Diamedix®
A Subsidiary of ERBA Diagnostics, Inc.

Rubella IgM Capture

Enzyme Immunoassay Test Kit

For *In Vitro* Diagnostic Use

Catalog No. 720-370 – 96 Tests

SUMMARY OF PROCEDURE

1. Prepare 1:101 dilutions of Cut-Off Calibrator, controls and patient samples in Sample Diluent. Mix well.
2. Add 100 µl of diluted Cut-Off Calibrator, Controls and patient samples into the wells.
   Reserve the first well for reagent blank (100 µl of Sample Diluent)
3. Incubate at 37 ± 3° C for 60 ± 5 min.
4. Prepare the Enzyme Tracer by adding 2.9 ml of Tracer Diluent and 0.1 ml (100 µl) 30 X Tracer to each vial of lyophilized antigen. Note that 1 vial of lyophilized antigen is sufficient for at least 2 strips.
5. After incubation, discard contents of the wells. Wash the wells 3 times with Wash Solution.
6. Add 100 µl of prepared Enzyme Tracer to each well, except the Blank.
7. Incubate at 37 ± 3° C for 60 ± 5 min.
8. Wash the wells as in #5 above.
9. Add 100 µl Substrate Solution to each well.
10. Incubate at 37 ± 3° C for 20 ± 2 min.
11. Add 100 µl Stop Solution to each well.
12. Read the absorbances at 450/600-630 nm.

INTENDED USE

For the qualitative detection of IgM antibodies to rubella in human serum by capture enzyme immunoassay as an aid in the presumptive diagnosis of current or recent infection with rubella.

SUMMARY AND EXPLANATION

Rubella (German or '3-day' measles) is a mild, contagious rash primarily of children and young adults. Acute rubella virus infection in a child or adult is usually a self-limited, benign disease, characterized by a low-grade fever, mild upper respiratory symptoms, an erythematous maculopapular rash and suboccipital lymphadenopathy. However, rubella can be a very serious disease early in pregnancy, leading to miscarriages, stillbirths or birth defects (congenital rubella syndrome, or CRS). Common manifestations of congenital rubella include deafness, ocular problems including cataracts and glaucoma, congenital heart disease and mental retardation.[1, 2]

The severity and risk of the effects of rubella virus on the fetus depend on the time during pregnancy when the rubella infection occurs. Up to 85% of infants infected in the first trimester will be found to be affected after birth and even an unapparent rubella infection in the mother can result in birth defects. After an attack of rubella or vaccination against rubella most mothers are protected against the disease for life. However, reinfection with rubella can occur.[3, 4] Reinfection occurs more frequently in vaccinated than in naturally immune individuals.[5] The overwhelming majority of these reinfections occur without symptoms. Rubella reinfection during pregnancy, however, rarely results in transmission of the virus to the unborn child.[2,3]

Since rubella vaccines were first licensed for use in 1969 the number of reportable cases has dropped dramatically. However, in recent years a moderate resurgence of rubella has occurred. Although rash is the most conspicuous feature of the disease, it is of such a variable character that it may be confused with that produced by other infectious diseases and even by drugs. Thus, diagnosis of rubella on clinical grounds may be somewhat inaccurate and there is a need for continued surveillance to identify susceptible individuals and reduce the risk of CRS.[2,3]

Serologic techniques for the detection of antibodies to rubella virus provide the approach of choice for the laboratory diagnosis of acute and congenital rubella infections and for the determination of rubella immune status. IgM antibodies are the first antibodies produced in response to rubella infection and become detectable 2-3 days after onset of symptoms. These antibodies reach peak levels by 14-21 days and then rapidly diminish in concentration over the next 4 to 8 weeks until antibody is no longer clinically detectable. The presence of IgM antibody in a single specimen suggests that the patient has recently experienced a rubella infection. IgG antibodies first appear several days after the IgM response, reach peak levels 14 to 21 days later, and then persist at varying levels for life.[6,7]

Historically, hemagglutination inhibition (HI or HAI) has been the most frequently used method of screening for the presence of rubella antibodies. The first enzyme immunoassay (EIA) for rubella was reported in 1975[8] and since then this method has gained widespread acceptance for detecting IgG and IgM antibodies. Capture EIAs offer the additional advantage of avoiding interference due to rheumatoid factor and competing IgG antibodies.

The Diamedix *immuno*simplicity® Is-Rubella IgM Capture Test Kit is a capture EIA procedure intended for the qualitative detection of IgM antibodies to rubella antigen.

PRINCIPLE OF THE PROCEDURE

The *Is*-Rubella IgM Capture Test Kit utilizes ELISA based on the antibody-capture technique. Diluted patient sera are incubated with goat antibody against human IgM bound to the solid surface of a microtiter well. Patient IgM is 'captured' by the surface bound antibody. Unbound serum components are washed away. The presence of anti-rubella IgM antibodies is 'detected' and bound by an immunocomplex, Enzyme Tracer, consisting of rubella antigen which is linked to a mouse monoclonal anti-rubella antibody conjugated to horseradish peroxidase. Enzyme substrate is then added and incubated. In the presence of bound enzyme the substrate is converted to an end product. The absorbance of this end product can be read spectrophotometrically at 450 nm (reference 600-630 nm) and is directly proportional to the concentration of IgM antibodies to rubella antigen present in the sample.

