VIDAS® RUB IgM (RBM)

IVD

VIDAS RUB IgM is a qualitative automated enzyme immunoassay for use on the VIDAS family instruments, for the detection of anti-rubella IgM (RBM) in human serum using the ELFA technique (Enzyme Linked Fluorescent Assay).

SUMMARY AND EXPLANATION

Rubella infection in children and adults is generally a mild, self-limiting disease of short duration. However, Rubella may cause severe congenital defects in the fetus, especially if infection occurs during the first trimester of pregnancy (1,5).

The diagnosis of a rubella infection is essentially based on serology and in particular on the detection of specific IgM (2,3): in a majority of cases, the presence of anti-rubella IgM is correlated to a recent primo-infection (4,5).

Capture immunoassay techniques are generally more sensitive and more specific for detecting anti-rubella IgM (2,3,4,5).

Detection of anti-rubella IgM is useful in the diagnosis of recent infections, particularly in pregnant women.

PRINCIPLE

The assay principle combines the enzyme immunoassay method by capture with a final fluorescent detection (ELFA). The Solid Phase Receptacle (SPR®) serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready-to-use and pre-dispensed in the sealed reagent strips.

All of the assay steps are performed automatically by the instrument. The reaction medium is cycled in and out of the SPR several times.

After sample dilution, the IgM are captured by the polyclonal antibodies coating the interior of the SPR. Anti-rubella IgM are specifically detected by the inactivated rubella antigen, which is itself revealed by an alkaline phosphatase-labeled anti-rubella monoclonal antibody (conjugate).

During the final detection step, the substrate (4-Methyl-ombelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyl-umbelliferone), the fluorescence of which is measured at 450 nm. At the end of the assay, an index is automatically calculated by the instrument in relation to the standard S1 stored in memory, and then printed out.

CONTENT OF THE KIT (30 TESTS):

30 RBM Strips	STR	Ready-to-use.
30 RBM SPRs 1 x 30	SPR	Ready-to-use. SPRs sensitized with anti-human µ chain antibodies (goat) purified by affinity.
RBM Positive Control 1 x 1 mL (liquid)	C1	Ready-to-use. Human* serum containing anti-Rubella IgM + 1 g/L sodium azide. MLE data indicate the index: confidence interval ("Control C1 (+) Test Value Range ").
Negative Control 1 x 1.9mL (liquid)	C2	Ready-to-use. Phosphate buffer + protein stabilizer of animal origin + preservatives.
RBM Standard 1 x 2 mL (liquid)	S1	Ready-to-use. Human* serum containing anti-Rubella IgM + 1 g/L sodium azide.
Specifications for the factory master data required to calibrate the test: • MLE data (Master Lot Entry) provided in the kit, or • MLE bar codes printed on the box label.		
1 Package Insert provided in the kit or downloadable from www.biomerieux.com/techlib.		

* This product has been tested and shown to be negative for HBs antigen, antibodies to HIV1, HIV2 and HCV. However, since no existing test method can totally guarantee their absence, this product must be treated as potentially infectious. Therefore, usual safety procedures should be observed when handling.

The SPR

The interior of the SPR is coated during production with anti-human µ chain antibodies (goat) purified by affinity. Each SPR is identified by the code RBM. Only remove the required number of SPRs from the pouch and **carefully reseal the pouch after opening.**

The Strip

The strip consists of 10 wells covered with a labeled, foil seal. The label comprises a bar code which mainly indicates the assay code, kit lot number and expiration date. The foil of the first well is perforated to facilitate the introduction of the sample. The first well in the strip is for the sample. The last well of each strip is a cuvette in which the fluorometric reading is performed. The wells in the center section of the strip contain the various reagents required for the assay.

