Instructions for Use

Rubella Virus IgG Avidity ELISA

Enzyme immunoassay for indicating Rubella-specific IgG avidity in human serum or plasma to differentiate between acute and past infection.

48 Determinations

ELS61245

Read the documentation

For Research Use Only

CONTENTS

1. INTRODUCTION

The presence of IgG antibodies to Rubella Virus indicates the occurrence of the infection but does not distinguish between recent and past infection. Virus-specific IgM antibodies are first detected approximately in ten days and peak at about four weeks post infection. They may persist for several months after acute infections. Based on the evidence that antibody avidity gradually increases after exposure to an immunogen, avidity of IgG antibodies can be used as a marker for distinguishing recent primary from long-term infections. Avidity describes the binding strength of a specific antibody to its antigen. Low-avidity IgG antibodies indicate a primary infection, whereas the presence of IgG antibodies with high avidity points to persistency or reactivation of infection.

2. INTENDED USE

The Rubella Virus IgG Avidity ELISA is intended to indicate the Rubella-specific IgG avidity in human serum or plasma (citrate, heparin) to differentiate between acute and past infection.

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microplates are coated with specific antigens to bind corresponding antibodies of the sample (dual pipetting). After washing the wells to remove all unbound sample material, one well is incubated with avidity reagent and the corresponding well with washing buffer. The avidity reagent removes the low-avidity antibodies from the antigens whereas the high-avidity ones are still bound to the specific antigens. After second washing step to remove the rest of avidity reagent and low-avidity antibodies, a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a third washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA microwell plate reader.

4. MATERIALS

4.1. Reagents supplied

- **Avidity Reagent:** 1 bottle containing 15 ml of a urea solution; coloured blue; ready to use; black cap.
- **Avidity Rubella Virus IgG Control Low:** 1 vial containing 2 ml control (human serum or plasma); coloured yellow; ready to use; blue cap.
- **Avidity Rubella Virus IgG Control High:** 1 vial containing 2 ml control (human serum or plasma); coloured yellow; ready to use; red cap.

For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

- 1 Instruction for use Rubella Virus IgG Avidity ELISA (Product Number: ELS61245)
- 1 Instruction for use Rubella Virus IgG ELISA (Product Number: ELS61244)
- 1 empty labelled bottle (white with white cap) for ready to use Washing Buffer

5. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

6.1. Avidity Reagent

If crystals have formed in the reagent warm up to 37°C e.g. in a water bath and mix gently until they disappear.

6.2. Washing Buffer

It is recommended to fill 15 ml ready to use Washing Buffer into supplied bottle (s. 4.2) to use it in step 5 of the test preparation.
Note: Ready to use Washing Buffer is stable for 5 days at room temperature (20…25 °C).

7. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) **Rubella IgG positive** samples with this assay.

Note: For samples with antibody concentrations greater than Standard D (100 IU/ml), appropriate higher dilutions should be used.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgG Sample Diluent. Dispense 10 µl sample and 1 ml IgG Sample Diluent into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

8.1. Test Preparation

Please read the instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three to five and the volume of Washing Buffer from 300 µl to 350 µl to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

For avidity determination dual pipetting of standards/controls and diluted samples is needed.

1. Dispense 100 µl standards/controls and diluted samples into their respective wells. Leave wells A1/A2 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µl of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µl of Avidity Reagent in wells B1, C1, D1, E1 etc, except for the Substrate Blank well A1.

   Dispense 100 µl of Washing Buffer in wells B2, C2, D2, E2 etc, except for the Substrate Blank well A2.
6. **Incubate for exactly 10 min at 37 ± 1 °C.**
7. Repeat step 4.
8. Dispense 100 µl Conjugate into all wells except in the blank wells (A1/A2).
9. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
10. Repeat step 4.
11. Dispense 100 µl TMB Substrate Solution into all wells.
12. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
13. Dispense 100 µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate, thereby a colour change from blue to yellow occurs.
14. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.2. Measurement

Adjust the ELISA microwell plate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA microwell plate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the **mean absorbance values** of all duplicates.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay to be considered valid, the following criteria must be met:

- **Substrate Blank** Absorbance value **< 0.100**
- **Control Low** Avidity (%): **< 45 %**
- **Control High** Avidity (%): **> 55 %**

If these criteria are not met, the test is not valid and must be repeated.

