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Immunoassay

REF **CMK0801/CMK0802/CMK0803/CMK0805**

50 tests*1/100 tests*1/100 tests*2/50 tests*2

Rubella IgG CLIA Microparticles

This assay is based on a chemiluminescent microparticle immunoassay (CLIA Microparticles) for the quantitative determination of Rubella IgG (IgG antibodies to rubella virus) in human serum or plasma.

Key to Graphical Symbols Used

LOT	batch code		use by
	manufacturer	Σ	contains sufficient for <n> tests
IVD	*in vitro* diagnostic medical device		temperature limitation
REF	catalogue number		consult instructions for use
EC REP	authorized representative in the European Community		

EC REP

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Introduction

Rubella is a disease caused by the rubella virus, and has a single stranded RNA genome.[1] It is caused by a virus that is spread through the air or by close contact. The illness follows a typically benign clinical course with rare complications and is subclinical in a large proportion of cases. Symptomatology is generally mild, characterized by fever, malaise, a maculopapular rash of three to five days duration and, possibly, coryza and conjunctivitis. The disease is usually accompanied by lymphadenopathy. Infection confers lifelong immunity. Infection from rubella virus is particularly disastrous if contracted during the first four months of gestation.[2] If not immunologically protected, women infected during pregnancy run a high risk of embryo fetal damage. Congenital rubella causes a wide range of severe defects, many of which are permanent and adversely affect later development (cataract, deafness, hepatosplenomegaly, psychomotor retardation, bone alterations, cardiopathies, neuropathies).[3,4] Rubella infection of children and adults is usually mild, self-limiting and often asymptomatic. The prognosis in children born with congenital rubella syndrome is poor. This test is helpful to determine if you have sufficient rubella antibodies to protect you from the rubella virus; to verify a past infection or detect a recent infection. The ELISA method for rubella antibody is most common and is the test done to see if a woman who is pregnant or planning to get pregnant is immune to rubella.[5]

Measurement Principle

This assay is based upon the two-step indirect method. The sample and natural rubella coated microparticles are combined. The rubella IgG present in the sample bind to the antigen coated on the microparticles. After washing, Enzyme Conjugate is added to the reaction mixture. During the incubation, the mouse anti-human IgG in the Enzyme Conjugate are allowed to react with the rubella IgG attached to the solid phase. Then a complex is generated between the solid phase, the rubella IgG in the sample and the mouse anti-human IgG in the Enzyme Conjugate by immunological reactions. The complex catalyzes substrate, resulting in a chemiluminescent reaction. The resulting chemiluminescent reaction is measured as RLUs. The RLU is proportional to the amount of rubella IgG in the sample.

Materials Provided

1. Calibrators

6 vials each containing 1.0 mL of calibrator A through F. The matrix is Tris-NaCl buffer containing BSA (bovine serum albumin). Contains sodium azide preservative.

Calibrators provided ready to use.

2. Reagent pack

Reagent pack provided ready to use.

Σ	50*1	100*1	100*2	50*2
Microparticles Solution	1.2 mL*1	2.3 mL*1	2.3 mL*2	1.2 mL*2
Enzyme Conjugate	5.5 mL*1	11.0 mL*1	11.0 mL*2	5.5 mL*2
Sample Diluent	5.5 mL*1	11.0 mL*1	11.0 mL*2	5.5 mL*2

- Enzyme Conjugate

Horseradish peroxidase labeled mouse anti-human IgG monoclonal antibodies in a Tris-HCl buffer containing 20% bovine serum. Contains ProClin 300® preservative.

- Microparticles Solution

Recombinant rubella antigen coated microparticles in PBS (phosphate buffered saline) buffer containing 1% casein. Contains ProClin 300® and sodium azide preservatives.

- Sample Diluent

Tris-HCl buffer containing 0.5% casein. Contains sodium azide preservative.

Assay Analyzers on which the kit can be used

- AutoLumo A2000 Plus
- AutoLumo A2000 Plus B
- AutoLumo A1000

The chemiluminescent microparticle immunoassay (CLIA Microparticles) is intended for use on Assay Analyzer which is AutoLumo A2000 Plus, AutoLumo A2000 Plus B or AutoLumo A1000.

