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Immunoassay

REF **CMK0301/CMK0302/CMK0303/CMK0305**

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

Rubella IgM CLIA Microparticles

This assay is based on a chemiluminescent microparticle immunoassay (CLIA Microparticles) for the quantitative determination of Rubella IgM (IgM antibodies to Rubella) 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-foetal 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. The detection of Rubella-specific IgM antibodies is used as an aid in the diagnosis of acute Rubella 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 capture method. In the first step, sample and mouse monoclonal anti-human IgM coated microparticles are incubated. During the incubation, the antibodies present in the sample bind to the anti-human IgM coated on the microparticles. After the washing, in the second step, Enzyme Conjugate is added to the reaction mixture. During the incubation, the HRP-conjugated Rubella antigen in the Enzyme Conjugate is allowed to react with the Rubella IgM already bound to the solid phase in the first step. After a second washing, a complex is generated among the solid phase, antibodies in the sample and enzyme-linked antigens 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 IgM in the sample.

Materials Provided

1. Calibrators

6 vials lyophilized calibrator A through F, the matrix is PBS (phosphate buffered saline) containing BSA (bovine serum albumin). Contains ProClin 300® preservative.

Reconstitute each lyophilized calibrator with 1.0 mL of distilled water. Invert the calibrator to mix it completely and then allow the reconstituted material to stand for at least 5 minutes.

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 Rubella antigens in a Tris-HCl buffer containing bovine serum and casein. Contains ProClin 300® preservative.

- Microparticles Solution

Mouse monoclonal anti-human IgM coated microparticles in PBS buffer containing casein. Contains ProClin 300® and sodium azide preservatives.

- Sample Diluent

Tris-HCl buffer containing BSA. Contains ProClin 300® and 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 cup(s) 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 measurand or analyte in the Rubella IgM calibrators is traceable to the manufacturer's working calibrators. Traceability process is based on EN ISO 17511. The assigned values were established and are specific to the assay methodologies of the reagents. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

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. 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. 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.
10. Do not use reagents beyond the labeled expiry date.
11. Do not mix or use components from kits with different batch codes.
12. When storing the calibrators, be certain the vials are securely sealed.

13. Ensure the microparticles are resuspended before loading it on the analyzer.
14. Do not substitute any reagent in this kit from other manufacturers or other lots.
15. 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 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 reconstituted calibrators at 2-8 ℃ immediately after the experiment, under which conditions the stability will be retained for 1 week, for longer use, store reconstituted calibrators in aliquots and freeze at -20℃, which can be stored up to 2 months. Avoid multiple freeze-thaw cycles, do not freeze-thaw more than 3 cycles.

Sample

1. Plasma samples collected in tubes containing EDTA, heparin or sodium citrate have been tested and may be used with this assay.
2. Collect serum samples in accordance with correct medical practices. After the blood collection, please follow the tube manufacturer's processing instructions for serum or plasma collection tubes.
3. Do not use samples with the following conditions:
   - heat-inactivated
   - pooled
   - grossly hemolyzed
   - obvious microbial contamination
   - cadaver samples or any other body fluids
   - sodium azide preservative
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 increased 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. Use caution when handling patient samples to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
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 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 cup(s) or tube(s) on the sample rack, and then add 10 μL of serum or plasma samples. 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 IgM in the sample
  - Discards the used reaction vessel
  - Calculates the result
- Refer to the Assay Analyzer's operation manual.

4. Calibration

- 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 Chemiluminescent Substrate 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 IgM value exceeding 160 AU/mL may be diluted manually. Human serum negative for Rubella IgM is used to dilute the samples. The concentration after dilution should be > 8 AU/mL. After dilution, multiply the result by the dilution factor. But please note: antibodies to Rubella are heterogenous. A non-linear dilution behavior is frequently observed.

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 acceptable range. 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.

Measurement Results

The sample test results are determined automatically by the system software. The amount of Rubella IgM 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 IgM CLIA Microparticles can be interpreted as follows:

Nonreactive: < 5 AU/mL

Equivocal: 5-8 AU/mL.

Reactive: ≥ 8 AU/mL

A nonreactive result cannot always rule out acute Rubella infection, because the infection maybe be in its very early stage and the patient is still unable to synthesize Rubella virus specific IgM.

An equivocal result may be indicative either of recent infection or of past infection with long-lasting Rubella virus IgM. A second sample should be collected within a reasonable period of time (e.g., within one week). Serological data from detection of additional Rubella virus markers may provide useful information for clinical interpretation of results.

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. For the samples who have received blood transfusions or other blood products in recent months, the positive result should be given careful analysis.
4. If the patient is immune-compromised or is receiving immune-suppressive therapy (for example, transplant recipients, AIDS patients), the reference value of their IgM antibodies serological detection is limited, and wrong medical explanation may be obtained.
5. Rubella IgM may be present for more than half a year in some patients' body; consequently, a positive result might not definitely indicate a recent infection. Moreover some reinfection patients will produce IgM antibody, so the additional IgG antibody avidity assays can be used to determine whether the primary infection or reinfection.
6. Samples from neonates, cord blood, pretransplant patients or body fluids other than serum and plasma, such as urine, saliva or amniotic fluid have not been tested.
7. This test measures concentrations within the range of 1-160 AU/mL. If Rubella IgM concentrations above the measuring range to be expected, it is recommended to dilute samples with human serum negative for Rubella IgM, the maximum dilution is 1:9 of this test, allowing samples to be quantitated up to approximately 1600 AU/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	Within-run	Total
				%CV	%CV
1	1	80	36.56	3.77	8.23
	2	80	26.41	2.49	5.32
	3	80	18.14	3.64	7.76
2	1	80	36.16	4.19	7.02
	2	80	25.72	2.27	6.62
	3	80	17.10	2.67	7.00
3	1	80	37.58	3.52	7.39
	2	80	26.27	2.62	4.81
	3	80	17.30	3.01	7.00
1	4	80	19.40	3.21	6.86
	5	80	41.07	2.49	7.06
	6	80	87.56	1.84	5.07
2	4	80	19.64	2.50	6.34
	5	80	41.70	2.56	5.68
	6	80	89.54	2.05	7.31
3	4	80	19.96	5.93	6.76
	5	80	43.58	1.93	4.34
	6	80	92.27	2.40	4.27