Description of the RBM Strip

Wells	Reagents
1	Sample well.
2	Sample diluent: TRIS (50 mmol/L) pH 7.4 + protein and chemical stabilizers + 1 g/L sodium azide (400 µL)
3	Pre-wash solution: TRIS (50 mmol/L) pH 7.4 + protein and chemical stabilizers + 1 g/L sodium azide (600 µL)
4 - 5 - 7 - 9	Wash solution: TRIS (50 mmol/L) pH 7.4 + protein and chemical stabilizers + 1 g/L sodium azide (600 µL)
6	Inactivated Rubella antigen + protein and chemical stabilizers + 1 g/L sodium azide (400 µL)
8	Alkaline phosphatase-labeled monoclonal anti-Rubella antibody (mouse) in **Fab' fragment** form + 1 g/L sodium azide (400 µL)
10	Reading cuvette with substrate: 4-methylumbelliferyl phosphate (0.6 mmol/L) + diethanolamine (DEA*) (0.62 mol/L or 6.6%, pH 9.2) + 1 g/L sodium azide (300 µL).

* Signal Word: **DANGER**

Hazard Statement

H318: Causes serious eye damage.

Precautionary Statement

P280:Wear protective gloves/protective clothing/eye protection/face protection.
P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

For further information, refer to the Material Safety Data Sheet.

MATERIALS AND DISPOSABLES REQUIRED BUT NOT PROVIDED

- Pipette with disposable tip to dispense 100 µL.
- Powderless, disposable gloves.
- For other specific materials and disposables, please refer to the Instrument User Manual.
- Instrument of the VIDAS family.

WARNINGS AND PRECAUTIONS

- **For *in vitro* diagnostic use only.**
- **For professional use only.**
- **This kit contains products of human origin. No known analysis method can 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 (see Laboratory Biosafety Manual - WHO - Geneva - latest Edition).**
- 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 or inhale).
- Do not use SPRs if the pouch is pierced.
- Do not use visibly deteriorated STRs (damaged foil or plastic).
- Do not use reagents after the expiration date indicated on the label.
- Do not mix reagents (or disposables) from different lots.
- Use **powderless gloves** as powder has been reported to cause false results for certain enzyme immunoassay tests.
- Kit reagents contain sodium azide which can react with lead or copper plumbing to form explosive metal azides. If any liquid containing sodium azide is disposed of in the plumbing system, drains should be flushed with water to avoid build-up.

- The substrate in well 10 contains an irritant agent (6.6% diethanolamine). Refer to the hazard statements "H" and the precautionary statements "P" above.
- Spills should be wiped up thoroughly after treatment with liquid detergent or a solution of household bleach containing at least 0.5% sodium hypochlorite. See the User Manual for cleaning spills on or in the instrument. Do not autoclave solutions containing bleach.
- The instrument should be regularly cleaned and decontaminated (see the User Manual).

STORAGE CONDITIONS

- Store the VIDAS RUB IgM kit at 2-8°C.
- **Do not freeze reagents.**
- **Store all unused reagents at 2-8°C.**
- After opening the kit, check that the SPR pouch is correctly sealed and undamaged. If not, do not use the SPRs.
- **Carefully reseal the pouch with the desiccant inside after use to maintain the stability of the SPRs and return the complete kit to 2-8°C.**
- If stored according to the recommended conditions, all components are stable until the expiration date indicated on the label.

SPECIMENS

Specimen Type and Collection

Human serum.
It is recommended that each laboratory checks the compatibility of used collection tubes.
The use of heat inactivated sera has not been validated.
Since the use of clearly hemolyzed, lipemic, icteric or inactivated samples has not been validated, it is recommended to collect a new specimen.

Specimen Stability

Samples can be stored at 2-8°C in stoppered tubes for up to 7 days, if longer storage is required, freeze the sera at -25 ± 6°C.
Avoid successive freezing and thawing.

INSTRUCTIONS FOR USE

For complete instructions, see the User Manual.

Reading Master Lot Data

Before each new lot of reagents is used, enter the specifications (or factory master data) into the instrument using the master lot entry (MLE) data.

If this operation is not performed **before initiating the tests**, the instrument will not be able to print results.

Note: the master lot data need to be entered only once for each lot.

It is possible to enter MLE data **manually or automatically** depending on the instrument (refer to the User Manual).