REAGENTS

Each Is-Rubella IgM Capture Test Kit contains reagents for 96 tests.

Anti-IgM Coated Wells	Twelve, 8-well microwell breakapart strips, color-coded orange, coated with goat anti-human IgM.
Cut-Off Calibrator	One vial with blue cap containing 0.25 ml of human serum preserved with 0.1% sodium azide, weakly reactive for rubella IgM antibodies. The Cut-Off Calibrator is used to determine the cut-off of the assay.
Low Positive Control	One vial with white cap containing 0.25 ml human serum preserved with 0.1% sodium azide. Assigned range printed on label. The Positive Control is used to control the low range of the assay.
Negative Control	One vial with black cap containing 0.25 ml of human serum preserved with 0.1% sodium azide, non-reactive for rubella IgM antibodies. The Negative Control is used to control the negative range of the assay.

Note: The Cut-Off Calibrator and Controls are prepared from different serum lots.

Lyophilized Antigen	Six vials of lyophilized rubella antigen (grade IV sucrose purified, strain HPV 77 produced in Vero cells).
30X Tracer	One vial with red cap containing 1.0 ml mouse monoclonal anti-rubella conjugated to horse-radish peroxidase (30X concentrate) in stabilizer. The mouse monoclonal antibody is designated R22T and recognizes an antigen involved in hemagglutination.
Tracer Diluent	One bottle with red cap containing 30 ml borate buffer. Also includes blocking agent, protein stabilizers and gentamycin and Proclin® 300 as preservatives. Color-coded pink.
Sample **A** Diluent	One bottle with blue cap containing 60 ml Phosphate buffer with protein stabilizers. Contains 0.2% sodium azide and Proclin® 300, 90 ppm active ingredient. Color-coded blue.
Wash **S** Concentrate (20X)	Two bottles with clear caps containing 50 ml of Phosphate buffered saline with Proclin® 300, 15 ppm active ingredient. Color-coded light blue/green. Each bottle is sufficient to make 1050 ml of wash solution.
Substrate **G**	One amber bottle with brown cap containing 25 ml buffered TMB solution (3,3',5,5' tetramethyl-benzidine).
Stop **M** Solution	One bottle with white cap containing 30 ml of 1 N Phosphoric and 1N Hydrochloric acids. CAUTION: Acids are corrosive. Avoid contact with skin or eyes. If contact is made, flush area with copious amounts of water. See Pre-cautions section.

Store these reagents at 2 to 8°C.

OTHER MATERIALS REQUIRED

Manual Users:

Wash bottle or automated microplate washer
Pipettors capable of dispensing appropriate volumes
Timer
One liter graduated cylinder
One liter wash solution reservoir
Deionized or distilled water
Absorbent toweling
Tubes or microwell plate for sample dilution
Reader capable of reading absorbance at 450 nm, reference at 600-630 nm.
Incubator capable of maintaining temperature of 37 ± 3°C

Automated EIA Processor Users:

One liter graduated cylinder

Deionized or distilled water

Pre-dilution cups, strips or plates.

ProbeClean™ Concentrate, or tip washing detergent solution, if applicable.

PRECAUTIONS

REAGENTS: For *In Vitro* Diagnostic Use.

1. Handle samples, calibrator, controls and the materials that contact them as potential biohazards. Each donor unit in the calibrator and controls has been found negative for Hepatitis B surface antigen, HCV and HIV-I and 2 antibodies by FDA-approved third generation tests. However, because no method can offer complete assurance that HIV-1 and 2, Hepatitis B virus or Hepatitis C virus, 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.
2. The reported 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.
3. Never pipette by mouth.
4. Avoid contact with open skin and mucous membranes.
5. Certain of the test reagents contain Proclin® 300 as a preservative. When disposing of reagents containing Proclin® 300, flush drains with copious amounts of water to dilute the active components below active levels.
6. Reagents containing Sodium Azide:
   (a) **CAUTION:** Some reagents in this kit contain Sodium Azide as preservative. Sodium Azide may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. For further information, refer to "Decontamination of Laboratory Sink Drains to Remove Azide Salts", in the Manual Guide – Safety Management No. CDC-22, issued by the Centers for Disease Control and Prevention, Atlanta, GA, 1976.

