POINTE SCIENTIFIC
A MEDTEST COMPANY

RUBELLA IgM

REF **T8004-96** 96-Test Set IVD

For in Vitro Diagnostic Use Only

Intended Use: For the qualitative detection of human IgM antibodies to rubella virus in human serum by enzyme immunoassay, to aid in the diagnosis of rubella infection. A positive result is presumptive for the detection of anti-rubella IgM antibodies and presumptive for the diagnosis of acute or recent rubella infection.

Summary of Test

1. Prepare 1:26 dilutions of Calibrator(s), Controls and samples in the test set Diluent. Mix well.
2. Place 100 µl of the dilutions in the Coated Wells; reserve one well for the reagent blank.
3. Incubate at room temperature for 30 ± 5 minutes.
4. Drain wells thoroughly. Wash wells 4 times with Wash Solution and drain.
5. Place 2 drops (or 100 µl) of Conjugate in wells.
6. Incubate at room temperature for 30 ± 5 minutes.
7. Drain wells thoroughly. Wash wells 4 times with Wash Solution and drain.
8. Place 2 drops (or 100 µl) of Substrate in wells.
9. Incubate at room temperature for 30 ± 5 minutes.
10. Stop the enzyme reaction with 2 drops (or 100 µl) of Stop Reagent.
11. Read absorbance at 405 nm against reagent blank.

Summary and Explanation of Test

Rubella infection, also known as three-day or German measles, usually results in mild or subclinical exanthemous disease in children or young adults. Infection during pregnancy however, particularly during the first trimester, can result in fetal death, or severe anatomical deformity and mental retardation (1). Therefore, the early detection of rubella infection is of utmost importance.
IgM antibodies directed against rubella virus, reach detectable levels within a few days, and peak seven to ten days following the onset of symptoms. They remain detectable at diminishing levels for four to five weeks (2). Thus the presence of IgM antibodies in the circulation, suggests active or recent infection. In the case of congenital rubella infection the newborn may produce rubella-specific IgM for several months (3).
The Rubella IgM EIA test is intended for the detection of IgM antibodies to rubella virus. Test results are obtained after one and one-half hours incubation time. They are objective and normalized as Index values, permitting uniformity of reporting.
Because enzyme immunoassays for IgM antibodies are performed with unfractionated serum, there are two potential sources of error. These include possible competition by rubella-specific IgG, leading to false negative results; and rheumatoid factor in the presence of rubella-specific IgG, leading to false positive results. The Rubella IgM EIA test has been designed to minimize the likelihood of errors due to these causes.

Principle of the Test

Diluted samples are incubated in antigen-coated wells. Absorbents have been included in the Diluent to neutralize the affects of rheumatoid factor and anti-rubella IgG antibody. Rubella antibodies (if present) are immobilized in the wells. Residual sample is eliminated by washing and draining, and conjugate (enzyme-labeled antibodies to human IgM) is added and incubated. If IgM antibodies to rubella are present, the conjugate will be immobilized in the wells. Residual conjugate is eliminated by washing and draining, and the enzyme-labeled substrate is added and incubated. In the presence of the enzyme, the substrate is converted to a yellow end product which is read photometrically.

Reagents

Coated Wells	Coated with rubella antigen, Strain: HPV 77. 12 eight-well strips.
Well Support	One.
Diluent*	25 mL (pink color). Phosphate-buffered saline with a protein stabilizer, and absorbents for rheumatoid factor and human IgG
Calibrator 1*	0.3 mL. Human serum. Contains high levels of rubella IgM antibody. Index value shown on vial label.
Calibrator 2*	0.3 mL. Human serum. Contains moderate to low levels of rubella IgM antibody. Index value shown on vial label.
Positive Control*	0.3 mL. Human serum. Reactive for rubella IgM antibodies. Index values shown on vial label.
Negative Control*	0.3 mL. Human serum. Non-reactive for rubella IgM antibodies.
Conjugate	12 mL (green color). Goat anti-human IgM labeled with alkaline phosphatase (calf).
Substrate	12 mL. p-nitrophenyl phosphate.

Note: The substrate may develop a slight yellow color during storage. One hundred microliters of substrate should yield an absorbance value less than 0.35, when read in a microwell against air or water.

Wash Concentrate*	30 mL. Tris-buffered saline with Tween 20, pH 8.0. Prepare Wash Solution by adding the contents of the Wash Concentrate bottle to 1 liter of distilled or deionized water.
Stop Reagent	12 mL. Trisodium Phosphate 0.5 M.

* Contains 0.1% sodium azide.

