Rubella is endemic worldwide (2). In countries without vaccination programs, 10–25% of women of childbearing age are seronegative and susceptible to infection (2). Extensive vaccination programs in the United States and the United Kingdom have greatly reduced the incidence of congenital rubella syndrome. Fewer than ten cases per year are now reported in the United States.

The presence of circulating maternal antibody indicates immunity to rubella and virtually excludes the possibility of transmission of rubella to the fetus (2, 5 and 6). If rubella is acquired during pregnancy, particularly during the first trimester, the fetus may be at risk of becoming infected (1). Acute rubella infection can be confirmed by simultaneously testing paired acute and convalescent sera and looking for seroconversion or a fourfold rise in titer, or by the presence of rubella specific IgM. The presence of rubella specific IgM in the neonate or the persistence of a high titer of IgG antibody for longer than expected for passively acquired antibody (6 months) confirms a diagnosis of congenital rubella (6).

Hemagglutination inhibition (HAI), the first widely used technique for detection of rubella antibody, has been the reference standard against which newer methods are measured (7). However, the HAI test is labor intensive and difficult to perform since serum samples must be pretreated to remove B-lipoprotein (6, 8). The ELISA (enzyme-linked immunosorbent assay) has been shown to be a sensitive and reliable procedure for detection of antibodies to rubella (8, 9 and 10). ELISA is less cumbersome than HAI and more applicable to screening large numbers of samples, since determinations are made on a single serum dilution which does not require pretreatment. Also, ELISA results are based on an objective absorbency reading which can be correlated with HAI titers (7, 8).

**TEST PRINCIPLE**

The Diagnostic Automation Inc. Rubella IgG ELISA test system is designed to detect IgG class antibodies to Rubella virus in human sera. Wells of plastic microwell strips are sensitized by passive absorption with Rubella antigen. The test procedure involves three incubation steps:

1. Test sera (properly diluted) are incubated in antigen coated microwells. Any antigen specific antibody in the sample will bind to the immobilized antigen.
2. Peroxidase conjugated goat anti-human IgG is added to the wells and the plate is incubated. The conjugate will react with IgG antibody immobilized on the plate.
3. The microwells containing immobilized peroxidase conjugate are incubated with a color developing solution. The color intensity of the solution is measured photometrically. The color intensity of the solution depends upon the antibody concentration in the original test sample.

**SPECIMEN COLLECTION AND PREPARATION**

1. It is recommended that specimen collection be carried out in accordance with NCCLS document M29: Protection of Laboratory Workers from Infectious Disease (Current Edition).
2. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.
3. Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures should be used in this assay (11, 14). Do not use if there are any added anticoagulants or preservatives. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
4. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2° and 8°C for no longer than 48 hours. If delay in testing is anticipated, store test sera at −20°C or lower. Avoid multiple freeze/thaw cycles that may cause loss of antibody activity and give erroneous results. It is the responsibility of the individual laboratory to use

### INTENDED USE

The Diagnostic Automation, Inc. Rubella ELISA Test System is designed for the qualitative and/or quantitative detection of IgG antibodies to rubella virus in human serum. The test system is intended to be used to evaluate single sera for immune status or paired sera to demonstrate seroconversion, and is for in vitro diagnostic use.

### SUMMARY AND EXPLANATION

Rubella is a mild, contagious viral infection that occurs primarily in children and young adults (1, 2). Rubella is characterized by an erythematous maculopapular rash that lasts two or three days. However, greater than 50% of rubella infections are not clinically apparent (2). Other symptoms of rubella may include low grade fever, mild upper respiratory symptoms, and suboccipital lymphadenopathy. Transient arthralgia and arthritis are common symptoms in young adults but more severe complications such as encephalitis or thrombocytopenic purpura are very uncommon (1). Although rubella infection in a child or adult is usually benign and self-limiting, infection of the fetus during the first trimester may cause spontaneous abortion, stillbirth or congenital birth defects (4). Infants infected in utero may be born with obvious birth defects or, more commonly, appear normal and either remain normal or develop later complications (1, 2).