   European Communities Hazardous Substance Risk Phrases (Regulation (EC) No 1272/2008)

   H300 –Fatal if swallowed.
   H310 – Fatal if contact with skin.
   EUH032 – Contact with acids liberates very toxic gas.
   H410 – Very toxic to aquatic life with long lasting effect.
   P264 – Wash all exposed external body areas thoroughly after handling.
   P302+P352 – IF ON SKIN: Wash with plenty of water and soap.
   P301+P310/P330 – IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician. Rinse mouth.
   P270 – Do not eat, drink or smoke when using this product.
   P501 – Dispose of contents/container as hazardous waste.
   P391 – Collect spillage.
   P273 – Avoid release to the environment. Refer to special instructions/ Safety Data Sheet.
   (b) Sodium Azide inhibits horseradish peroxidase activity. Care must be taken to ensure that azide is not carried over from other reagents into conjugate and substrate steps.
7. Avoid contamination of the TMB substrate solution with conjugate or other oxidants which will cause the solution to change color prematurely.
8. Do not interchange reagents from different reagent lots except for Sample **A** Diluent, Wash **S** Concentrate, Substrate **G** and Stop **M** Solution.
9. Do not use reagents beyond their expiration date. Expiration dates are printed on the reagent labels.
10. Store unused reagents at 2 to 8°C.
11. Incubations above or below the recommended temperatures or times may give erroneous results.
12. The capture ELISA method is a very sensitive technique. Maintain consistent pipetting technique, incubation times, and temperature conditions throughout the test procedure. Cross contamination between reagents can invalidate the test.
13. Coated microwells should be stored with the desiccant in the resealable bag provided and returned to the refrigerator immediately after use.
14. (*Manual Procedure Only*) The washing procedure is very important and requires special attention. (Please refer to the Procedure section.)

NOTE: *Improperly washed wells may give erroneous results.*

SPECIMEN COLLECTION

Whole blood should be collected by accepted medical techniques. Separated serum should remain at 22°C for no longer than 8 hours. If assays are not completed within 8 hours, serum should be refrigerated (2-8°C). If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at -20°C. Avoid multiple freeze-thaw cycles. Prior to testing, bring frozen sera to room temperature slowly and mix gently, avoiding foam formation. Specimens containing visible particulate matter should be clarified by centrifugation before testing. Grossly contaminated, hemolyzed, lipemic, or icteric specimens should not be used. The CLSI, formerly NCCLS, provides recommendations for collecting and storing blood specimens.[10]

CAUTION: *Serum samples must not be heat-inactivated prior to use.*

PROCEDURE

Allow all test components and patient samples to warm to room temperature before use. Invert reagent bottles gently several times before use. Return promptly to the refrigerator after use.

Prepare Wash Solution by adding 50 ml of Wash Concentrate (20X) to one liter of deionized or distilled H_2O.

Each vial of antigen is sufficient for at least 2 strips. Reconstitute only the number of vials required. Discard any unused Enzyme Tracer after the day's testing is completed.

MANUAL USERS:

1. Prepare 1:101 dilutions of the Cut-Off Calibrator (in triplicate), controls and patient samples in Sample Diluent. (e.g., by addition of 2 µl sample to 200µl Sample Diluent or 5 µl sample to 500 µl Sample Diluent).
2. Mix sample dilutions gently by withdrawing and expelling in a pipette tip 2 or 3 times or by vortex mixing for 2 or 3 seconds. Transfer 100 µl of diluted Calibrator, controls and patient samples, to the antigen wells. Avoid formation of bubbles when transferring diluted samples.
   Note : Include one well which contains 100 µl of Sample Diluent as a reagent blank. This will ultimately be used to "zero" the photometer before reading test results. ***DO NOT ADD ENZYME TRACER TO THE BLANK WELL.***
3. Allow the wells to incubate uncovered at 37± 3° for 60 ± 5 minutes.
4. As soon as the sample incubation has commenced, prepare the Enzyme Tracer by adding 2.9 ml of Tracer Diluent to each vial of lyophilized antigen. Mix until all the lyophilized material is reconstituted. Then add 100 µl of 30 X Tracer to each antigen vial and mix well. Allow the prepared Enzyme Tracer to sit at room temperature (18-30°C) for at least 30 minutes.
5. Aspirate or discard the contents of the wells. Remove any excess moisture in the wells by tapping on paper toweling. Wash the wells by rinsing 3 times with at least 300 µl each of Wash Solution. After adding the 3rd volume of Wash Solution, allow the wells to "soak" for at least one minute prior to final aspiration/emptying. When using an automated washer, follow the manufacturer's instructions and set up the same wash procedure as described.
6. Place 100 µl of Enzyme Tracer into each well (*except the Blank*), avoiding bubble formation.
7. Add 100 µl of Sample Diluent to the Blank well.
8. Allow the wells to incubate uncovered at 37 ± 3° for 60 ± 5 minutes.
9. Wash the wells as described in Step 5 above.
10. Place 100 µl of Substrate into each well, avoiding bubble formation.
11. Allow the wells to incubate uncovered at 37 ± 3° for 20 ± 2 minutes.
12. Place 100 µl of Stop Solution into each well, avoiding bubble forma-

tion.

13. Read the absorbance of each well at 450 nm using a reference wavelength of 600-630 nm. The plate should be read within 30 minutes of adding Stop Solution.

Automated EIA Processor Users:

When using an Automated EIA Processor, refer to the Operator's Manual for the test setup and procedures.

NOTE: *Automated EIA Processor users must validate their equipment to demonstrate that the results obtained are equivalent to those obtained using manual assay.*

QUALITY CONTROL

1. The Low Positive and Negative Controls must be included in each test run.
2. The absorbance of the Blank must be < 0.100.
3. The absorbance of the Cut-off Calibrator must be ≥ 0.150 when read against the Blank.
4. The Low Positive and Negative Controls must be within their assigned ranges.