Store these reagents according to the instructions on the bottle labels. Do not allow them to contact the skin or eyes. If contact occurs, wash with copious amounts of water.

Other Materials Required

1. Microplate washer
2. Pipettors for dispensing 8, 100 and 200 µl
3. Timer
4. 1 or 2 liter container for Wash Solution
5. Distilled or deionized water
6. Dilution tubes or microwells
7. Microwell reader capable of reading absorbance at 405 nm. Dual or single wavelength readers may be used.

Warnings and Precautions

1. For in vitro diagnostic use.
2. Test samples, Calibrator(s), Controls and the materials that contact them, should be handled as potential biohazards. The calibrators and controls have been found to be negative for HIV, hepatitis B surface antigen and HCV antibodies by FDA licensed tests. However, because no method can offer complete assurance that HIV, hepatitis B virus, HCV or other infectious agents are absent, these materials should be handled at the Biosafety Level 2 as recommended for any potentially infectious serum or blood specimen in the Centers for Disease Control/National Institutes of Health Manual "Biosafety in Microbiological and Biomedical Laboratories", 1993, or latest edition.
3. Avoid contact with open skin.
4. Never pipet by mouth.
5. Certain of the test reagents contain sodium azide. Azides are reported to react with lead and copper in plumbing to form compounds that may detonate on percussion. When disposing of solutions containing sodium azide, flush drains with large volumes of water to minimize the build-up of metal-azide compounds.

Xn HARMFUL
0.1% NaN_3
R 21/22: Harmful in contact with skin and if swallowed.
S24/25 - 36/37/39: Avoid contact with skin and eyes. Wear suitable protective clothing, gloves and eye/face protection.
For further information, refer to product MSDS.

6. Do not interchange reagents from different reagent lots, except for Wash Concentrate, Substrate and Stop Reagent.
7. Do not use reagents beyond their stated expiration date.
8. Incubation times recommended in the Test Procedure section should be adhered to.
9. Unused Coated Wells should be kept in their resealable bag with dessicant, and stored in the refrigerator.

Specimen Collection

Sera should be separated from clotted blood. If specimens are not tested within 8 hours, they should be stored at 2 to 8° C for up to 48 hours. Beyond 48 hours specimens should be stored at -20° C or below. Multiple freeze-thaw cycles should be avoided. Samples containing visible particulate matter should be clarified by centrifugation; and hemolyzed, icteric or grossly contaminated samples should not be used. Samples should not be heat-inactivated before testing.

Test Procedure

Allow all reagents and patient samples to reach room temperature before use. Return them promptly to refrigerator after use. The test procedure follows:

1. Prepare 1:26 dilutions of test samples, Calibrator(s), Positive and Negative Controls, in the test set Diluent. For example: add 8 µl of sample to 200 µl of Diluent in a dilution well or tube, and mix well.
Note: A single Calibrator (Calibrator 2) may be used; or Calibrator 1 and Calibrator 2 may be used to prepare a calibration curve.
2. Place an appropriate number of Coated Wells in the Well Support.
Note: For combination testing (multiple assays per plate), the strips should be assembled on a white background with good lighting. Be sure to note the placement of each strip and the corresponding color.
3. Transfer 100 µl of each diluted Calibrator, Control and patient sample to the wells.
Note: Include one well which contains 100 µl of Diluent only. This will serve as the reagent blank and will be ultimately used to zero the photometer before reading the test results, or it may be used as the zero point in a calibration curve.
4. Incubate the wells at room temperature (20 to 25° C) for 30 ± 5 minutes.
5. Wash wells four times with at least 250 µL/well/wash. Do not allow the wells to soak between washes. Drain thoroughly after the last wash.
6. Place 2 drops (or 100 µl) of Conjugate into each well.
7. Incubate the wells at room temperature for 30 ± 5 minutes.
8. Wash wells four times with at least 250 µL/well/wash. Do not allow the wells to soak between washes. Drain thoroughly after the last wash.
9. Place 2 drops (or 100 µl) of Substrate into each well.
10. Incubate at room temperature for 30 ± 5 minutes.
11. Place 2 drops (or 100 µl) of Stop Reagent into each well.
12. Read and record the absorbance of the contents of each well at 405 nm against the reagent blank.
Note: Adjust the photometer to zero absorbance at 405 nm against the reagent blank. Readings should be made within 2 hours after the reactions have been stopped.

Calculation of Results

Test results may be calculated using a single calibrator (Calibrator 2), or using a calibration curve.