Congenital rubella syndrome has long been recognized and is characterized by congenital heart disease, cataracts, neurosensory deafness, mental retardation, and intrauterine growth retardation (1, 4). Following an epidemic of rubella in 1964, other clinical manifestations of congenital rubella were recognized and include neonatal thrombocytopenic purpura, hepatitis, bone lesions and meningoencephalitis (3). Also, diabetes mellitus and progressive rubella panencephalitis are late-emerging manifestations of congenital rubella infection that have recently been recognized (1).

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1. Test sera (properly diluted) are incubated in antigen coated microwells. Any antigen specific antibody in the sample will bind to the immobilized antigen.
2. Peroxidase conjugated goat anti-human IgG is added to the wells and the plate is incubated. The conjugate will react with IgG antibody immobilized on the solid phase in step 1. The wells are washed to remove unreacted conjugate.
3. The microwells containing immobilized peroxidase conjugate are incubated with a color developing solution. The color intensity of the solution is measured photometrically. The color intensity of the solution depends upon the antibody concentration in the original test sample.

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all available references and/or its own studies to determine stability criteria for its laboratory (15).

MATERIALS AND COMPONENTS

Materials provided with the test kits

Each kit contains the following components in sufficient quantities to perform the number of tests indicated on packaging label. Note: All reactive reagents contain sodium azide as a preservative at a concentration of < 0.1% (w/v): Controls, Calibrators and Sample Diluent.

1. Plate: 96 wells configured in twelve 1x8-well strips coated with inactivated Rubella virus antigen. The strips are packaged in a strip holder and sealed in an envelope with desiccant.
2. Conjugate: Conjugated (horseradish peroxidase) goat anti-human IgG (Fc chain specific). One, 15 mL vial with a white cap. Ready to use.
3. Positive Control (Human Serum): One, 0.35 mL vial with a red cap.
4. Calibrator (Human Serum): One, 0.5 mL vial with a blue cap.
5. Negative Control (Human Serum): One, 0.35 mL vial with a green cap.
6. Sample Diluent: One, 30 mL bottle (green cap) containing Tween-20, bovine serum albumin and phosphate-buffered-saline. Ready to use.

NOTE: The Sample Diluent will change in color in the presence of serum.

7. TMB: One, 15 mL amber bottle (amber cap) containing 3,3',5,5'-tetramethylbenzidine (TMB). Ready to use.
8. Stop solution: One, 15 mL bottle (red cap) containing 1M H₂SO₄, 0.7M HCl. Ready to use.
9. Wash buffer concentrate (10X): dilute 1 part concentrate + 9 parts deionized or distilled water. One 100 mL bottle (clear cap) containing a 10X concentrated phosphate-buffered-saline and Tween-20 solution (blue solution). NOTE: 1X solution will have a pH of 7.2 ± 0.2.

NOTES:
1. The following components are not kit lot number dependent and may be used interchangeably with the Diagnostic Automation, Inc ELISA System: TMB, Stop Solution, and Wash Buffer.
2. The Kit also contains an insert for instruction and a COA report in each package.

Materials required but not provided

1. ELISA microwell reader capable of reading at a wavelength of 450nm.
2. Pipettes capable of accurately delivering 10 to 200μL.
3. Multichannel pipette capable of accurately delivering (50-200μL).
4. Reagent reservoirs for multichannel pipettes.
5. Wash bottle or microwell washing system.
6. Distilled or deionized water.
7. One liter graduated cylinder.
8. Serological pipettes.
9. Disposable pipette tips.
11. Laboratory timer to monitor incubation steps.
12. Disposal basin and disinfectant. (example: 10% household bleach, 0.5% sodium hypochlorite.)

