VIDAS®RUB IgG II (RBG)

IVD

VIDAS RUB IgG II (RBG) is an automated quantitative test for use on the VIDAS family instruments, for the quantitative measurement of immunoglobulins G (IgG) directed against the Rubella virus in human serum or plasma (heparin or EDTA) 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. If it is contracted by an expectant mother (1), Rubella may cause severe congenital defects in the fetus, especially if infection occurs during the first trimester of pregnancy. Due to the serious complications that may arise from congenital rubella infection, it is important to determine the immune status of women of childbearing age, pregnant women, or individuals such as health care workers who may have close contact with contaminated individuals (3).

Anti-rubella IgG detection aids in diagnosing rubella infection and determining immune status (2) (4) of patients with regards to this virus. If the result of the anti-Rubella IgG assay is positive, the diagnosis (current or convalescent infection) must be confirmed using a second specimen collected 3 weeks later, by testing for an increase in or stabilization of the IgG titer. It is recommended to test the initial specimen in the same run as the second specimen, using the same lot. A significant rise in IgG is evidence of an evolving rubella infection. However, no increase in the IgG level does not necessarily exclude the possibility of active rubella infection. Only by performing other biological tests (anti-rubella IgM, IgG avidity, viral culture, etc.) can diagnosis of a current rubella infection be confirmed or dismissed.

In patients who have been vaccinated against rubella, the virus can be detected in respiratory secretions for at least 4 weeks following injection of the vaccine.

The antibodies induced by vaccination would appear to be detectable in serum for at least 16 years and probably during the whole life span of 95% of vaccinated individuals.

PRINCIPLE

The assay principle combines a 2-step enzyme immunoassay sandwich method 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 dilution, the sample is incubated with the SPR. Anti-Rubella IgG antibodies present in the specimen will bind to the Rubella antigen coating the interior of the SPR. Unbound components are eliminated during the preliminary wash step.

A second incubation step is then performed using alkaline phosphatase-labeled monoclonal anti-human IgG antibodies (mouse), followed by a second wash step.

During the final detection step, the substrate (4-Methyl-umbelliferyl 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.

The intensity of the fluorescence is proportional to the concentration of antibodies present in the sample. At the end of the assay, results are automatically calculated by the instrument in relation to the calibration curve stored in memory, and then printed out.

CONTENT OF THE KIT (60 TESTS):

60 RBG strips	STR	Ready-to-use.
60 RBG SPRs 2 x 30	SPR	Ready-to-use. SPRs coated with Rubella antigen.
RBG positive control 1 x 1.5 mL (liquid)	C1	Ready-to-use. MLE data indicate the confidence interval in IU/mL (international unit per milliliter) ("Control C1 (+) Dose Value Range").
Negative control 1 x 1.9 mL (liquid)	C2	Ready-to-use. Phosphate buffer + protein stabilizer of animal origin + preservatives.
Calibrator 1 x 2 mL (liquid)	S1	Ready-to-use. Human serum* containing anti-Rubella IgG and calibrated against the WHO standard “International Reference Preparation of anti-Rubella serum” (1 IU = 0.14595 mg) + 1 g/L sodium azide. MLE data indicate the concentration in IU/mL ("Calibrator (S1) Dose Value") and the confidence interval in "Relative Fluorescence Value ("Calibrator (S1) RFV Range).
Specifications for the factory master data required to calibrate the test: • MLE data (Master Lot Entry) provided in the kit, or • MLE bar code 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 Rubella virus antigen. Each SPR is identified by the RBG code. Only remove the required number of SPRs from the pouch and **carefully reseal the pouch after opening**.

The Reagent 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 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 strip

Wells	Reagents
1	Sample well.
2	Sample diluent: TRIS buffer (50 mmol/L) pH 7.4 + protein and chemical stabilizers + 0.9 g/L sodium azide (600 µL).
3	Pre-wash solution: TRIS (50 mmol/L) pH 7.4 + protein and chemical stabilizers + 0.9 g/L sodium azide (600 µL).
4 - 5 - 7 - 8	Wash solution: TRIS (50 mmol/L) pH 7.4 + protein and chemical stabilizers + 0.9 g/L sodium azide (600 µL).
6	Conjugate: Alkaline phosphatase-labeled monoclonal anti-human IgG antibodies (mouse) + 0.9 g/L sodium azide (400 µL).
9	Serum diluent: Tris buffer (50 mmol/L) pH 7.4 + protein and chemical stabilizers + 0.9 g/L sodium azide (400 µL).
10	Cuvette with substrate: 4-Methyl-umbelliferyl phosphate + diethanolamine* (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.
- VIDAS family instrument.