Single Calibrator (Calibrator 2)
Determine the Index value for each test sample (or Control) using the following formula:

$$\frac{\text{Calibrator Index}}{\text{Calibrator Absorbance}} \times \text{Test Sample Absorbance} = \text{Test Sample Index}$$

If the Calibrator is run in duplicate, use the average absorbance value to calculate results.

Calibration Curve
Alternatively, test results may be calculated from a three-point curve comprised of: Calibrator 1 (high-point), Calibrator 2 (mid-point) and the reagent blank (zero / origin), using a point-to-point curve fit.

Quality Control

1. The Calibrator(s), Positive and Negative Controls must be included in each test run.
2. The absorbance value of Calibrator 1 must be at least 0.4 when read against the reagent blank.
3. The absorbance value of the reagent blank should be less than 0.35.
4. The Negative Control must have an Index value less than 0.9.
5. The Positive Control must have an Index value within the range printed on the label. Users may supply an alternative Positive Control if they wish.
6. The Negative and Positive Controls are intended to monitor for substantial reagent failure. The Positive Control will not ensure precision at the assay cutoff. Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations. For guidance on appropriate quality control practices, please refer to NCCLS document C24-A, Internal Quality Control Testing: Principles and Definitions.

Interpretation of Results

Index Value	Interpretation
< 0.9	Negative for rubella IgM antibody
$\geq 0.9 < 1.1$	Equivocal
≥ 1.1	Positive for rubella IgM antibody

Report results as positive or negative. The presence of IgM antibody to rubella suggests recent or current infection.
The Rubella IgM EIA cut-off values were based on statistical analyses, i.e. mean + 2 standard deviations, of 90 normal serum specimens, including 77 from women of childbearing age (18 to 45 years, mean age: 32). They were challenged in tests of positive and negative specimens (see Performance Characteristics).
Specimens which yield absorbance values above the range of the test set calibrator(s), may be pre-diluted in the test set Diluent and reassayed. The resulting Index value must be multiplied by the dilution factor. Example: If the specimen has been pre-diluted 1:5 before testing, the resulting Index value should be multiplied by 5.
The concentrations of anti-rubella IgM in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity.
The magnitude of the reported IgM level cannot be correlated to an endpoint titer. The magnitude of the assay result above the cut-off is not an indicator of the total antibody present.
Specimens collected too early during the course of the disease may not contain anti-rubella IgM antibody. Furthermore, some individuals may not produce a detectable IgM response to rubella infection.

Limitations

Rubella IgM test results are intended as an aid to the diagnosis of active or recent infection. They should however, be interpreted in conjunction with other clinical findings and diagnostic procedures.
Rheumatoid factor in the presence of specific IgG may contribute to false positive results. The absorbent in the Rubella IgM EIA Diluent is intended to neutralize the effects of rheumatoid factor. Studies have indicated that this absorbent was able to neutralize up to ninety-eight percent of the activity in a sample known to contain 3,328 IU/mL of rheumatoid factor activity.
It has been suggested that samples containing high levels of rubella specific IgG, and low levels of rubella specific IgM, may yield false negative results. These conditions may exist in the sera of congenitally infected newborns due to the presence of maternal IgG. The absorbent in the sample diluent has been designed to block IgG antibodies, and studies performed at Laboratory C have shown that the diluent is capable of completely blocking up to 73 IU/mL of rubella IgG antibody. This possibility however, cannot be completely ruled out.
Specimens obtained too early, or too late, during the course of infection, may not demonstrate detectable levels of IgM antibody. Therefore, the absence of detectable rubella-specific IgM does not necessarily rule out the possibility of current or recent infection. In cases of suspected rubella infection with negative test results, a second specimen should be obtained 5 to 7 days later, and the test should be repeated.
The assays performance characteristics with matrices other than human serum have not been established.
The assays performance characteristics with newborn specimens or with cord blood have not been established.
Results obtained for immunosuppressed individuals should be interpreted with caution.
Patients with heterophil antibody positive mononucleosis, have been reported to have heterotypic rubella IgM responses. This may be due to polyclonal stimulation of B lymphocytes by Epstein-Barr virus (4).
The performance characteristics of the Rubella IgM test with automated analyzers have not been established.

