (10mL): One vial containing phosphate buffered solution with stabilisers, detergent, antimicrobial agent (yellow in colour).

MATERIALS REQUIRED BUT NOT PROVIDED
- Oral fluid collection device (e.g. Oracol) and transport medium.
- Good quality deionised or distilled water.
- Tubes suitable for diluting serum specimens and microtitre plate sealer.
- Micropipettes and disposable tips capable of delivering 1000 μL, 100 μL 10μL and 5 μL volumes.
- Waste discard container with disinfectant.
- EIA plate reader capable of reading optical density at 450nm (and 620-650nm).
- Incubator, 37 ± 2°C.

REAGENT PREPARATION

Dilute the rRA, 10x (Reagent 6) in Antigen Diluent (Reagent 10) before use. For example, add 100μL of the rRA, 10x to 900μL of Antigen Diluent. On dilution, the solution should turn green. The diluted antigen may be stored up to 7 days at 2..8°C.

Warm the Wash buffer 10x (Reagent 3) to re-dissolve any salts that may have formed on storage. Prepare working strength wash buffer by adding 1 part Wash Buffer, 10x to 9 parts distilled or deionised water. It is recommended that working strength buffer be prepared as required on the day of use. Remaining Wash Buffer, 10x should be stored at 2..8°C. Enough Wash Buffer, 10x has been provided to enable four washes of each well.

All other reagents are provided ready to use.

WARNINGS AND PRECAUTIONS

- The Positive Control serum and Negative Control serum are not reactive for antibodies to HIV 1 and 2, HCV or Hepatitis B surface antigen. The controls should be handled and disposed of as though potentially infectious.

- The TMB substrate solution containing 3,3', 5,5'-tetramethylbenzidine has been reported to be non-carcinogenic. Contact with skin and mucous membranes should be avoided. Wear latex gloves when dispensing and using this reagent. If TMB comes into contact with skin and mucous membranes, rinse with copious amounts of water.

- The Stop Solution contains hydrochloric acid (0.5M). Contact with skin and mucous membranes should be avoided. If the Stop Solution comes into contact with these sites, rinse with copious amounts of water.

- Wear disposable gloves when handling clinical specimens and kit components. Treat all clinical specimens and controls and any materials coming into contact with them as potentially infectious.

- Dispose clinical material and potentially infected materials in accordance with local regulations.

- Do not mix components of one lot of kits with components from other lots.

- Avoid microbial contamination of reagents. Do not use reagents that show signs of contamination.

- Good laboratory procedure should be employed to avoid cross contamination of samples and reagents. Take out only the required volume of reagent from the original container (usually 0.9-1.0mL per strip) for dispensing into wells. Discard unused reagents - do not return to containers.

SPECIMEN COLLECTION

Handle all oral fluid, blood, serum and plasma as potentially infectious material.

Oral fluid specimens should be collected and processed as described in the product literature for the oral fluid collection devices. Optimal performance is obtained with specimens taken between seven days and up to four weeks after onset of symptoms.

Serum and plasma (EDTA, citrated or heparinised) samples are suitable specimens for the test and should be obtained using standard procedure.

STABILITY AND STORAGE

When stored at 2..8°C, the kit is stable up to the expiration date printed on the kit label.

ENZYME IMMUNOASSAY PROCEDURE

1. Bring all reagents to room temperature (18..25°C) before use.

2. Dilute serum/plasma samples 1/201in Serum Diluent (Reagent 2). Dispense 5 μL of specimen into a labelled tube and add 1mL of Serum Diluent. **Oral Fluid samples should not be diluted.**

3. Remove and assemble the required number of microwell strips to perform the test. A minimum of two wells is required for each sample and controls. A suggested format for the assay is as shown below.

4. Pipette 100 μL/well of the positive (Reagent 4) and negative (Reagent 5) controls in duplicate to assigned wells. Two wells for the positive control (reagent 4) and two wells for the negative control (Reagent 5).

5. Pipette 100 μL/well of the oral fluid or diluted serum specimens to assigned wells in duplicate (see Figure 1). Only test the number of samples, in a single test run, that can be dispensed within ten minutes. Alternatively use a low binding microtitre plate to pre-dispense samples and then transfer to a test plate using a multichannel pipette. Cover microtitre plate with lid or sealing tape.