If any one of these criteria is not met, the results are invalid and the test should be repeated.

NOTES: Additional controls may be tested according to guidelines or requirements of local, state or federal regulations or accrediting organizations. For guidance on appropriate quality control practices, please refer to CLSI, formerly NCCLS, document C24-A2, Statistical Quality Control for Quantitative Measurements: Principles and Definitions.

RESULTS

1. Calculation

Calculate the mean absorbance of the Cut-Off Calibrator. Note: When calculating the mean absorbance value for the Cut-Off Calibrator exclude any absorbance value that deviates by more than 20% from the mean of the three absorbance values. Calculate the mean absorbance value from the two remaining absorbances. Exclusion of more than one of the 3 absorbance values invalidates the run. Determine the Index Value for each patient sample or control using the following formula:

$$\frac{\textbf{Absorbance of Sample}}{\textbf{Mean Absorbance of Cut-off Calibrator}} = \textbf{Index value}$$

Example: *Absorbance values obtained for the Cut-Off Calibrator: 0.356, 0.345, 0.368 (after subtraction of the Blank)*
Mean Absorbance of the Cut-Off Calibrator = 0.356
Sample Absorbance = 0.959
Index value = 2.69

When using an Automated EIA Processor (e.g. MAGO® Plus Automated EIA Processor), results are calculated and expressed as Positive, Equivocal or Negative.

2. Interpretation of Results

Index Value	*Interpretation*
< 0.90	Negative for anti-rubella IgM
0.90 – 1.09	Equivocal for anti-rubella IgM*
≥ 1.10	Positive for anti-rubella IgM

Note that when using the assay qualitatively the magnitude of the Index Value has no significance and results should be reported as under 'Interpretation' above.

* When equivocal results are obtained, another specimen should be collected ten to fourteen days later and tested in parallel with the initial specimen. If the second sample is also equivocal, the patient is negative for primary or recent infection, and equivocal for antibody status. If the second sample is positive, the patient may be considered to have a primary infection.

3. Reporting Results

When reporting results the following statement should be included : "The following results were obtained with the Diamedix ***immuno***simplicity *Is*-Rubella IgM Capture EIA Test System. The magnitude of the measured result, above the cut-off, is not indicative of the total amount of antibody present. "

CUT-OFF ESTABLISHMENT

The Diamedix *Is*-Rubella IgM Capture Test Kit cut-off value has been established to optimally discriminate those individuals with, from those individuals without, IgM antibodies to rubella. The optimal cut-off value was determined by statistical analysis of 200 normal sera shown to be negative for rubella IgM antibodies in other test methods. The mean and standard deviation of the absorbance values for these sera were 0.0567 and 0.0518 respectively. The cut-off was determined as being equal to the mean plus 3 standard deviations, 0.0567 + (3 X 0.0518) = 0.2121. The Cut-Off Calibrator has been titrated to equal this result. Therefore, the mean value of the Cut-Off Calibrator will be equal to the cut-off for the assay. To account for the inherent variation in any EIA method, an equivocal range of +/- 10% has been included.

The appropriateness of the cut-off value was verified by applying the principles from Receiver-Operator Characteristics (ROC) Curves to 246 sera assayed manually by Diamedix in the Is-Rubella IgM Capture Test Kit and another commercially available test method. At the optimized cut-off level, the Diamedix *Is*-Rubella IgM Capture Test Kit has a relative sensitivity of 95% and a relative specificity of 96% based on comparison to the marketed test. Comparable values were obtained for MAGO Plus results.

LIMITATIONS

1. The results obtained with the *Is*-Rubella IgM Capture Test Kit serve only as an aid to diagnosis of current or recent infection. They should be interpreted in conjunction with other clinical findings and diagnostic procedures.
2. Rubella IgM ELISA assays are not intended to replace virus isolation and/or identification.
3. Rubella vaccination usually results in elevated levels of specific IgM antibodies which may persist for several months.
4. Low levels of IgM antibodies may occasionally persist for more than 12 months post-infection. This response may be distinguished from the early IgM response to infection by testing samples two weeks later to ascertain if there is a change in IgM antibody level.
5. Assay performance characteristics have not been established for visual result determination.
6. Performance of this assay has not been established on spectrophotometry utilizing a single wavelength.
7. The test should be performed on serum. The use of whole blood or plasma has not been established.
8. A negative result does not always exclude the possibility of active rubella infection. The sample may have been collected before appearance of IgM antibody. If infection is suspected, a second sample should be collected at least 10 days after onset of rash and tested.
9. An IgM response can sometimes accompany re-infections.
10. The performance characteristics have not been established for newborns using cord blood.
11. The results on serum from immunosuppressed individuals must be interpreted with caution.
12. The performance characteristics of the Diamedix Is-Rubella IgM Test Kit with automated equipment other than the MAGO® Plus Automated EIA Processor have not been established.