PRECAUTIONS

1. For In Vitro Diagnostic Use.
2. Normal precautions exercised in handling laboratory reagents should be followed. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
3. The wells of the ELISA plate do not contain viable organisms. However, the strips should be considered POTENTIALLY BIOHAZARDOUS MATERIALS and handled accordingly.
4. The human serum controls are POTENTIALLY BIOHAZARDOUS MATERIALS. Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, these products should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual “Biosafety in Microbiological and Biomedical Laboratories”: current edition; and OSHA’s Standard for Bloodborne Pathogens (4).
5. Adherence to the specified time and temperature of incubations is essential for accurate results. All reagents must be allowed to reach room temperature (20-25°C) before starting the assay. Return unused reagents to refrigerated temperature immediately after use.
6. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual wash solution; (e.g., by blotting or aspiration) before adding Conjugate or Substrate. Do not allow the wells to dry out between incubations.
7. The Sample Diluent, controls, wash buffer, and conjugate contain sodium azide at a concentration of 0.1% (w/v). Sodium azide has been reported to form lead azides in laboratory plumbing which may cause explosions on hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing sodium azide.
8. The Stop Solution is TOXIC. Causes burns. Toxic by inhalation, in contact with skin and if swallowed. In case of accident or if you feel unwell, seek medical advice immediately.
9. The TMB Solution is HARMFUL. Irritating to eyes, respiratory system and skin.
10. The Wash Buffer concentrate is an IRRITANT. Irritating to eyes, respiratory system and skin.
11. Wipe bottom of plate free of residual liquid and/or fingerprints that can alter optical density (OD) readings.
12. Dilution or adulteration of these reagents may generate erroneous results.
13. Reagents from other sources or manufacturers should not be used.
14. TMB Solution should be colorless, very pale yellow, very pale green, or very pale blue when used. Contamination of the TMB with conjugate or other oxidants will cause the solution to change color prematurely. Do not use the TMB if it is noticeably blue in color.
15. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
16. Avoid microbial contamination of reagents. Incorrect results may occur.
17. Cross contamination of reagents and/or samples could cause erroneous results.
18. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
19. Avoid splashing or generation of aerosols.
20. Do not expose reagents to strong light during storage or incubation.
21. Allow the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
22. Collect the wash solution in a disposal basin. Treat the waste solution with disinfectant (i.e.: 10% household bleach - 0.5% Sodium Hypochlorite). Avoid exposure of reagents to bleach fumes.
23. Caution: Liquid waste at acid pH should be neutralized before adding to bleaching solution.
24. Do not use ELISA plate if the indicator strip on the desiccant pouch has turned from pin to pink.
25. Do not allow the conjugate to come in contact with containers or instruments that may have previously contained a solution utilizing sodium azide as a preservative. Residual amounts of sodium azide may destroy the conjugate’s enzymatic activity.
26. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors from bleach-containing solutions. Trace amounts of bleach (sodium hypochlorite) may destroy the biological activity of many of the reactive reagents within this kit.

ASSAY PROCEDURE

Diagnostic Automation/ Cortez Diagnostics, Inc.
21250 Califa St, Suite 102 and 116, Woodland Hills, CA 91367 USA Phone: 818-591-3050, Fax: 818-591-6383
Email: one@rapicdect.com Website: www.rapidtest.com
1. Remove the individual components from storage and allow them to warm to room temperature (20-25°C).
2. Determine the number of microwells needed. Allow for six Control/Calibrator determinations (one Blank, one Negative Control, three Calibrators and one Positive Control) per run. A Reagent Blank should be run on each assay. Check software and reader requirements for the correct Controls/Calibrator configurations. Return unused strips to the resealable pouch with desiccant, seal, and return to storage between 2° and 8°C.

<table>
<thead>
<tr>
<th>EXAMPLE PLATE SET-UP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
</tr>
<tr>
<td>A</td>
</tr>
<tr>
<td>B</td>
</tr>
<tr>
<td>C</td>
</tr>
<tr>
<td>D</td>
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<td>E</td>
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<td>F</td>
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<tr>
<td>G</td>
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<tr>
<td>H</td>
</tr>
</tbody>
</table>

3. Prepare a 1:21 dilution (e.g.: 10µL of serum + 200µL of Sample Diluent. NOTE: Shake Well Before Use) of the Negative Control, Calibrator, Positive Control, and each patient serum. NOTE: The Sample Diluent will undergo a color change confirming that the specimen has been combined with the diluent.

4. To individual wells, add 100µL of each diluted Control, Calibrator and patient specimen. Ensure that the samples are properly mixed. Use a different pipette tip for each sample.
5. Add 100µL of Sample Diluent to well A1 as a reagent blank. Check software and reader requirements for the correct reagent blank well configuration.
6. Incubate the plate at room temperature (20-25°C) for 25±5 minutes.
7. Wash the microwell strips 5X.

