Rubella virus IgM (CLIA) PACKAGE INSERT

[INTENDED USE]

Rubella virus IgM (CLIA) is a Chemiluminescence Immunoassay test for the qualitative detection of rubella virus IgM antibody (Rubella IgM) in human serum or plasma, which is mainly used clinically as an aid in the diagnosis of rubella virus infection.

The test must be performed on the ACCRE series analyzers (including ACCRE 6, ACCRE 8, ACCRE 90, ACCRE 100, ACCRE 120, and all other ACCRE series analyzers).

[SUMMARY]

Rubella virus (RV), a single-stranded positive RNA virus, belongs to the Rubivirus genus of the Togaviridae family. Rubella is an acute respiratory infectious disease caused by RV with mild or absent clinical features and is easily neglected. RV infection in pregnant women, especially during the first trimester, can cause intrauterine death, miscarriage, or premature birth. Pre-pregnancy RV screening can prevent neonatal infections by providing a base value for comparison of test results during pregnancy and helping to determine whether the pregnancy was a first-time or recurrent infection. Positive levels of RV IgM strongly suggest that patients recently had an acute rubella virus infection.

[ASSAY PRINCIPLES]

The Rubella virus IgM (CLIA), hereinafter also referred to as the "Rubella IgM" is a two-step sandwich immunoassay to determine the level of Rubella virus IgM antibody.

Reagents for the assay are ready-to-use and pre-dispensed in the sealed reagent cartridge. All the assay steps are performed automatically by the ACCRE analyzer.

Firstly, pre-diluted sample, sample treatment solution, Rubella virus antigen coated magnetic particles were mixed thoroughly together, incubating and washing. The second step, alkaline phosphatase-labeled anti-human IgM antibody was added to it, and incubated for immune reaction to form a double-antibody sandwich immune complex; the complex was washed after the end of the reaction. The addition of a substrate triggers a chemiluminescence reaction, the result of which is measured in relative light units (RLUs) by a photomultiplier tube (PMT). The concentration of the substance to be detected in the sample is proportional to the luminescence intensity. Finally, the ACCRE analyzer automatically calculates the result based on the stored calibration curve.

[CONTENT OF THE KIT]

Package: 60 tests/kit with quality controls, 36 tests/kit with quality controls.

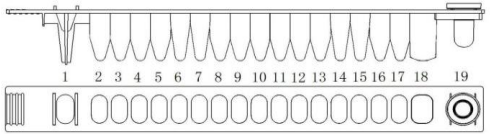
Traceability: The calibrators of this kit can be traced to the internal reference substance. For specific calibrator concentration values, see the Calibrator Barcode Sheet for each lot.

Contents	Quantities		Notice
	REF W305A	REF W305C	
Rubella IgM reagent cartridge	60 strips	36 strips	Ready to use
Rubella IgM Calibrator 1 (CAL 1)	1.0mL×1 vial	1.0mL×1 vial	Tris buffer, ProClin300. BND,
Rubella IgM Calibrator 2 (CAL 2)	1.0mL×1 vial	1.0mL×1 vial	Tris buffer, ProClin300, BND, Rubella IgM antibody.
Rubella IgM Quality Control level 1 (QC 1)	1.0mL×1 vial	1.0mL×1 vial	Tris buffer, ProClin300, BND.
Rubella IgM Quality Control level 2 (QC 2)	1.0mL×1 vial	1.0mL×1 vial	Tris buffer, ProClin300, BND, Rubella IgM antibody.
Instruction for Use	1	1	N/A
Reagent Registration Card	1	1	N/A
Calibrator Barcode Sheet	1	1	N/A
Quality Control Sheet	1	1	N/A

Note: Refer to the target value list of the controls from Quality Control Sheet within the kit.

The Reagent Cartridge

The strip consists of 19 wells. The 1st well is the sample well and the last 19th well is the reading well for chemiluminescent signal. From the 2nd to the 18th well are all covered with a labeled, aluminum foil seal. The label comprises a barcode which mainly indicates the reagent assay information, reagent lot number and expiration date. All reagents components needed for the assay are ready-to-use and pre-dispensed in the cartridges. And please do not interchange integral component from different reagents or lots.