Materials Required but not Provided

1. Assay Analyzer
2. Reaction vessel(s) for sample and reagent reaction
3. Sample cups or tube(s) for sample containing
4. Diluent Universal
5. Chemiluminescent Substrate
6. System Wash for washing the pipetting needles
7. Wash Buffer used in the washing procedure
8. Distilled water or deionized water

Metrological Traceability of Calibrators

The product calibrators are signal matched to our working calibrators, which are also signal matched to an international conventional calibrator purchased from WHO (The World Health Organization) RUBI-1-94, at each concentration level.

Warnings and Precautions

1. For professional use only.
2. Follow the instruction for use carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this instruction for use.
3. Refer to the material safety data sheet and product labeling for any chemical hazards that may be present in this assay.
4. Handle the potentially contaminated materials and wastes safely according to local requirement.
5. CAUTION: the calibrators contain material of human origin, which has been tested and found non-reactive for HBsAg, HIV-1 and HIV-2, HCV and syphilis. This assay contains materials of animal origin. Bovine components originate from countries where BSE has not been reported.
6. Some reagents contain ProClin 300® may cause sensitization by skin contact, which must be avoided to contact with skin. This material and its container must be disposed in a safe way. If swallowed, seek medical advice immediately and show this container or label.
7. Do not smoke, drink, eat or use cosmetics in the working area.
8. Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.
9. Use caution when handling patient samples to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
10. Conduct the assay away from bad ambient conditions. e. g. ambient air containing high concentration corrosive gas such as sodium hypochlorite acid, alkaline, acetaldehyde and so on, or containing dust.
11. Do not use reagents beyond the labeled expiry date.
12. Do not mix or use components from kits with different batch codes.
13. Ensure the microparticles are re-suspended before loading it on the analyzer.

14. Avoid foam formation in all reagents and sample types (samples, calibrators and controls).
15. Do not substitute any reagent in this kit from other manufacturers or other lots.
16. When any damage to the protective packaging or any change of analytical performance is observed, do not use the kit.

Storage

1. Store the kit at 2-8℃. Do not freeze. Avoid strong light. When stored as directed, all reagents are stable until the expiration date.
2. Refrigerate the reagent pack at 2-10 ℃ for a minimum of 2 hours prior to use.
3. Store the unsealed reagents pack upright on the analyzer or 2-10℃ for a maximum of 28 days. After 28 days, the reagent pack must be discarded. Once they are removed from the analyzer, store them at 2-10 ℃ in an upright position.
4. Seal and return the remaining calibrators at 2-8 ℃ immediately after the experiment, under which conditions the stability will be retained for 2 months.

Sample

1. Collect samples in accordance with correct medical practices.
2. Plasma samples collected in tubes containing EDTA, heparin or sodium citrate have been tested and may be used with this assay.
3. Do not use heat-inactivated samples and samples with obvious microbial contamination. Do not use sodium azide preservative in samples.
4. Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit in creased clotting time. If the sample is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results. Be sure that the samples are not decayed prior to use.
5. Prior to shipment, it is recommended that samples be removed from the clot, serum separator or red blood cells.
6. Insufficient processing of the sample or disruption of the sample during transportation may cause depressed results.
7. Avoid grossly hemolytic, lipemic or turbid samples.
8. Cap and store the samples at 18-25 ℃ for no more than 8 hours, for longer use samples should be capped and stored at 2-8℃ up to 48 hours. Or freeze the samples that need to be stored or transported for more than 48 hours at -20℃. Avoid multiple freeze-thaw cycles. Mix thawed samples thoroughly by low speed vortex or by inverting 10 times. Visually inspect the samples, if layering or stratification is observed, continue mixing until samples are visibly homogeneous. After thawing, bring to room temperature and mix well by gently shaking.
9. Centrifuge the thawed samples containing red blood cells or particulate matter, or which are hazy or cloudy in appearance prior to use to ensure consistency in the results.
10. Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
11. If proper sample collection and preparation cannot be verified, or if samples have been disrupted due to transportation or sample handling, an additional centrifugation step is recommended. Centrifugation conditions should be sufficient to remove particulate matter.
12. For optimal results, inspect all samples for bubbles. Remove bubbles with a tip prior to analysis. Use a new tip for each sample to prevent cross contamination.