Expected Values

Studies performed with a similar enzyme immunoassay procedure (6), on 116 specimens obtained at various times after the onset rubella symptoms, yielded the following results:

Day from Onset	No. Tested	No. Positive (%)

< 0	12	0 (0%)
0	12	0 (0%)
1-2	16	10 (63%)
3-4	9	9 (100%)
5-40	53	53 (100%)
≥ 40	14	2 (14%)

In the same study, 1,192 (99.3%) of 1,200 normal blood donors were negative for IgM rubella antibodies. Two specimens gave equivocal results, and 6 gave positive results. Rubella specific IgM was confirmed in one of the two equivocal samples, and in 5 of the 6 positive samples, by another method.
Studies performed with specimens obtained in the U.S., and in the United Kingdom, using the Rubella IgM EIA test, revealed the following: The incidence of anti-rubella IgM antibody among 90 normal, asymptomatic donors in Miami, FL, including 82 female donors between the ages of 18 and 45, was 1 in 90, or 1.1 %. In 84 patients presenting with one or more rubella-like symptoms (rash, lymphadenopathy, fever) in Oxford, England, 82 (97.6 %) were positive for rubella IgM antibodies and 2 (2.4 %) were negative.

Table 1. Results of Tests of 90 Archival Specimens (frozen), from Normal South Florida Donors, Including 82 from Women of Childbearing Age. The Assays were performed at Laboratory C, Miami, FL, Using the Rubella IgM EIA Test.

Index Value Ranges	Specimens	
< 1.1	88 {80}	97.8 %
≥ 1.1 to < 2.4	2 {2}	2.2 %
≥ 2.4 to < 4.8	0	0 %
≥ 4.8	0	0 %

{ } Number of female donors of childbearing age.

Eighty-four specimens from individuals whose clinical presentation was consistent with rubella infection, were identified in the clinical studies. Of these, 82 (97.6 %) were positive, 2 (2.4 %) were equivocal, and 0 (0 %) were negative, when tested by the Rubella IgM EIA test. The ranges of values obtained for these individuals are shown below in Table 2.

Table 2. Results of Rubella IgM EIA Tests of 84 Serum Specimens Obtained from Individuals Whose Clinical Presentation was Consistent With Rubella Infection. These Specimens Were Obtained and Assayed at Laboratory A, Oxford, England.

Index Value Ranges	Specimens	
< 1.1	2 {1}	2.4 %
≥ 1.1 to < 2.4	16 {16}	19.0 %
≥ 2.4 to < 4.8	61 {55}	72.6 %
≥ 4.8	5 {4}	6.0 %

{ } Number of female donors of childbearing age.

Performance Characteristics

Comparative Testing

The results of Rubella IgM EIA tests correlate well with other commercial serological tests. Serum specimens obtained from asymptomatic normal donors and patients whose clinical presentations were consistent with rubella infection, were assayed for the presence of anti-rubella antibody using the Rubella IgM EIA test and two other commercial serological assays. The assays were performed at two independent laboratories (Lab A, Oxford, England and Lab B, Raleigh, NC), and at Laboratory C (Miami, FL). The results obtained in these studies are shown below in Tables 3, 4 and 5, respectively.

Table 3. Results of Tests of 111 Archival Patient Specimens Obtained and Tested at a Public Health Laboratory (Laboratory A) Oxford, England, Using the Rubella IgM EIA Test and Another Commercial Test.

Comparative	Rubella IgM EIA		
Test #1	Positive	Equivocal	Negative
Positive	75	0	0
Negative	15	5	16

Agreement excluding equivocal results, 85.8 %

Table 4. Results of tests of 97 Archival Specimens, Obtained from Pregnant Women, Individuals Presenting With Rubella Symptoms, Individuals Who Came in Contact with Rubella Infection and Asymptomatic Normal Individuals. The Tests were Performed at Laboratory B (Raleigh, NC), Using the Rubella IgM EIA Test and Another Commercial Test.

Comparative	Rubella IgM EIA		
Test #2	Positive	Equivocal	Negative
Positive	29	1	4
Equivocal	1	0	0
Negative	5	8	49

Agreement excluding equivocal results, 89.7 %

Table 5. Results of Tests of 91 Archival Specimens, Including: 49 Patient Specimens Obtained at a Public Health Laboratory (Oxford, England), and 42 Specimens Obtained from Serum Brokers in the U.S. The Tests Were Performed at Laboratory C (Miami, FL), Using the Rubella IgM EIA Test and Another Commercial Test.

Comparative	Rubella IgM EIA		
Test #1	Positive	Equivocal	Negative
Positive	46	0	0
Negative	2	3	40

Agreement excluding equivocal results, 97.7 %

Eighty-four of the patients in the study groups examined in Table 3, presented with one or more rubella-like symptoms, e.g. rash, lymphadenopathy, fever, etc. Follow-up serological testing was performed at a public health laboratory.