Description of the reagent cartridge

Well	Component	Content	Volume
5	Enzyme Label	ALP (alkaline phosphatase) labeled anti-human IgM antibody, MES Buffer, ProClin300, BND.	150μL
7	Magnetic Particles	Rubella virus antigen coated magnetic particles, Tris buffer, ProClin300, BND.	75μL
9	Sample Treatment Solution	Tris buffer, ProClin300, BND.	75μL
2, 3, 10,1 1, 12	Wash Solutions	Tris buffer, ProClin300, BND.	500μL
16	Substrate Solution	Disodium [(4-chlorophenyl) sulfanyl] (10-methyl-9(10H)-acridinylidene) methyl phosphate.	200μL
18	Sample diluent	Sodium chloride	420μL

Note: Well 1,4,6,8,13,14,15,17 are empty.

[MATERIALS AND DISPOSABLES REQUIRED BUT NOT PROVIDED]

- Sample diluent (manufactured by Tisenc)
- Pipette with disposable tip to dispense 200 μL.
- Powderless and disposable gloves.
- Disposable tips for ACCRE analyzer.
- For other specific materials and disposables, please refer to the Instrument User Manual.
- Instruments of the ACCRE series analyzers.

[WARNINGS AND PRECAUTIONS]

- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (do not ingest; do not inhale).
- Do not use reagents after the expiration date indicated on the box label.
- Do not use if the packaging of reagent cartridge is damaged or leaked.
- Do not use the reagents if the quantity is obviously insufficient.
- If any component adheres to inside wall of wells or aluminum film of the reagent cartridge, please gently switch the reagent cartridge to let the component back the wells.
- Do not reverse the reagent cartridge. Otherwise, the result will be unreliable.
- Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.
- Use powderless gloves, as powder has been reported to cause false results for certain enzyme immunoassay tests.
- When using this kit, it is important to follow all laboratory reagent handling precautions; skillful technique and strict adherence to the instructions are necessary to obtain reliable results.
- Do not use mixed samples, heat-inactivated samples, or samples that are visibly contaminated with microorganisms.
- Due to methodological or antibody specificity reasons, testing of the same sample with reagents from different manufacturers may yield different results, and results obtained with different reagents should not be compared directly with each other to avoid erroneous medical interpretations; it is recommended that laboratories indicate the characteristics of the reagents used in the test reports sent to clinicians.

[STORAGE]

- Store the Rubella virus IgM (CLIA) upright at 2-8 °C, kit can be stored for 18 months from manufactured date. After the reagent cartridge is taken out from the refrigerator to equilibrate, the testing should be completed within 2 hours.
- Store the calibrators and quality controls at 2-8 °C till expiry date. When opened, the calibrators and/or controls will be stable for30 days at 2-8 °C.
- Keep away from sunlight.
- Do not freeze reagents cartridge.

[SAMPLES]

The sample volume for Rubella virus IgM (CLIA) assay is 30μL, please note that at least 55μL of sample has to be pipetted manually into the reagent cartridge.

Sample type and collection

Human serum or plasma (heparin,lithium heparin sodium, sodium citrate, EDTA-K_2, EDTA-K_3) are recommended

Types of tubes:
- Plastic tube with clot activator;
- Plastic tube with clot activator and separation gel,
- Plastic tube with lithium heparin,
- Plastic tube with sodium heparin,
- Plastic tube with sodium citrate,
- Plastic tube with EDTA-K_2,
- Plastic tube with EDTA-K_3.

It is recommended to validate collection tubes before using them as some may contain substances which interfere with test results.

Note: Blood sampling tube results may vary from one manufacturer to another depending on the materials and additives used.

It is the responsibility of each laboratory to validate the type of sample tube used and to follow the manufacturer's recommendations for use.

Sample preparation

The current revision of the WHO/DIL/LAB/99.1 document provides recommendations for the preparation of samples. For use of the sample tubes, follow the tube manufacturer's instructions for use.

The pre-analytical step, including the preparation of blood samples, is an essential part of medical analyses. In accordance with Good Laboratory Practice, this step is performed under the responsibility of the laboratory manager.

Insufficient clot time can result in the formation of fibrin with micro-clots that are invisible to the naked eye. The presence of fibrin, red blood cells, or suspended particles can lead to erroneous results.

Samples containing suspended fibrin particles or

erythrocyte stroma should be centrifuged before testing.
If there is a lipid layer over the sample after centrifuging, please transfer the clear sample into a new sample tube without transferring the lipid layer.
Severe hemolysis samples or heat-inactivated samples are not recommended for applying on the reagents.
Preparation of frozen-stored samples: after thawing, these samples must be thoroughly mixed before testing.
Mix using a vortex-type mixer. If necessary, clarify samples by centrifuging before testing.

Sample stability
Samples (serum and plasma) can be stored at room temperature(15-30°C) for 8 hours or at 2-8 °C for 7 days; if longer storage is required, freeze the serum or plasma at -20 °C or below -20 °C, without exceeding 3 times freeze/thaw cycle.