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 the 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 and 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).
- Turbidity may be observed for bottles S1, C1 and C2 before opening, but this does not affect the results.

STORAGE CONDITIONS

- Store the VIDAS RUB IgG II 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 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:

Serum or plasma (lithium heparin or EDTA).
Samples containing impurities must be centrifuged before analysis.
It is recommended that each laboratory checks the compatibility of used collection tubes.
None of the following factors have been found to significantly influence this assay:

- hemolysis (after spiking samples with hemoglobin: 0-96 µmol/L),
- lipemia (after spiking samples with lipids: 0-5 mg/mL equivalent in triglycerides),
- bilirubinemia (after spiking samples with bilirubin: 0-400 µmol/L).

However, it is recommended not to use samples that are clearly hemolyzed, lipemic or icteric and, if possible, to collect a new sample.
Do not inactivate samples.

Specimen Stability

Samples can be stored at 2-8°C in stoppered tubes for up to 1 week; if longer storage is required, freeze the sera or plasma at -25 ± 6°C.
A study performed on frozen samples over a period of 2 months, showed that the quality of results is not affected.
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 calibrator 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 calibrator identified by S1, must be tested in **duplicate** (see User Manual). The calibrator value must be within the set RFV "Relative Fluorescence Value" range. If this is not the case, recalibrate.

Procedure

1. **Only remove the required reagents from the refrigerator and allow them to come to room temperature for at least 30 minutes.**
2. Use one "RBG" strip and one "RBG" SPR for each sample, control or calibrator 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 "RBG" code on the instrument. The calibrator 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 calibrator, controls and samples using a vortex-type mixer (for serum or plasma separated from the pellet).
5. **For this test, the calibrator, control, and sample test portion is 100 µL.**
6. Insert the "RBG" SPRs and "RBG" 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. Restopper the vials and return them to 2–8°C after pipetting.
9. The assay will be completed within approximately 40 minutes. After the assay is completed, remove the SPRs and 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 incubating the substrate 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 results are automatically calculated by the instrument using calibration curves which are stored by the instrument (4-parameter logistic model). The concentrations are expressed in « IU/mL » (WHO standard), and interpretations are printed on the result sheet.

The interpretation, depending on the test value, is as follows:

Titer	Interpretation
< 10 IU/mL	Negative
10 ≤ Titer < 15 IU/mL	Equivocal
≥ 15 IU/mL	Positive

Samples with anti-Rubella IgG titers > 400 IU/mL should be reassayed after dilution “1/3” in saline solution.

If the dilution factor has not been entered when the Work List was created (see User Manual), multiply the result by the dilution factor to obtain the sample concentration.

For serological monitoring, it is recommended to test the initial specimen in the same run as the second one, using the same lot.
Equivocal samples should be reassayed. If the sample repeats as equivocal, test a new specimen collected at least two to three weeks after the first one.
Interpretation of test results should be made taking into consideration the patient's history, and the results of any other tests performed.

QUALITY CONTROL

One positive control and one negative control are included in each VIDAS Rub IgG II 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 taking into consideration the patient's history, and the results of any other tests performed.
VIDAS RUB IgG II performance characteristics have not been established on immunosuppressed populations.
VIDAS RUB IgG II has not been validated for use with cord blood, or specimens other than human serum and plasma.
In the case of congenital rubella, the antibodies may disappear around 3 or 4 years of age. A negative serological test for a child over the age of 3 does not therefore exclude the possibility of congenital rubella.
Since a few rare cases of reinfection with rubella have been reported in previously vaccinated or infected women, certain authors have suggested revaccinating subjects at risk when the level of anti-rubella IgG is less than 25 IU/mL (5).

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.

PERFORMANCE

Studies performed using VIDAS RBG IgG II gave the following results:
Relative sensitivity and relative specificity were established for each study in comparison with two commercialized reagents.
For each serum, the result taken as a reference is the one obtained with at least two of the three techniques used (rule of two out of three).
Uninterpretable sera are eliminated. A sample is considered as uninterpretable if one of the methods gives an equivocal result, and the two others give discrepant results.

Study A

338 preselected sera were composed as follows:
- 85 pregnant women,
- 112 with Rubella IgG titers < 10 IU/mL by another EIA technique,
- 51 children aged < 3 years,
- 19 seroconversions,
- 19 patients infected with congenital Rubella,
- 22 potentially interfering sera,
- 30 miscellaneous sera.