A. **Manual Wash Procedure:**
   - a. Vigorously shake out the liquid from the wells.
   - b. Fill each microwell with Wash Buffer. Make sure no air bubbles are trapped in the wells.
   - c. Repeat steps a. and b. for a total of 5 washes.
   - d. Shake out the wash solution from all the wells. Invert the plate over a paper towel and tap firmly to remove any residual wash solution from the wells. Visually inspect the plate to ensure that no residual wash solution remains. Collect wash solution in a disposable basin and treat with disinfectant at the end of the day’s run.

B. **Automated Wash Procedure:**
   If using an automated microwell wash system, set the dispensing volume to 300-350µL/well. Set the wash cycle for 5 washes with no delay between washes. If necessary, the microwell plate may be removed from the washer, inverted over a paper towel and tapped firmly to remove any residual wash solution from the microwells.
8. Add 100µL of the Conjugate to each well, including reagent blank well, at the same rate and in the same order as the specimens.
9. Incubate the plate at room temperature (20-25°C) for 25±5 minutes.
10. Wash the microwells by following the procedure as described in step 7.
11. Add 100µL of TMB to each well, including reagent blank well, at the same rate and in the same order as the specimens.
12. Incubate the plate at room temperature (20-25°C) for 10 to 15 minutes.
13. Stop the reaction by adding 50µL of Stop Solution to each well, including Reagent Blank well, at the same rate and in the same order as the TMB. Positive samples will turn from blue to yellow. After adding the Stop Solution, tap the plate several times to ensure that the samples are thoroughly mixed.
14. Set the microwell reader to read at a wavelength of 450nm and measure the optical density (OD) of each well against the Reagent Blank. Read the plate within 30 minutes of the addition of the Stop Solution.

### RESULTS

**A. Calculations:**
1. **Correction Factor**
   A cutoff OD value for positive samples has been determined by the manufacturer and correlated to the Calibrator. The correction factor (CF) will allow you to determine the cutoff value for positive samples and to correct for slight day-to-day variations in test results. The correction factor is determined for each lot of kit components and is printed on the Component label located in the kit box.

2. **Cutoff OD Value**
   To obtain the cutoff OD value, multiply the CF by the mean OD of the Calibrator determined above.
   
   \[(CF \times \text{Mean OD of Calibrator} = \text{Cutoff OD value})\]

3. **Index Values or OD Ratios**
   Calculate the Index Value or OD Ratio for each specimen by dividing its OD value by the Cutoff OD from step 2.

   **Example:**
   
   - Mean OD of Calibrator = 0.793
   - Correction Factor (CF) = 0.25
   - Cut off OD = 0.793 x 0.25 = 0.198
   - Unknown Specimen OD = 0.432
   - Specimen Index Value or OD Ratio = 0.432 / 0.198 = 2.18

**B. Interpretations:**

Index Values or OD ratios are interpreted as follows:

<table>
<thead>
<tr>
<th>Index Value or OD Ratio</th>
<th>Negative Specimens</th>
<th>Equivocal Specimens</th>
<th>Positive Specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤0.90</td>
<td>0.91 - 1.09</td>
<td>≥1.10</td>
</tr>
</tbody>
</table>

1. An OD ratio ≤0.90 indicates no detectable antibody to rubella virus. A negative result indicates no current or previous infection with rubella virus. Such individuals are presumed to be susceptible to primary infection. However, specimens taken too early during a primary infection may not have detectable levels of IgG antibody. If a primary infection is suspected, another specimen should be taken in 8-14 days and tested concurrently in the same assay with the original specimen to look for seroconversion.

2. An OD ratio ≥1.10 is positive for IgG antibody to rubella virus. A positive value indicates a current or previous infection with rubella virus. Such individuals are...
presumed to be at risk of transmitting rubella virus infection, but are not necessarily currently contagious.
3. Specimens with OD ratio values in the equivocal range (0.91-1.09) should be retested in duplicate.
4. To evaluate paired (acute and convalescent) sera, both samples must be tested in the same assay. If the acute specimen is negative and the convalescent specimen is positive, seroconversion has taken place and a primary rubella virus infection is indicated.