[INSTRUCTIONS FOR USE]

For complete instructions, see the Instrument User Manual.
When using the assay for the first time. Register the assay using the registration code in the reagent kit before any assay is used in the ACCRE analyzer for the first time. Calibration, QC and sample test are only available after assay was registered successfully.
When opening a new lot of reagents. Each new lot of reagent must be registered by using the registration code in Reagent Registration Card within the reagent kit.

Calibration
Calibration must be performed with the reagents and calibrators from Manufacturer.
Calibration is necessary under the following conditions:
(1). New reagent lot number applied;
(2). Same lot of reagents used more than 4 weeks;
(3). QC out of control;
(4). When necessary, for example: system maintenance or repair that may affect the analytic performance.

Test Procedure
1. Remove the kit from storage at 2-8 °C and take out the required number of reagent cartridges. Allow the reagent cartridges, specimens, calibrators, and/or controls to equilibrate to room temperature (15-30°C) prior to testing.
2. Use each assay strips for each sample, calibrator or control respectively.
3. Place the reagent cartridges into the reagent rack.
4. Manual sample dilution: Use the sample diluent to dilute the sample with a proportion of 1:15 (for example, a 10uL sample is evenly mixed with 140μL sample diluent).
Note: the calibrator, control has been pre- diluted and can be used directly.
5. Manually pipette the Sample, Calibrator, or Control into the sample well of each reagent cartridge.
Please note that at least 55μL sample has to be pipetted manually into the reagent cartridge and 30μL sample is needed for each test.
** Before pipetting: Mix the samples using a vortex-type mixer if necessary, and ensure that the samples and sample diluent (if applicable) DO NOT have any bubbles. The same for calibrators and controls.*
6. Put the sample rack into the reagent chamber of ACCRE analyzer.
7. Place the assay tips into the reagent chamber of ACCRE analyzer.
8. Input Sample ID, sample type, dilution etc. into the software as directed by USER MANUAL.
9. Rubella virus IgM (CLIA) assay will be performed automatically by the ACCRE analyzer.
10. After assay is completed, remove the reagent rack from the ACCRE analyzer. Dispose the used reagent cartridge into an appropriate recipient.

Dilution
Samples with concentrations above 500.00 AU/mL can be diluted with sample diluent manually. The recommended dilution is 1:10. The concentration of the diluted sample must be >50.00 AU/mL. After manual dilution, multiply the result by the dilution factor (10x). ACCRE analyzer cannot perform automatic dilution for samples.

Quality control
Quality control should be performed in accordance with local regulations or requirements related to accreditation, as well as requirements defined in the laboratory's quality control procedure.

[RESULTS AND INTERPRETATION]

Calculation of Results
The results are automatically calculated by the ACCRE analyzer using stored calibration curve which generate by 2-point calibration, and calculated according to a predefined mathematical model, the results expressed in AU/mL.

Interpretation of Results
Positive judgment values were determined by the method of subject operating characteristic curve (ROC):
A test result <5.00 AU/mL was judged negative;
5.00 AU/mL ≤ a test result < 8.00 AU/mL is considered suspicious;
If the test result is ≥8.00 AU/mL, it is considered positive.
Since there are some differences in different sample groups in different regions, it is recommended that each laboratory establish its own positive judgment value according to the actual situation.
A result of <5.00 AU/mL suggests a non-recent infection, but does not exclude the absence of RV infection because the time from RV infection to antibody production varies from person to person, and it is possible that the body may not have produced enough antibodies to be detected at the time of testing.
A result of ≥ 5.00 AU/mL and < 8.00 AU/mL is considered a gray zone result, and it is recommended that the test be resampled in 1-2 weeks or confirmed by other testing methods.
A result of ≥8.00 AU/mL suggests a possible recent infection and is recommended to be combined with the results of IgG antibody and IgG antibody affinity testing.
** Please note that results may differ between laboratories due to variations in population and test method. If necessary, each laboratory should establish its own reference range.*
In interpreting the results, patient's clinical condition including symptoms, diseases history, and other relevant data and information should be considered.