EXPECTED VALUES

The prevalence of rubella infection can vary depending on a number of factors such as age, gender, vaccination history, geographic location, socio-economic status, race, type of test used, specimen collection and handling procedures and clinical and epidemiological history of individual patients. In the present study two hundred sera from South Florida blood donors (102 female and 98 male) were evaluated in the Is-Rubella IgM Capture Test Kit. Of these samples, one hundred and ninety-five (97.5%) were negative, two (1%) were positive and three (1.5%) were equivocal. Comparable results were obtained using the MAGO Plus. TABLE 1 provides age distribution, geographic location and prevalence. FIGURE 1 is a histogram showing the distribution of Index values obtained for this population. FIGURE 2 shows the distribution of Index values for the positive samples tested by Diamedix Corporation (see Performance Characteristics).

TABLE 1
Age Distribution and Prevalence of Is-Rubella IgM in a Normal S. Florida Population

	Number of Donors	% Seronegative	% Seropositive	% Equivocal
Total Number	200			
Geographic Location: S. Fla	200	97.5% (195)	1.0% (2)	1.5% (3)
Age: 10 – 19	18	100.0% (18)	0.0% (0)	0.0% (0)
20 – 29	47	100.0% (47)	0.0% (0)	0.0% (0)
30 – 39	74	98.6% (73)	0.0% (0)	1.4% (1)
40 – 49	40	92.5% (37)	2.5% (1)	5.0% (2)
50 – 59	11	91.0% (10)	9.0% (1)	0.0% (0)
60 – 69	9	100.0% (9)	0.0% (0)	0.0% (0)
>70	1	100.0% (1)	0.0% (0)	0.0% (0)
Gender				
Males	98	97.0% (97)	2.0% (2)	1.0% (1)
Females	102	98.0% (98)	0.0% (0)	2.0% (2)

FIGURE 1
Distribution of *Is*-Rubella IgM Results in a Normal Population

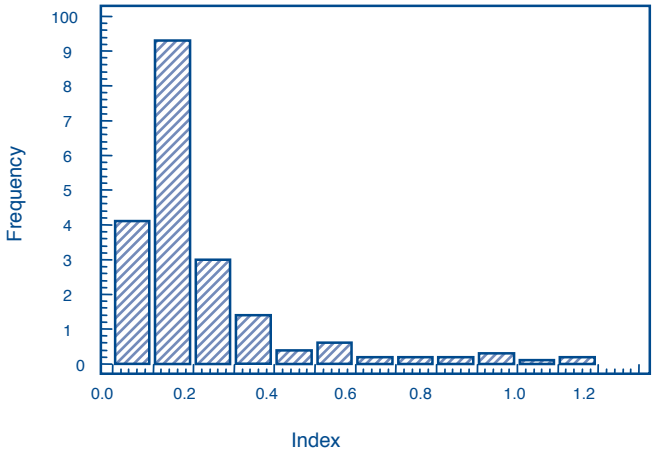
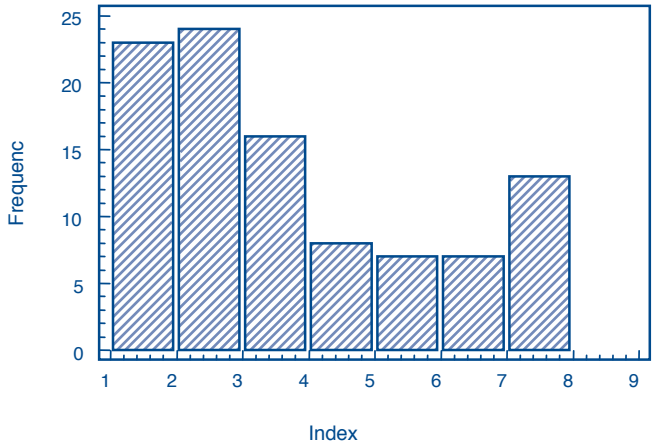


FIGURE 2
Distribution of *Is*-Rubella IgM Results in a Positive Population



PERFORMANCE CHARACTERISTICS

A. Comparison Testing

A total of four hundred and ninety-eight sera were tested for the presence of rubella IgM antibodies using the Diamedix *Is*-Rubella IgM Capture Test Kit and two other marketed tests at two independent sites, (site #1, California and site #2, New York) as well as Diamedix Corp., Miami, FL (site #3). At site #3 testing was performed both manually and using the MAGO® Plus Automated EIA Processor.

Site #1, a large commercial laboratory in California, not affiliated with the manufacturer, tested 127 samples. These consisted of 101 fresh samples submitted to the laboratory for Rubella IgM testing and 26 frozen samples which had previously tested positive for Rubella IgM antibodies. Samples came from a variety of geographic locations and from patients with ages ranging from 1 day to 62 years old. For the fresh samples, 23 were from males and 77 from females. The remaining sample was not identified as regards gender. TABLE 2 compares the results obtained for the *Is*-Rubella IgM Capture Test Kit and their currently used EIA testing method.