Figure 1. A suggested format for dispensing specimens and controls

	1	2	3	4
A	PC	PC	S7	S7
B	NC	NC	S8	S8
C	S1	S1	S9	S90
D	S2	S2	S10	S10
E	S3	S3	S11	S11
F	S4	S4	S12	S12
G	S5	S5	S13	S13
H	S6	S6	S14	S14
	Test well Add diluted rRA to wells in column 1	**Control well.** Add Antigen Diluent (Reagent 10)	**Test well** Add diluted rRA to wells in column 3	**Control well**. Add Antigen Diluent (Reagent 10)

S1, S2 etc are either diluted serum samples or undiluted oral fluid samples. PC and NC are the ready to use positive (Reagent 4) and negative controls (Reagent 5) respectively.

6. Incubate at 37 ± 2°C in a moist chamber for 30 ± 2 minutes.

7. Wash wells four times with diluted wash buffer (see reagent preparation). The wash cycle is carried out as follows: Aspirate the contents of the well and dispense 350 μL/well of diluted wash buffer, leave to soak for approximately 30seconds and aspirate. Repeat the wash cycle three further times. Alternatively an automatic plate washer may be used.

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8. After washing, tap the wells dry on absorbent paper. For each of the controls and samples to be tested, pipette 100 μL/well of the diluted rRA antigen solution (green) to one of the sample wells, and pipette 100 μL of Antigen Diluent (yellow, Reagent 10) to the other duplicate sample well. Cover plate and incubate at $37 \pm 2°C$ in a moist chamber for 120 ± 2 minutes.

9. Wash the wells four times with wash buffer as in step 7. Tap the wells dry on absorbent paper.

10. Pipette 100 μL/well of the conjugate (Reagent 7, blue in colour) to all the wells, cover plate and incubate at $37 \pm 2°C$ in a moist chamber for 30 ± 2 minutes.

11. Wash wells four times with wash buffer as in step 7. Tap the wells dry on absorbent paper.

12. Pipette 100 μL/well of the TMB substrate (Reagent 8). This is best performed with a multichannel pipette.

13. Incubate for 20 ± 1 minutes at room temperature (18..25°C) protected from strong light.

14. Pipette 100 μL/well of the Stop Solution (Reagent 9). The stop solution should be added using the same timing and sequence used to add the substrate solution.

15. Read optical density at 450nm (set the reference wavelength at 620, or between 615 and 650nm, if available on the spectrophotometric plate reader).

CALCULATIONS

Calculate **T-C** for each specimen and for the positive and negative control as follows

$\quad$ **T-C**= $OD_{450/620}$ in rRA antigen (**Test**) well - $OD_{450/620}$ in diluent (**Control**) well

For example the OD values obtained for the Positive Control sample was

$OD_{450/620}$ was 0.858 in well to which rRA antigen was added= **T**(e.g. well A1 in table, Page 5)
$OD_{450/620}$ was 0.075 in diluent well = **C** (e.g. well A2 in table, Page 5)

T-C for positive control = 0.858 – 0.075 = 0.783

QUALITY CONTROL
$\quad$ **T-C** for the Positive Control should be ≥ 0.4
$\quad$ **T-C** for the Negative Control should be ≤ 0.20

INTERPRETATION OF RESULTS

The following criteria are required for a specimen to be identified as Rubella specific IgM Positive, Negative or Equivocal.

Rubella specific IgM Detected ("Positive")

        Serum :   **T-C** of specimen is **≥ 0.35**

        Oral Fluid   **T-C** of specimen is **≥ 0.20**

Rubella specific IgM Not Detected ("Negative")

        Serum :   **T-C** of specimen is **< 0.20**

        Oral Fluid   **T-C** of specimen is **< 0.10**

Equivocal for Rubella specific IgM

        Serum :   **T-C** of specimen is >**0.20 and < 0.35**

        Oral Fluid   **T-C** of specimen is >**0.10 and < 0.20**

A sample giving an equivocal result should be re-tested. If the equivocal status cannot be resolved on re-testing, follow up samples taken between 7 and 21 days after the initial sample should be tested in parallel with a further retest of the first sample. If an equivocal result is obtained on re-testing a follow up sample, it should be interpreted as Rubella IgM negative.

LIMITATIONS OF THE TEST

Although a positive rubella specific IgM result is indicative or recent infection or vaccination, occasionally some patients produce long persisting specific IgM antibody after past infection or vaccination.

Specimens giving high readings in the diluent only wells (i.e. OD values >0.25) indicate the presence of non-specific interfering factors, possibly rheumatoid factors (RF) and positive results on these specimens are unreliable and other confirmatory tests should be used. If RF is suspected, remove the RF using a commercially available RF absorbent and retest in the Microimmune Rubella IgM capture EIA.

Oral fluid samples with low total immunoglobulin concentration (<1ug/mL) are not suitable for use in this test and may give rise to false negative results.

Microbiological contamination of the specimens may lead to erroneous results.

Patient's profile, epidemiological data and the test results should be considered before interpreting the results of the IgM test.