[LIMITATIONS]

1. The results of this kit are for clinical reference only and should not be used as the sole basis for clinical diagnosis and treatment. Clinical diagnosis of a patient should be considered in conjunction with his/her symptoms/signs, medical history, epidemiology, and other laboratory tests (e.g., pathogenetic tests). Since false positive Rubella IgM results may cause adverse consequences, it is recommended that clinicians should consider positive Rubella IgM results in conjunction with clinical history and other test results, and should not use them as the sole basis for diagnosis of acute rubella virus infection.
2. In the early stages of infection, Rubella IgM-specific antibodies are not produced or are below the limit of detection, which can lead to negative results. If pathogen infection is suspected, the patient should be prompted to review the test over a period of time, e.g., more than 2 weeks, and a second sample should be drawn and tested when the same conditions as the first sample are present, to determine whether there is a seroconversion of the initial infection or a significant elevation of the Rubella-specific IgM antibodies.
3. High titers of Rubella-specific IgG antibodies will compete with specific IgM antibodies for antigen-binding sites, which will make the test less sensitive and may result in falsely low or negative specific IgM antibody results.
4. Screening for Rubella IgM antibodies in asymptomatic pregnant women is not recommended due to the high risk of false positives in laboratory tests for Rubella IgM antibodies in pregnant women and the inability to reliably identify the risk of fetal disease. The results of this reagent should not be used alone as a basis for termination of pregnancy.
5. Antibody and antibody affinity testing in patients who are immunocompromised or receiving immunosuppressive therapy, such as patients with human immunodeficiency virus (HIV) infection or patients receiving immunosuppressive therapy after organ transplantation, is of limited reference value and may lead to erroneous medical interpretations.
6. Positive test results should be analyzed with caution in those who have been treated with blood transfusions or other blood products within the last few months.
7. Rubella-specific IgM antibodies are not only present in primary infection, but may also be present when secondary and recurrent infections occur. Rubella-specific IgM antibodies in an individual can be detected long after the initial infection, so caution should be exercised in interpreting positive specific IgM antibodies in determining the time of initial infection.
8. Heterophilic antibodies in human serum may bind to immunoglobulins in the reagent and interfere with the test results. People who have frequent contact with animals or animal serum products should be alerted to the possibility of abnormal interference with the results.
9. Human anti-mouse antibodies (HAMA) may be present in samples from patients treated with mouse monoclonal antibodies. For test reagents with mouse-derived antibodies in the kit composition, the presence of HAMA in the sample to be tested may affect the accuracy of the test results.

[PERFORMANCE CHARACTERISTICS]

Detection Limit
Limit of Detection (LoD): 1.0 AU/mL
The Limit of Detection was determined in accordance with the CLSI® EP17-A2 recommendations.

Precision
The precision study was performed according to CLSI® EP05-A3 recommendations.
The within-lot precision CV% is less than 10%.

Interfering Substances
Potential interference substances were studied according to CLSI ® EP07-A3 recommendations.
Total IgG, total IgM, bilirubin, hemoglobin, triglycerides, antinuclear antibodies, rheumatoid factor, HAMA had no significant effect on the test results.

Cross-reactivity
No cross-reactivity with positive samples for Rubella virus IgG antibody (Rubella IgG), Cytomegalovirus IgM antibody (CMV IgM), Toxoplasma gondii IgM antibody (Toxo IgM), Herpes simplex virus type I IgM (HSV-1 IgM) antibody, herpes simplex virus type II IgM antibody (HSV-2 IgM), EB virus IgM antibody, hepatitis B virus IgM antibody, hepatitis A virus IgM antibody, varicella-zoster virus IgM antibody, and Mycoplasma pneumoniae IgM antibody.

[WASTE DISPOSAL]

Dispose used or unused reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.
It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

[INDEX OF SYMBOLS]

Symbol	Meaning	Symbol	Meaning	Symbol	Meaning
	Consult instructions for use		Contains sufficient for <n> tests		Date of manufacture
	Temperature limit		Use-by date		Do not re-use
	Keep away from sunlight	LOT	Batch code	REF	Catalogue number
	Do not roll		Keep dry		Manufacturer
	Biological risks		Do not use if package is damaged		Contains biological material of animal origin
	Upright		Caution	CONTROL	Control
	Fragile, handle with care				

[REFERENCES]

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2. Klaus-Peter Wandinger et al. Diagnosis of recent primary rubella virus infections: Significance of glycoprotein-based IgM serology, IgG avidity and immunoblot analysis[J]. Journal of Virological Methods, 2011, 174(1-2): 85-93.
3. Thabelo Makhupane and DSK Habedi. Rubella epidemiology in Lesotho after vaccine introduction: a five-year review, 2018–2022[J]. BMC Public Health, 2024, 24(1) : 2874-2874.
4. Gulimov M. K. et al. Long non-coding RNAs — regulators of rubella virus infection and antiviral response[J]. Russian Journal of Infection and Immunity, 2024, 14(3) : 581-585.

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