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

2. Dilution Test

It has been demonstrated that IgM-capture assays may present sample-dependent dilution tests and may be affected by different matrixes used to dilute the neat samples[6]. The table below shows three well-responsive serum samples containing high Rubella virus IgM concentrations tested as such and after serially diluted with human serum negative for Rubella IgM. Rubella virus IgM concentrations measured versus concentrations expected were analyzed by linear regression. The correlation coefficients (r) ranged from 0.995 to 0.999.

Dilution	Expected Concentration, AU/mL	Measured Concentration, AU/mL	% Recovery
1:32	3.80	4.18	109.87
1:16	7.60	8.05	105.86
1:8	15.20	16.93	111.35
1:4	30.40	35.53	116.88
1:2	60.80	61.13	100.54
neat	/	121.60	/

Dilution	Expected Concentration, AU/mL	Measured Concentration, AU/mL	% Recovery
1:32	2.67	3.00	112.31
1:16	5.34	6.75	126.35
1:8	10.69	13.03	121.90
1:4	21.37	24.04	112.47
1:2	42.74	42.05	98.39
neat	/	85.48	/

Dilution	Expected Concentration, AU/mL	Measured Concentration, AU/mL	% Recovery
1:32	5.22	4.63	71.93
1:16	10.43	9.02	90.25
1:8	20.87	22.27	90.68
1:4	41.74	39.04	86.68
1:2	83.48	83.28	87.71
neat	/	166.96	/

Please note: the examples shown should not be considered totally representative either of behaviour of different samples diluted in the same sample diluent or of the same samples diluted in different dilution matrixes.

3. Analytical Sensitivity

Analytical sensitivity represents lowest measurable analyte level that can be distinguished from zero, is ≤1 AU/mL.

A 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.

4. Analytical Specificity

Cross reaction: the specificity of the Rubella IgM CLIA Microparticles was evaluated by testing a total of 117 samples for potential cross-reactivity (HSV-1, HSV-2, Toxoplasma gondii, HEV, Mycoplasma Pneumoniae, Chlamydia Pneumoniae, HIV, Treponema pallidum, HCV, Anti-HBs, Parvovirus B19, RF, ANA). The data are summarized in the following table.

Category	N	Rubella IgM CLIA Microparticles		
		Reactive	Nonreactive	Equivocal
HSV-1 IgM antibodies	4	0	4	0
HSV-2 IgM antibodies	3	0	3	0
hCMV IgM antibodies	9	0	9	0
Toxoplasma gondii IgM antibodies	9	0	9	0
HEV IgM antibodies	10	0	10	0
Mycoplasma Pneumoniae IgM antibodies	10	0	10	0
Chlamydia Pneumoniae IgM antibodies	5	0	5	0
HIV antibodies	10	0	10	0
Treponema pallidum antibodies	10	0	10	0
HCV antibodies	10	0	10	0
Anti-HBs	10	0	10	0
Parvovirus B19 IgM antibodies*	5	0	4	1
RF*	15	0	14	1
ANA*	12	0	12	0

*One sample was repeatedly equivocal with Rubella IgM CLIA Microparticles and was negative tested with reference assay;

*RF: Rheumatoid factor; one sample was repeatedly equivocal with Rubella IgM CLIA Microparticles and was negative tested with reference assay;

*ANA: anti-nuclear antibody.

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).

5. Clinical Specificity

Pre-selected negative samples

In 227 pre-selected Rubella IgM negative samples, 226 negative and 1 equivocal results were found with Rubella IgM CLIA Microparticles.

Routine samples

A total of 1236 samples obtained from clinical routine were tested at 2 different sites in comparison to commercially available Rubella IgM assay. Sample with inconsistent results were re-tested with other two commercially available Rubella IgM assays. The relative specificity was as follows:

N	Relative specificity	Lower 95% confidence limit
1236	99.35% (1214/1222)	98.89%

7 samples which were found reactive with Rubella IgM CLIA Microparticles assay and the comparison test, 6 samples giving unconfirmed results determined by abovementioned rules, and 1 sample which could not be further examined were excluded from the calculation of specificity.

In total of 1222 samples were analyzed. 8 samples among which were found reactive or equivocal with the Rubella IgM CLIA Microparticles assay were found nonreactive with the comparison test and other two reference

assays; 8 samples which were found nonreactive with the Rubella IgM CLIA Microparticles assay and other two reference assays were found reactive or equivocal with the comparison test.

6. Clinical Sensitivity

Pre-selected positive sample

In total of 98 Rubella IgM positive samples that suspected of Rubella virus infection were collected, and were tested with Rubella IgM CLIA Microparticles assay and three commercially available Rubella IgM assays. 60 among the samples were reactive for at least 3 reagents. 38 samples with inconsistent test results were excluded. The results of 60 positive samples were as follows:

Rubella IgM CLIA Microparticles		Comparison Rubella IgM reagent	
Reactive	Non-reactive	Reactive	Nonreactive
57	3	57	3
Relative sensitivity: 95%		Relative sensitivity: 95%	

Literature References

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