9.2. Calculation of Results

For each sample or control calculate the ratio between the absorbance of the well dispensed with Avidity Reagent and the absorbance of the well dispensed with Washing Buffer multiplied by 100:

$$\frac{\text{Absorbance (sample or control) Avidity Reagent}}{\text{Absorbance (sample or control) Washing Buffer (diluted 1+19)}} \times 100 = \text{Avidity (\%)}$$

Note: For samples with antibody concentrations greater than Standard D (100 IU/ml), appropriate higher dilutions should be used.

9.3. Interpretation of Results

Result	Avidity	Interpretation
Low-avidity IgG	< 45 %	An avidity index of less than 45 % indicates a primary infection acquired within the past 2 months.
Equivocal	45 – 55 %	No interpretation can be deduced from an equivocal result. It is recommended to take a second sample within an appropriate period of time (e.g. 2 weeks) and repeat testing. If the result of the repeated test is still equivocal, precise statements regarding the time of infection cannot be made.
High-avidity IgG	> 55 %	The presence of high-avidity IgG indicates a past infection or reinfection.
A result of high avidity cannot exclude the possibility of a recent infection.		

9.3.1. Antibody Isotypes and State of Infection

IgG	IgM	IgG-Avidity	Probable result
+	-	low	Vague, further investigation necessary
+	-	high	Indicatives of a past infection
+	+	low	Suggests a current or very recent infection
+	+	high	Suggests a past infection with persisting IgM or reactivation of infection

10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

10.1. Diagnostic Performance

The evaluation of the diagnostic performance of the Rubella Virus IgG Avidity assay was performed in comparison to well defined samples. The resulting relative agreement was 98.6 %.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

12. PRECAUTIONS AND WARNINGS

- The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the test kits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the samples.
- For research use only.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel who are familiar with good laboratory practice.

12.1. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. ORDERING INFORMATION

Prod. No.: ELS61245 Rubella Virus IgG Avidity ELISA (48 Determinations)

BIBLIOGRAPHY

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Banatvala, J. E.; Brown, D. W.G. (2004): Rubella. In *The Lancet* 363 (9415), pp. 1127–1137. DOI: 10.1016/S0140-6736(04)15897-2.

Inouye, Sakae; Hasegawa, Ayako; Matsuno, Shigeo; Katow, Shigetaka (1984): Changes in Antibody Avidity After Virus Infections: Detection by an Immunosorbent Assay in Which a Mild Protein-Denaturing Agent Is Employed. In *Journal of Clinical Microbiology* 20 (3), pp. 525–529.

SYMBOLS KEY

LOT	Lot Number
	Expiration Date
	Storage Temperature
REF	Catalogue Number
	Consult Instructions for Use
REAG AVI	Avidity Reagent
CONTROL L	Control Low
CONTROL H	Control High
	Contains sufficient for “n” tests

SUMMARY OF TEST PROCEDURE

SCHEME OF THE ASSAY

Rubella Virus IgG Avidity ELISA

Test Preparation

Prepare reagents and samples as described.
Establish the distribution and identification plan for all samples and standards/controls on the plate layout supplied in the kit.
Select the required number of microtiter strips or wells and insert them into the holder.

Assay Procedure

	Substrate Blank A1/A2	Avidity Control Low B1	Avidity Control Low B2	Avidity Control High C1	Avidity Control High C2	Sample (diluted 1+100) e.g. D1	Sample (diluted 1+100) e.g. D2
Avidity Control Low	-	100 µl	100 µl	-	-	-	-
Avidity Control High	-	-	-	100 µl	100 µl	-	-
Sample (diluted 1+100)	-	-	-	-	-	100 µl	100 µl
Cover wells with foil **Incubate for 1 h at 37 °C** Wash each well three times with 300 µl of Washing Buffer							
Avidity Reagent	-	100 µl	-	100 µl	-	100 µl	-
Washing Buffer	-	-	100 µl	-	100 µl	-	100 µl
Incubate for exactly 10 min at 37°C Wash each well three times with 300 µl of Washing Buffer							
Conjugate	-	100 µl	100 µl	100 µl	100 µl	100 µl	100 µl
Incubate for 30 min at room temperature (20...25 °C) Do not expose to direct sunlight Wash each well three times with 300 µl of Washing Buffer							
TMB Substrate solution	100 µl	100 µl	100 µl	100 µl	100 µl	100 µl	100 µl
Incubate for exactly 15 min at room temperature (20...25 °C) in the dark							
Stop Solution	100 µl	100 µl	100 µl	100 µl	100 µl	100 µl	100 µl
Photometric measurement at 450 nm (reference wavelength: 620 nm)							