Calibration

Calibration, using the standard provided in the kit, must be performed each time a new lot of reagents is opened, after the master lot data have been entered. Calibration should then be performed every 14 days. This operation provides instrument-specific calibration curves and compensates for possible minor variations in assay signal throughout the shelf-life of the kit.
The standard, identified by "S1", must be tested **in duplicate** (see User Manual). The standard value must be within the set RFV (Relative Fluorescence Value). If this is not the case, recalibrate.

Test Procedure

1. **Only remove the required reagents from the refrigerator and allow them to come to room temperature for 30 minutes before use.**
2. Use one "RBM" strip and one "RBM" SPR for each sample, control or standard to be tested. **Make sure the storage pouch has been carefully resealed after the required SPRs have been removed.**
3. The test is identified by the "RBM" code on the instrument. The standard must be identified by "S1", and tested **in duplicate**. If the positive control is to be tested, it should be identified by "C1". If the negative control needs to be tested, it should be identified by C2.
4. Mix the standard, controls and samples using a vortex -type mixer (for serum separated from the pellet).
5. **For this test, the standard, control, and sample test portion is 100 µL.**
6. Insert the "RBM" SPRs and "RBM" Reagent Strips into the instrument. Check to make sure the color labels with the assay code on the SPRs and the Reagent Strips match.
7. Initiate the assay as directed in the User Manual. All the assay steps are performed automatically by the instrument.
8. Reclose the vials and return them to 2–8°C after pipetting.
9. The assay will be completed within approximately 60 minutes. After the assay is completed, remove the SPRs and the strips from the instrument.
10. Dispose of the used SPRs and strips into an appropriate recipient.

RESULTS AND INTERPRETATION

Once the assay is completed, results are analyzed automatically by the computer. Fluorescence is measured twice in the Reagent Strip's reading cuvette for each sample tested. The first reading is a background reading of the substrate cuvette before the SPR is introduced into the substrate. The second reading is taken after the substrate has been incubated with the enzyme remaining on the interior of the SPR. The RFV (Relative Fluorescence Value) is calculated by subtracting the background reading from the final result. This calculation appears on the result sheet.
The instrument calculates a test value (index) for each sample, which is the ratio between its RFV and that of the memorized standard.

This index and its interpretation appear on the result sheet.

Index i	Interpretation
i < 0.80	Negative
0.80 ≤ i < 1.20	Equivocal
i ≥ 1.20	Positive

Equivocal samples must be retested. If the interpretation remains equivocal, a new sample must be collected 2 to 3 weeks later.

Interpretation of test results should be made taking into consideration the patient's history, and the results of any other tests performed.

As no international standard is available for the determination of anti-Rubella IgM, the VIDAS RUB IgM reagent is calibrated against collection sera.

QUALITY CONTROL

One positive control and one negative control are included in each VIDAS RUB IgM kit.

These controls must be performed immediately after opening a new kit to ensure that reagent performance has not been altered. Each calibration must also be checked using these controls. The instrument will only be able to check the control values if they are identified by C1 and C2.

Results cannot be validated if the control values deviate from the expected values.

Note

It is the responsibility of the user to perform Quality Control in accordance with any applicable local regulations.

LIMITATIONS OF THE METHOD

Interference may be encountered with certain sera containing antibodies directed against reagent components. For this reason, assay results should be interpreted as part of a complete clinical profile.

VIDAS RUB IgM has not been validated for use with neonatal specimens (cord blood, etc.), or specimens other than human serum.

PERFORMANCE

Studies performed using VIDAS RUB IgM gave the following results:

Specificity and Sensitivity Study

281 sera were tested using VIDAS in comparison with another EIA technique.

Discrepant sera were tested using two other commercially available capture techniques; the greatest number of concordant results were considered as the reference result.

Equivocal results were not included in the performance calculation.

Specificity

Out of the 243 negative sera (202 were taken from pregnant women for a screening of rubella immunity, and 41 sera were selected for possible interference), 239 were found to be negative using the VIDAS RUB IgM test, giving a specificity of 98.35% for this study (95% confidence interval: 95.77-99.37%).Out of the 4 samples found to be positive with the VIDAS RUB IgM test, and negative using the other techniques, 1 serum showed residual IgM.