Measurement Procedure

1. Check the consumable materials
- Verify adequate volume of consumable materials is present prior to running the test.
- Refer to the Assay Analyzer's operation manual.

2. Load the kit
- Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the analyzer. Avoid foam formation in all reagents. Don't invert the open (punctured) packs. If necessary, shake gently to mix horizontally after the first loading.
- Read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Refer to the Assay Analyzer's operation manual.

3. Order tests
- Place the sample cups or tubes on the sample rack, and then add 30 μL of serum or plasma sample which will be automatically diluted by Diluent Universal and mixed well. But consider the sample container and 150 μL of system dead volumes, which can be refer to the appropriate Assay Analyzer manuals for the minimum sample volume required.
- Load the sample rack and input the sample information on the system software interface.
- Select "run" to start the test, the analyzer automatically operates tests. It performs the following functions:
  - Moves the sample to the set point
  - Loads a reaction vessel into the process path
  - Aspirates and transfers Diluent Universal and sample into the reaction vessel and mixed well
  - Aspirates and transfers the diluted sample to the reaction vessel
  - Adds Microparticles Solution and Sample Diluent to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Enzyme Conjugate to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Chemiluminescent Substrate
  - Measures chemiluminescent emission to determine the quantity of Rubella IgG in the sample
  - Discards the used reaction vessel
  - Calculates the result
- Refer to the Assay Analyzer's operation manual.

4. Calibrate the curve
- Analyzer can read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Transfer the calibrators into the sample cups or tubes and place them on the sample rack. Conduct duplicate detection on the system.
- Load the sample rack and input calibrators' information on the system software interface.
- Select "run" to start the test and generate the calibration curve, calibration is required every 28 days.
- Once a calibration curve is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - Controls are out of range after repeated measurements
  - A reagent kit and substrate solution with new batch code is used
  - Beyond the expiration date of a calibration curve
  - Important parts of the analyzer are replaced or repaired.
- Refer to the Assay Analyzer's operation manual.

5. Dilute the sample

Samples with a Rubella IgG value exceeding 400 IU/mL may be diluted manually. Diluent Universal is used to dilute the samples. The concentration of the sample after dilution should not be less than 10 IU/mL. After dilution, multiply the result by the dilution factor. Antibodies to Rubella are heterogenous. A non-linear dilution behavior is frequently observed.

Measurement Results

The sample test results are determined automatically by the system software. The amount of Rubella IgG in the samples is determined from the measured light production by means of the stored calibration data. Refer to the Assay Analyzer's operation manual on reviewing the stored data.

Interpretation of Results

Results obtained with the Rubella IgG CLIA Microparticles can be interpreted as follows:

Non-reactive: < 5 IU/mL

Equivocal: 5-10 IU/mL

Reactive: ≥10 IU/mL

A non-reactive result indicates that the specific immunity has not been acquired but cannot rule out the early stage of acute infection. Patients with non-reactive results are still suspected the exposure to *rubella virus* should be retested within 2 weeks.

A reactive result indicates either early acute infection or past exposure to the pathogen. If it is suspected to be early acute infection, a rubella IgM test or other serological method for detection of additional *rubella virus* markers, such as a rubella IgG Avidity test, could be performed.

For equivocal result, a second sample should be taken and repeated rubella IgG testing no less than one or two weeks later. Serological data from detection of additional rubella virus markers may provide useful information for clinical interpretation of results.

Control Procedure

The recommended control requirement for this assay is to purchase control materials separately and test them together with the samples within the same run. The result is valid if the control values fall within the concentration ranges printed on the labels. When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results may be invalid and may require retesting. Assay recalibration may be necessary. It is recommended that each laboratory establish its accepted range to ensure proper test performance.