Site #2, a commercial reference laboratory in New York, not affiliated with the manufacturer, tested 125 samples. These samples consisted of 50 fresh and 65 frozen samples submitted to the laboratory for Rubella IgM testing, as well as an additional 10 frozen samples procured from a vendor based on their supposed positive serostatus. Samples were obtained from various geographic regions and from patients with ages ranging from 2 years to 57 years old. Twenty-four samples were from males and ninety-six from females. The remainder were not identified as regards gender. Thirty-six of the females were identified as pregnant. TABLE 3 compares the results obtained for the *Is*-Rubella IgM Capture Test Kit and their currently used EIA testing method. TABLE 3a shows the performance for the pregnant female samples.

TABLE 2
***Is*-Rubella IgM Capture – Site #1**

		Positive	Negative	Equivocal
Other EIA	Positive	21	4	1
	Negative	3	95	0
	Equivocal*	0	3	0

				** *95% CI*
Overall Agreement	116/123	=	94.3%	88.6 – 97.7

TABLE 3
***Is*-Rubella IgM Capture – Site #2**

		Positive	Negative	Equivocal
Other EIA	Positive	7	13	0
	Negative	2	102	1
	Equivocal*	0	0	0

				** *95% CI*
Overall Agreement	109/124	=	87.9%	82.2 – 93.6

TABLE 3a: Pregnant Samples
***Is*-Rubella IgM Capture - Site #2**

		Positive	Negative	Equivocal
Other EIA	Positive	2	3	0
	Negative	2	29	0
	Equivocal*	0	0	0

				** *95% CI*
Overall Agreement	31/36	=	86.1%	70.5 – 95.3%

* Equivocal results were excluded from calculations.

** 95% Confidence Intervals (CI) calculated by the Exact Method.[9]

For Site #1, further resolution of the discordant samples was performed by testing such samples in a referee capture EIA method. Of the four samples that were negative in the *Is*-Rubella IgM Capture Test Kit and positive in the other EIA, three were negative and one was positive in the referee capture EIA method. The three samples positive in the Is-Rubella IgM Capture and negative in the other EIA were negative in the referee capture EIA.

For Site #2, further resolution of the discordant samples was performed in a similar manner. However, in this case, two referee test methods were used. The first was a capture EIA and the second was a non-capture EIA. Of the thirteen samples that were negative in the *Is*-Rubella IgM capture Test Kit and positive in the other EIA, nine were negative and four were positive in the capture EIA. For the non-capture EIA, all 13 samples were negative. For the two samples that were positive in the *Is*-Rubella IgM Test Kit and negative in the other EIA, both were negative in either referee method.

Note that the tabulated data were not recalculated after retesting of

discordant samples

Site #3 (Diamedix Corp.) tested 246 samples (all frozen) by both the manual and the automated method. Of these samples 111 were from normal S. Florida blood donors. Fifty-six of the samples were obtained following an outbreak in the UK and were either serum pairs, seroconversion samples or had IgM detected by other methods. Fifty-five of the sera were from an outbreak in Japan. Seventeen sera were obtained post-vaccination and the remaining 7 sera were a commercially available seroconversion panel. TABLES 4 and 5 compare the results obtained for the *Is*-Rubella IgM Capture Test Kit and another marketed capture EIA method.

TABLE 4
***Is*-Rubella IgM Capture**
Site #3: Manual

		Positive	Negative	Equivocal
Other EIA	Positive	94	3	6
	Negative	6	127	1
	Equivocal*	1	7	1

			** 95% CI
Overall Agreement	221/230 =	96.1%	96.1 – 99.6

TABLE 5
***Is*-Rubella IgM Capture**
Site #3: MAGO Plus

		Positive	Negative	Equivocal
Other EIA	Positive	100	2	1
	Negative	5	126	3
	Equivocal*	2	7	0

			** 95% CI
Overall Agreement	226/233 =	97.0%	97.8 – 100.0

* Equivocal results were excluded from calculations.

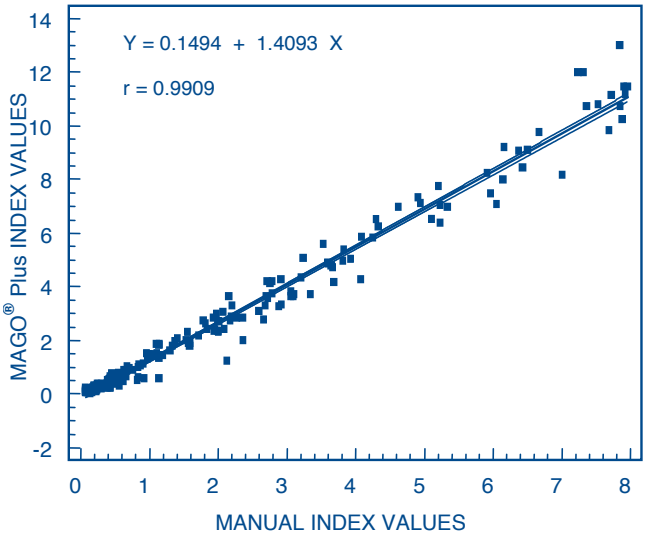
** 95% Confidence Intervals (CI) calculated by the Exact Method.[9]

For Site #3 (manual testing), further resolution of the discordant sera revealed that of the 3 sera negative in the *Is*-Rubella IgM Capture Test Kit but positive in the other EIA, one was negative and two were positive by a referee EIA method. For the six sera positive in the *Is*-Rubella IgM Capture Test Kit and negative by the other EIA, 3 were positive and 3 were negative in the referee EIA. For MAGO® Plus testing, of the two sera that were negative in the *Is*-Rubella IgM Capture Test Kit but positive in the other EIA, one was negative and one was positive in the referee EIA method. For the 5 sera that were positive in the *Is*-Rubella IgM Capture Test Kit and negative in the other EIA, 3 were positive and 2 were negative in the referee EIA.