		2 EIA*			
		Results 2/3			
		+	Equiv.	-	Total
VIDAS	+	184	0	1	185
	Equiv.	3	0	0	3
	-	4	0	134	138
	Total	191	0	135	326

* Product registered by the Agence du Médicament.

12 uninterpretable sera

Sensitivity: 97.87%

(95% confidence interval: 94.57% - 99.18%).
Specificity: 99.26%
(95% confidence interval: 95.81% - 99.87%).

Study B

		2 EIA*			
		Results 2/3			
		+	Equiv.	-	Total
VIDAS	+	210	0	1	211
	Equiv.	4	0	0	4
	-	10	0	76	86
	Total	224	0	77	301

* Products registered by the Paul-Ehrlich-Institut.

4 uninterpretable sera

Sensitivity: 95.45%
(95% confidence interval: 91.74% - 97.54%).
Specificity: 98.70%
(95% confidence interval: 92.98% - 99.97%).

Study C

		EIA and IHA*			
		Results 2/3			
		+	Equiv.	-	Total
VIDAS	+	207	0	3	210
	Equiv.	0	1	0	1
	-	0	0	87	87
	Total	207	1	90	298

* Products registered by the Paul-Ehrlich-Institut.

7 uninterpretable sera

Sensitivity: 100 %
(95 % confidence interval: 98.10% - 100.00%).
Specificity: 96,67 %
(95 % confidence interval: 90.57 % - 99.31%).

Studies B and C

305 preselected sera composed as follows:
- 202 pregnant women,
- 74 with serum titers < 10 IU/mL by another EIA technique,
- 4 children aged < 3 years,
- 4 seroconversions,
- 21 potentially interfering sera.

Study on Post-Vaccinal Seroconversion Panels

Two commercial post-vaccinal seroconversion panels were tested. For these 2 panels, the IgG titer was ≥ 15 IU/mL respectively 19 and 24 days after injection of the vaccine.

Precision

Within-Run Reproducibility:

3 samples were tested 30 times in the same run.

	Number	Mean Titer IU/mL	CV %
S1	30	2.5	12.2
S2	30	20.1	10
S3	30	236	7.5

Between-Run Reproducibility

3 samples were tested singly in 15 different runs on the same VIDAS instrument.

	Number	Mean Titer IU/mL	CV %
S1	15	2.5	12.8
S2	15	25.5	5.7
S3	15	311.3	11.5

CROSS REACTIVITY AND RELEVANT INTERFERENTS

Potential interferent	Site 1	Site 2	total
Parvovirus B19 IgG	3	0	3
CMV IgG	8	11	19
EBV IgG	9	10	19

No bias linked to the presence of these potential interferents was revealed.

WASTE DISPOSAL

Dispose of used or unused reagents as well as any other other contaminated disposable material following procedures for infectious or potentially infectious products. It is the responsibility of each laboratory to handle waste and effluents produced according to their type 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. Pathol-Biol, 1992, 40, 706-10.
2. NCCLS. Evaluation and performance criteria for multiple component test products intended for the detection and quantitation of rubella IgG antibody. NCCLS Document I/LA6-7, 1992, 12.
3. GALAZKA A. Rubella in Europe. Epidemiol. Infect., 1991, 107 : 43-54.
4. GRANGEOT KEROS L., SICE J., PILLOT J. D. Détermination de l'immunité anti-rubéolique. Comparaison des résultats obtenus par agglutination de particules de latex, inhibition d'hémagglutination et immunoenzymologie. Feuillets de biologie, 1991, 32, n° 183.
5. Matter L., Kogelschatz K., Germann D. – Serum levels of rubella virus antibodies indicating immunity: response to vaccination of subjects with low or undetectable antibody concentrations – *J Infect Dis*. – 1997, vol.175, p 749-755.

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	08588H	Administrative	INDEX OF SYMBOLS REVISION HISTORY
		Technical	CONTENT OF THE KIT (60 TESTS) WARNINGS AND PRECAUTIONS
2015/06	08588I	Technical	CONTENT OF THE KIT (60 TESTS) – RECONSTITUTION OF REAGENTS INSTRUCTIONS FOR USE
2016/10	08588J	Technical	CONTENT OF THE KIT (60 TESTS) – RECONSTITUTION OF REAGENTS

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