Limitations of the Procedure

1. This assay is intended as an aid for the clinical diagnosis. Conduct this assay in conjunction with clinical examination, patient's medical history and other test results.
2. If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
3. Rheumatoid factors in samples may interfere with test results.
4. If the patient is immune-compromised or is receiving immunosuppressive therapy (for example, transplant recipients, AIDS patients), the reference value of their IgG antibodies serological detection is limited, and wrong medical explanation may be obtained.
5. For pregnant women, it is recommended that give followed detection for the IgG antibodies during pregnancy.
6. A negative result, however, does not always rule out the possibility of rubella infection. Because different people have different times from rubella infection to produce antibodies, maybe the infections in its very early stage and the patient may be still unable to synthesize enough rubella specific IgG. If clinical exposure to rubella is suspected despite a negative finding, a second sample should be collected and tested on less than one week later.
7. Samples from neonates, cord blood, pre-transplant patients or body fluids other than serum and plasma, such as urine, saliva or amniotic fluid have not been tested.
8. For the samples who have received blood transfusions or other blood products in recent months, the positive result should be given careful analysis.
9. This test measures concentrations within the range of 0.5- 400 IU/mL. If rubella IgG concentrations above the measuring range to be expected, it is recommended to dilute samples with Diluent Universal, the maximum dilution is 1:9 of this test, allowing samples to be quantitated up to approximately 4000 IU/mL.

Performance Characteristics

1. Measurement Precision

3 clinical samples (1, 2 and 3) and 3 quality controls (4, 5 and 6) were assayed, using 3 lots of reagent, in replicates of two at two separate times per day for 20 testing days. Data from this study are summarized in the following table.

Lot	Panel Member	n	Mean (IU/mL)	Within-run	Total
				%CV	%CV
1	1	80	23.50	2.55	9.38
	2	80	62.47	2.45	7.35
	3	80	165.28	2.22	6.04
2	1	80	23.01	3.13	8.27
	2	80	58.22	3.03	6.39
	3	80	150.39	2.89	6.12
3	1	80	24.53	2.82	8.73
	2	80	63.84	3.09	5.86
	3	80	175.72	2.84	6.84
1	4	80	11.11	2.32	5.79
	5	80	37.84	2.16	4.73
	6	80	66.15	2.14	5.94
2	4	80	11.04	1.92	4.93
	5	80	35.54	2.41	4.48
	6	80	60.85	2.29	4.48
3	4	80	11.82	3.14	4.96
	5	80	40.37	2.31	5.06
	6	80	70.71	2.49	5.53

*Representative data; results in individual laboratories may vary from these data.

2. Analytical Sensitivity

Analytical sensitivity represents lowest measurable analyte level that can be distinguished from zero, is 0.5 IU/mL.

The study was conducted on 3 reagent batches using 5 human serum-based panels which were prepared at target concentrations. The panel were assayed in replicates of 3 over 4 days for a total of 60 replicates per batch.

3. Analytical Specificity

Cross reaction: This assay is tested to have no cross reactivity with the HSV-2 IgG, Rubella IgG, CMV IgG, EBV IgG, VZV IgG and parvovirus B19 IgG antibodies.

Interference: Controlled studies of potentially interfering substances or conditions showed that the assay performance was not affected by

anticoagulants (sodium citrate, EDTA, heparin), bilirubin (up to 20 mg/dL), hemoglobin (up to 1000 mg/dL), triglyceride (up to 3000 mg/dL).

4. Clinical Study

Relative Sensitivity and Relative Specificity: A study was performed where samples were tested using this assay and a reference Rubella IgG assay which as already available on the market. Equivocal or inconsistent results after comparison shall be tested by another reference assay, the result can be determined by at least 2 assays with the same results. Exclude the unconfirmed or equivocal samples in the calculation of relative sensitivity and relative specificity. Data for relative sensitivity and relative specificity are summarized in the following table.

Rubella IgG CLIA Microparticles					
Site	Number of Sample	Relative Sensitivity	Lower 95% CI limit	Relative Specificity	Lower 95% CI limit
Site 1	643	100%	99.90%	100%	99.75%
Site 2	767	100%	99.91%	100%	99.75%
Site 3	147	100%	99.01%	100%	99.82%
Total	1557	100%	99.93%	100%	99.87%

* CI denotes Confidence Interval

Literature References

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