B. Correlation of Manual and MAGO Plus Results

The *Is*-Rubella IgM Capture Test Kit has been developed for automated as well as manual use. To demonstrate the equivalence of the manual and MAGO Plus procedures, the results of the 246 samples tested above were compared. A scattergram and regression line of the results obtained with 95% confidence intervals is shown in FIGURE 3.

FIGURE 3
Manual vs. MAGO Plus Correlation



C. Cross-Reactivity Data

The specificity of the *Is*-Rubella IgM Capture Test Kit was verified by testing a number of sera containing relatively high levels of IgM antibody to other viruses as determined using commercially available test kits. A total of 25 known IgM-positive sera were tested. In addition, the effects of potential interference from rheumatoid factor (RF), anti-nuclear antibody (ANA), viral-specific IgG and heterophile antibodies were assessed by testing an additional 30 characterized sera. These data are shown in TABLE 6. TABLE 7 shows the lack of interference from samples containing high levels of IgG antibodies and low levels of IgM antibodies before and after removal of the IgG-class antibody.

TABLE 6

Specificity	# of Positive Samples	# Positive in *Is*-Rubella IgM Capture
EBV IgM	7	0
Lyme IgM	3	0
HSV IgM	5	0
CMV IgM	5	0
Toxoplasma IgM	5	0
Heterophile Antibody	4	0
RF	5	0
ANA	10	0
Rubella IgG	11	0

TABLE 7

Sample #	Before IgG Removal		After IgG Removal	
	IgG IU/ml	IgM Index	IgG IU/ml	IgM Index
1	48.9	2.389	0.0	2.071
2	46.2	1.585	0.0	1.382
3	55.3	2.499	0.0	2.359
4	35.8	2.829	0.0	2.567
5	44.5	2.053	0.0	1.788
6	38.7	2.027	0.0	1.737
7	36.4	1.826	0.0	1.613

IgG Pos ≥ 10 IU/ml IgM Pos ≥ 1.10

D. Verification of IgM Specificity

To confirm that the *Is*-Rubella IgM Capture Test Kit specifically detects IgM-class antibodies, twelve samples positive for Rubella IgM in the *Is*-Rubella IgM Capture Test Kit were treated with dithiothreitol (DTT) to destroy the IgM and were then retested in the *Is*-Rubella IgM Capture Test Kit. The results in TABLE 8 show that these samples were rendered negative following treatment with DTT confirming the specificity of the *Is*-Rubella IgM Capture Test Kit for detecting IgM-class antibodies.

TABLE 8

Sample	Untreated		Treated with DTT	
	Is-Rubella IgM Capture Index	Interp	*Is*-Rubella IgM Capture Index	Interp
1	2.922	POS	0.227	NEG
2	4.683	POS	0.640	NEG
3	3.666	POS	0.418	NEG
4	3.278	POS	0.371	NEG
5	4.096	POS	0.565	NEG
6	4.872	POS	0.821	NEG
7	2.860	POS	0.394	NEG
8	4.384	POS	0.539	NEG
9	3.786	POS	0.499	NEG
10	2.325	POS	0.364	NEG
11	3.821	POS	0.628	NEG
12	1.736	POS	0.247	NEG

E. Precision

Six serum samples, as well as the kit Controls, were tested to assess the precision of the *Is*-Rubella IgM Capture Test Kit. Sites #1 and #2 tested samples in triplicate in three separate runs on three different days. Site #3 (Diamedix Corp.) tested samples in triplicate in two separate runs on three different days both manually and using the MAGO® Plus Automated EIA Processor. The results obtained are shown in TABLES 9-12.

TABLE 9
Site #1- Intra-Assay and Interassay Precision

SERUM	INTRA-ASSAY DAY 1			INTRA-ASSAY DAY 2			INTRA-ASSAY DAY 3			INTERASSAY (n=9)		
	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%
R1	0.185	0.01	5.41	0.220	0.01	4.55	0.195	0.005	25.64	0.200	0.029	14.50
R2	1.085	0.06	5.53	1.219	0.03	2.46	1.231	0.02	1.62	1.178	0.078	6.62
R3	1.590	0.12	7.55	1.588	0.08	5.04	1.490	0.04	2.68	1.556	0.088	5.66
R4	0.164	0.01	6.10	0.210	0.03	14.29	0.200	0.02	10.00	0.191	0.027	14.14
R5	2.547	0.24	9.42	2.645	0.14	5.29	2.460	0.10	4.07	2.551	0.168	6.59
R6	3.635	0.08	2.20	3.883	0.04	1.03	3.582	0.07	1.95	3.700	0.150	4.05
LPC	1.158	0.02	1.73	1.364	0.07	5.13	1.396	0.05	3.58	1.306	0.120	9.19
NC	0.262	0.01	3.82	0.273	0.00	3.66	0.245	0.01	4.08	0.260	0.013	5.00