TEST PERFORMANCE

The performance of the Microimmune Rubella IgM capture EIA (MI) was evaluated on the following panels of sera.

Set 1. 73 sera from Ethiopia previously tested by a competitor indirect rubella IgM EIA.

Set 2. 20 sera received for routine surveillance in the Virus Reference Department, Health Protection Agency, Colindale, U.K. and tested positive for measles IgM by a commercial EIA.

Set 3. 18 sera positive for rheumatoid factor by a latex agglutination test.

Set 4. 18 sera that formed part of the Acupanel (Quest Biomedical, U.K) and consisted of 5 specimens each for measles, varicella zoster, rubella and three for mumps.

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EVALUATION OF MICROIMMUNE RUBELLA IgM CAPTURE EIA ON SERUM SAMPLES

Table 1. Evaluation of Rubella specific IgM on sera from Ethiopia

Competitor EIA result	Microimmune Rubella IgM Capture EIA			
	POS	**NEG**	**EQV**	**TOTAL**
POS	62	2	0	64
NEG	0	0	0	0
EQV	2	7	0	9
TOTAL	64	9	0	73

Of the two MI negative and Competitor EIA positive subjects, one was a five year old boy and the sample was taken four days after onset of symptoms. The IgG in the serum from this subject was of high avidity (69%) suggesting that the case was unlikely to be an acute rubella infection. IgG was not detected in the second subject, an eight year old female, and this may be a false negative in the MI test.

Of the nine subjects giving equivocal results in the competitor test, seven were negative in the MI test. For four of these subjects IgG avidity results were available and all four had high IgG avidity (>72%). Two further samples were from infants under the seven months of age. Two subjects giving an equivocal result in the competitor test and positive result in the MI test were from 3 year old and 7 year old males and sera were collected eight and one day post onset of symptoms. IgG was not detected serum from the seven year old and IgG avidity was not determined in the serum from the 3 year old.

Overall, the sensitivity of the MI rubella IgM capture EIA on serum samples compared to the competitor test was 96.9% (95% CI 98.2% to 99.6%). The positive predictive value for the MI test was 100% (95%CI 94.2% - 100.0%).

Specificity of the MI test on serum samples (sets 2-4)

Specimen Category	MI rubella IgM result		
	POS	NEG	EQV
Measles IgM Positive (by indirect EIA)	0	20	0
RF Positive	0	18	2
VZM (Acupanel)	0	5	0
MSM (Acupanel)	0	5	0
MPM (Acupanel)	0	3	0
RBM (Acupanel)	3	2	0

VZM= varicella zoster IgM panel, MSM= measles IgM Panel, MPM= Mumps IgM Panel and RBM= rubella IgM Panel.

Excluding the two RF sera that gave equivocal results in the MI, the specificity of the test was 100% (53/53; 95%CI 93.2 to 100.0%).

EVALUATION OF THE MICROIMMUNE RUBELLA IgM CAPTURE EIA ON ORAL FLUIDS

Test performance with oral fluids was evaluated using the following specimens.

Set1: Matched oral fluids were received from 55 subjects for whom rubella IgM results on serum or blood spot were available. These were tested in the MI rubella IgM capture EIA. The samples were collected from four regions in Turkey between May and June 2003 during a rubella outbreak and were mostly taken between 10 and 14 days post onset of rash symptoms.

Set 2: 37 oral fluid specimens positive for measles IgM were tested in the rubella IgM capture EIA

Table 3: Results of tests on matched oral fluids from Set 1.

Competitor rubella IgM test result on Serum	Microimmune Result on Matched Oral Fluid		
	POS	NEG	TOTAL
POS	42	2	44
NEG	1	9	10
EQV	0	1	1
TOTAL	43	13	55

An overall agreement of 92.7% (51/55) was obtained for oral fluid results compared to serum / blood spot results. Clinical details were insufficient to interpret the discordant result in this cohort. The sensitivity of the oral fluid test compared to results on the matched serum using a competitor serum test was 95.5% (42/44; 95%CI 84.5 to 99.4%).

Of the 37 measles IgM positive oral fluid specimens tested, 33 were negative and four positive in the MI rubella IgM capture EIA. Of the four positive oral fluid samples, three oral fluids were from subjects that had recently received MMR vaccine and are likely to be true positive. The one other oral fluid sample was from a 13 month old infant, collected 2 days after onset of rash, and was a high measles IgM positive and measles PCR positive. This infant was reported as not having received MMR. Assuming that this particular sample was a MI rubella IgM false positive, the specificity of the MI test on oral fluids was 97.1%;(33/34; 95% CI 84.7 to 99.9%).

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WARRANTY

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