Sensitivity

Out of the 38 positive sera tested, 38 were found to be positive with the VIDAS RUB IgM test, giving a sensitivity of 100.00% for this study (95% confidence interval: 90.75-100.00%).

Precision

Within-Run Reproducibility:

3 samples were tested 30 times in the same run.

Sera	No. of Assays	Mean Index	Standard Deviation	CV % Intra-Assay
S1	30	7.56	0.17	2.3%
S2	30	2.19	0.06	2.7%
S3	30	0.16	0.01	6.3%

Between-Run Reproducibility

2 samples were tested singly in 10 runs on the same VIDAS instrument.

Sera	No. of Assays	Mean Index	Standard Deviation	CV % Inter-Assay
S2	10	2.14	0.06	2.8%
S3	10	0.15	0.004	2.9%

CROSS REACTIVITY AND RELEVANT INTERFERENTS

41 negative sera were selected for possible interference, notably because they were positive in IgM directed against a micro-organism other than the rubella virus, or because of the presence or rheumatoid factors. They were all found negative using VIDAS RUB IgM.

41 selected samples	VIDAS RUB IgM result
6 positive for rheumatoid factors	negative
6 positive for antinuclear antibodies	negative
9 positive for anti-EBV IgM	negative
9 positive for anti-CMV IgM	negative
3 positive for anti-HSV IgM	negative
4 positive for anti-mycoplasma IgM	negative
2 positive for anti-Parvovirus IgM	negative
1 positive for anti-measles IgM	negative
1 positive for anti-mumps IgM	negative

RANGE OF EXPECTED VALUES

According to surveys, in western Europe, between 6 and 11% of young adults are not immune to rubella. In France, an estimated 94% of pregnant women possess antibodies produced following infection or vaccination.

Despite the vaccination programs launched in numerous countries, the risk of congenital rubella remains; in France the incidence of congenital rubella is approximately 1 to 4 for 10,000 pregnancies.

WASTE DISPOSAL

Dispose of 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.

LITERATURE REFERENCES

1. GRANGEOT-KEROS L.: Rubella and pregnancy Path. Biol. 1992, 40, 706-710.
2. TEDDER R.S., YAO J.L., ANDERSON M.J.: The production of monoclonal antibodies to rubella haemagglutinin and their use in antibody-capture assays for rubella-specific IgM. J.Hyg.Camb.1982, 88, 335-350.
3. BELLAMY K., ROUSSEAU S.A., GARDNER P.S.: The development of an M antibody capture ELISA for rubella IgM. J. Virol. Meth. 1986, 14, 243-251.
4. GRANGEOT-KEROS L., PILLOT J., DAFFOS F., FORESTIER F.: Prenatal and postnatal production of IgM and IgA antibodies to rubella virus studied by antibody capture immunoassay. J. Inf. Dis. 1988, 158, 138-143.
5. GRANGEOT-KEROS L. Virus de la rubéole. in Les virus transmissibles de la mère à l'enfant. Paris: John Libbey Eurotext, 1999: 345-364.

INDEX OF SYMBOLS

Symbol	Meaning
REF	Catalog number
IVD	*In Vitro* Diagnostic Medical Device
	Manufacturer
	Temperature limit
	Use by date
LOT	Batch code
	Consult Instructions for Use
	Contains sufficient for <n> tests
	Date of manufacture

WARRANTY

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REVISION HISTORY

Change type categories:

N/A	Not applicable (First publication)
Correction	Correction of documentation anomalies
Technical change	Addition, revision and/or removal of information related to the product
Administrative	Implementation of non-technical changes noticeable to the user

Note: *Minor typographical, grammar, and formatting changes are not included in the revision history.*

Release date	Part Number	Change Type	Change Summary
2015/01	07108J	Administrative	INDEX OF SYMBOLS REVISION HISTORY
		Technical	CONTENT OF THE KIT (30 TESTS) WARNINGS AND PRECAUTIONS INSTRUCTIONS FOR USE
2016/10	07108K	Technical	CONTENT OF THE KIT (30 TESTS)

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