Cross-reactivity

Of thirty-four specimens which were unreactive in the Rubella IgM EIA test, 4 were shown to contain moderate to high levels of IgM antibody directed against cytomegalovirus, 1 against varicella zoster virus, 1 against Epstein-Barr virus (VCA), 4 against herpes simplex virus, 4 against toxoplasma, 8 against type A influenza virus, 10 against measles, and 1 against parvovirus.

Precision

Eight serum specimens (2 negative and 6 positive) and the Rubella IgM EIA positive and negative controls, were assayed in triplicate, on three separate occasions.

The precision experiments were performed manually at two independent laboratories (Lab A and Lab B) and at Laboratory C. These results are shown below in Tables 6, 7, 8 and 9 respectively.

Table 6. Results of Intra-assay and Interassay Precision Tests Performed at Lab A. Values were calculated from Rubella IgM EIA Index Values.

	INTRA-ASSAY			INTERASSAY		
SAMPLE	MEAN INDEX	S.D	C.V. %	MEAN INDEX	S.D	C.V. %
Pos. Control	2.0	0.115	5.9	1.9	0.112	5.9
Neg. Control	0.4	0.058	NA	0.4	0.050	NA
1	0.6	0.000	NA	0.6	0.088	NA
2	0.4	0.058	NA	0.3	0.053	NA
3	1.8	0.153	8.6	1.5	0.249	16.4
4	2.9	0.058	2.0	2.8	0.260	9.3
5	2.0	0.231	11.7	1.8	0.424	23.8
6	1.3	0.100	7.7	1.2	0.154	12.9
7	1.0	0.153	14.8	0.9	0.141	15.7
8	2.0	0.252	12.4	1.8	0.298	16.4

Table 7. Results of Intra-assay and Interassay Precision Tests Performed at Lab B. Values were calculated from Rubella IgM EIA Index Values.

	INTRA-ASSAY			INTERASSAY		
SAMPLE	MEAN INDEX	S.D	C.V. %	MEAN INDEX	S.D	C.V. %
Pos. Control	1.7	ND	ND	1.7	0.058	3.3
Neg. Control	0.8	0.171	NA	0.7	0.137	NA
1	0.8	0.058	NA	0.7	0.071	NA
2	0.4	0.058	NA	0.5	0.053	NA
3	1.0	0.115	11.2	1.1	0.109	10.1
4	3.6	0.404	11.3	3.4	0.400	11.8
5	2.0	0.100	5.0	1.9	0.105	5.5
6	1.0	0.058	6.0	1.0	0.071	7.3
7	0.8	0.115	15.1	0.8	0.067	8.6
8	1.3	0.153	11.5	1.4	0.186	13.0

Table 8. Results of Intra-assay and Interassay Precision Tests Performed at Lab C. Values were calculated from Rubella IgM EIA Index Values.

	INTRA-ASSAY			INTERASSAY		
SAMPLE	MEAN INDEX	S.D	C.V. %	MEAN INDEX	S.D	C.V. %
Pos. Control	1.2	0.058	4.7	1.3	0.122	9.2
Neg. Control	0.1	0.058	NA	0.1	0.033	NA
1	0.2	0.000	NA	0.2	0.000	NA
2	0.2	0.000	NA	0.2	0.000	NA
3	1.4	0.058	4.0	1.5	0.105	7.1
4	1.2	0.058	4.9	1.2	0.071	5.9
5	1.0	0.058	6.0	1.0	0.087	8.4
6	1.5	0.000	0	1.6	0.105	6.6
7	1.3	0.000	0	1.3	0.067	5.0
8	1.8	0.058	3.3	2.8	0.067	3.7

Table 9. Results of Interlaboratory Precision Tests Performed at Three Different Sites. Values were calculated from Rubella IgM EIA Index Values.

SAMPLE	LAB A INDEX	LAB B INDEX	LAB C INDEX	MEAN INDEX	S.D	C.V. %
Pos. Control	1.9	1.7	1.3	1.6	0.097	6.0
Neg. Control	0.4	0.7	0.1	0.4	0.073	NA
1	0.6	0.7	0.2	0.5	0.053	NA
2	0.3	0.5	0.2	0.3	0.035	NA
3	1.5	1.1	1.5	1.4	0.154	11.3
4	2.8	3.4	1.2	2.5	0.244	9.9

5	1.8	1.9	1.0	1.6	0.205	13.1
6	1.2	1.0	1.6	1.3	0.110	8.7
7	0.9	0.8	1.3	1.0	0.092	9.2
8	1.8	1.4	1.8	1.7	0.184	11.0

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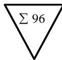
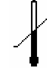


96 Tests

Catalog No.

In vitro diagnostic use only



Temperature Limitations

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