TABLE 10
Site #2 - Intra-Assay and Interassay Precision

SERUM	INTRA-ASSAY DAY 1			INTRA-ASSAY DAY 2			INTRA-ASSAY DAY 3			INTERASSAY (n=9)		
	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%
R1	0.277	0.04	14.44	0.288	0.06	20.83	0.435	0.10	22.99	0.333	0.097	29.13
R2	1.382	0.07	5.07	1.287	0.08	6.22	1.274	0.04	3.14	1.314	0.077	5.86
R3	1.722	0.15	8.71	1.431	0.15	10.48	1.574	0.08	5.08	1.576	0.170	10.79
R4	0.279	0.02	7.17	0.184	0.02	10.87	0.416	0.06	14.42	0.293	0.106	36.18
R5	2.330	0.02	0.86	2.042	0.19	9.30	2.531	0.15	5.93	2.301	0.246	10.69
R6	3.413	0.08	2.34	2.950	0.02	0.68	2.963	0.07	2.36	3.108	0.235	7.56
c/o CAL	0.944	0.03	3.18	0.719	0.06	8.34	0.901	0.04	4.44	0.854	0.110	12.88
LPC	1.168	0.04	3.42	1.195	0.12	10.04	1.281	0.18	14.05	1.215	0.121	9.96
NC	0.175	0.03	17.14	0.174	0.04	22.99	0.468	0.15	32.05	0.272	0.168	61.76

TABLE 11
Site #3-Intra-Assay and Interassay Precision (Manual)

SERUM	INTRA-ASSAY DAY 1			INTRA-ASSAY DAY 2			INTRA-ASSAY DAY 3			INTERASSAY (n=18)		
	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%
A	0.315	0.073	23.17	0.268	0.029	10.82	0.250	0.017	6.80	0.278	0.052	18.71
B	0.275	0.070	25.45	0.213	0.026	12.21	0.208	0.042	20.19	0.232	0.056	24.14
C	1.247	0.028	2.25	1.220	0.066	5.41	1.251	0.070	5.60	1.240	0.056	4.52
D	1.487	0.109	7.33	1.626	0.120	7.38	1.588	0.187	11.78	1.567	0.147	9.38
E	2.179	0.120	5.51	2.209	0.167	7.56	2.027	0.077	3.80	2.138	0.144	6.74
F	4.195	0.231	5.51	4.359	0.480	11.01	4.549	0.504	11.08	4.368	0.425	9.73
c/o CAL	1.060	0.074	6.98	0.995	0.113	11.36	0.967	0.080	8.27	1.007	0.094	9.33
LPC	2.032	0.250	12.30	1.698	0.108	6.36	1.766	0.105	5.95	1.832	0.217	11.84
NC	0.246	0.063	25.61	0.163	0.039	23.93	0.167	0.021	12.57	0.192	0.057	29.69

TABLE 12
Site #3- Intra-assay and Interassay Precision (MAGO Plus)

SERUM	INTRA-ASSAY DAY 1			INTRA-ASSAY DAY 2			INTRA-ASSAY DAY 3			INTERASSAY (n=18)		
	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%
A	0.17	0.046	27.06	0.16	0.054	33.75	0.17	0.040	23.53	0.17	0.04	23.53
B	0.15	0.034	22.67	0.17	0.034	20.00	0.14	0.021	15.00	0.15	0.03	20.00
C	1.35	0.078	5.78	1.39	0.055	3.96	1.38	0.091	6.59	1.37	0.07	5.11
D	1.99	0.218	10.95	2.32	0.270	11.64	2.21	0.234	10.59	2.17	0.27	12.44
E	3.10	0.296	9.55	3.64	0.200	5.49	3.42	0.352	10.29	3.38	0.36	10.65
F	4.39	0.269	6.13	4.64	0.272	5.86	4.41	0.288	6.53	4.48	0.28	6.25
c/o CAL	1.14	0.054	4.74	1.34	0.188	14.03	1.35	0.151	11.19	1.28	0.17	13.28
LPC	1.90	0.160	8.42	2.12	0.273	12.88	1.90	0.210	11.05	1.97	0.23	11.68
NC	0.15	0.029	19.33	0.13	0.036	27.99	0.14	0.024	17.14	0.14	0.03	21.43

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Diamedix Corporation • A Subsidiary of ERBA Diagnostics, Inc.
14100 NW 57th Court – Miami Lakes, Florida 33014 - USA
(305) 324-2300 / (800) 327-4565
www.erbadiagnostics.com

EC REP Delta Biologicals S.r.l., Via Nicaragua 12/14, 00071 - Pomezia, Rome Italy
Telephone #: +39-06-91190.1 Fax #: +39-069105244

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