C. Conversion of OD Ratio to IU/mL
As an option, OD ratios may be converted to IU/mL by multiplying the OD ratio by 9.091 IU/mL values may then be interpreted as follows:

<table>
<thead>
<tr>
<th>OD Ratio</th>
<th>IU/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Specimens</td>
<td>≤ 0.18</td>
</tr>
<tr>
<td>Positive Specimens</td>
<td>≥ 10.0</td>
</tr>
<tr>
<td>Equivocal Specimens</td>
<td>8.19 to 9.99</td>
</tr>
</tbody>
</table>

Interpretation criteria for positive, negative, and equivocal specimens are as stated above. NOTE: The assay is linear and correlates well with the WHO Standard between 0 and 20 IU/mL. Specimens producing a result >20 IU/mL should be reported as “positive”, or “>20 IU/mL”. If greater accuracy is required, the specimen must be diluted and re-tested. The final result may be found by multiplying the resulting IU/mL value by the dilution factor.

Example: Initial Result: Ratio = 2.68 = 24.34 IU/mL
Dilute 1:2 in Sample Diluent; then, 1:2 as the procedure indicates.
Re-test Result: Ratio = 1.88 = 17.09 IU/mL x 2 = 34.18 IU/mL

QUALITY CONTROL
1. Each time the assay is performed the Calibrator must be run in triplicate. A Reagent Blank, Negative Control, and Positive Control must also be included in each assay.
2. Calculate the mean of the three Calibrator wells. If any of the three values differ by more than 15% from the mean, discard that value and calculate the mean using the remaining two wells.
3. The mean OD value for the Calibrator, Positive Control, and Negative Control should fall within the following ranges:

<table>
<thead>
<tr>
<th>OD Range</th>
<th>Negative Control</th>
<th>Calibrator</th>
<th>Positive Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 0.250</td>
<td>≥ 0.300</td>
<td>≥ 0.500</td>
<td></td>
</tr>
</tbody>
</table>

a. The OD of the Negative Control divided by the mean OD of the Calibrator should be ≤ 0.9.
b. The OD of the Positive Control divided by the mean OD of the Calibrator should be ≥ 1.25.
c. If the above conditions are not met the test should be considered invalid and should be repeated.
4. The Positive Control and Negative Control are intended to monitor for substantial reagent failure and will not ensure precision at the assay cut-off.
5. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

LIMITATIONS OF PROCEDURE
1. The antibody titer of a single serum specimen cannot be used to determine recent infection. Paired samples (acute and convalescent) should be collected and tested concurrently to demonstrate seroconversion
2. Test results should be interpreted in conjunction with the clinical evaluation and the results of other diagnostic procedures.
3. Samples collected too early in the course of an infection may not have detectable levels of IgG. In such cases, a second sample may be collected after 2-7 weeks and tested concurrently with the original specimen to look for seroconversion or, an IgM specific assay should be performed.

4. A positive rubella IgG test in neonates should be interpreted with caution since passively acquired maternal antibody can persist for up to 6 months. However, a negative test for IgG antibody in the neonate may help exclude congenital infection (12).

EXPECTED VALUES

Seroepidemiologic studies indicate that in most countries 80 - 90% of the adult population have detectable antibodies to rubella (2).

STORAGE

1. Store the unopened kit between 2° and 8°C.
2. Coated microwell strips: Store between 2° and 8°C. Extra strips should be immediately resealed with desiccant and returned to proper storage. Strips are stable for 60 days, as long as the indicator strips on the desiccant pouch remains blue.
3. Conjugate: Store between 2° and 8°C. DO NOT FREEZE.
4. Calibrator, Positive Control and Negative Control: Store between 2° and 8°C.
5. TMB: Store between 2° and 8°C.
6. Wash Buffer concentrate (10X): Store between 2° and 8°C.
7. Sample Diluent: Store between 2° and 8°C.
8. Stop Solution: Store between 2° and 25°C.
9. TMB: Store between 2° and 8°C. Extra strips should be immediately resealed with desiccant and returned to proper storage. Strips are stable for 60 days, as long as the indicator strips on the desiccant pouch remains blue.
10. Sample Diluent: Store between 2° and 8°C.
11. Stop Solution: Store between 2° and 25°C.
12. Sample Diluent: Store between 2° and 8°C.

REFERENCES