研究用試薬

Avidity determination of IgG antibodies against Rubella viruses
Test instruction

ORDER NO.	ANTIBODIES AGAINST	IG-CLASS	SUBSTRATE	FORMAT
EI 2590-9601-1 G	Rubella viruses	IgG	Ag-coated microplate wells	96 x 01 (96)

Intended Use: This test kit is intended for the avidity determination of IgG class antobodies against Rubella virus in human serum or plasma.

Background

The differentiation between fresh and long-standing infections is one of the greatest challenges in serology. Until now this was based mainly on determination of specific antibodies of the immunoglobulin class IgM, which generally only appear initially. However, the detection of these antibodies is often unreliable and problematic due to interfering factors such as persistence of the IgM response, too weak or delayed IgM production, and unspecific IgM production through polyclonal B-cell stimulation.

In recent years additional determination of the antibody avidity has become an established method for identifcation of primary infections. The immune system reacts to an infection by first forming low-avidity antibodies. With continued disease duration, IgG that are more precisely adapted to the antigens are produced – the avidity increases. If high-avidity IgG are detectable in the serum, it can be assumed that the infection is at a late stage.

Contents of the test system: EI 2590-9601-1 G

Component	Colour	Format	Symbol
1. Test kit Anti-Rubella ELISA (IgG, order number EI 2590-9601 G)	---		
2. Positive control HA High-avidity anti-Rubella (IgG, human), ready for use	red	1 x 1.3 ml	POS CONTROL HA
3. Positive control LA Low-avidity anti-Rubella (IgG, human), ready for use	blue	1 x 1.3 ml	POS CONTROL LA
4. Urea solution for Anti-Rubella ELISA, ready for use	yellow	1 x 12 ml	UREA
5. Phosphate buffer ready for use	light blue	1 x 12 ml	PBS BUFFER
6. Test instruction	---	1 booklet	

LOT Lot description
IVD In vitro diagnostics
CE 0197
Storage temperature
Unopened usable until

Storage and stability: The test kit has to be stored at a temperature between +2°C to +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Waste disposal: Patient samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All reagents must be disposed of in accordance with local disposal regulations.

Updates with respect to the previous version are marked in grey.

Preparation and stability of the reagents

Note: All reagents must be brought to room temperature (+18°Cto +25°C) approx. 30 minutes before use. After first use, the reagents are stable until the indicated expiry date if stored at +2°C to +8°C and protected from contamination, unless stated otherwise below.

- **Controls:** Ready for use. The reagents must be mixed thoroughly before use.

- **Urea solution:** Ready for use.

- **Phosphate buffer:** Ready for use.

Warning: The calibrators and controls used have been tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2 using enzyme immunoassays and indirect immunofluorescence methods. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the toxic agent sodium azide. Avoid skin contact.

Preparation and stability of the patient samples

Sample material: Human serum or EDTA, heparin or citrate plasma.

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Sample dilution: Patient samples are diluted **1:101** in sample buffer. For example: dilute 10 µl serum to 1.0 ml sample buffer and mix well by vortexing (sample pipettes are not suitable for mixing).

NOTE: The controls are prediluted and ready for use, do not dilute them.

Incubation

Sample incubation: (1. step)
Transfer 100 µl controls or diluted patient samples into the individual microplate wells according to the pipetting protocol. Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Wash:
Manual: Empty the wells and subsequently wash **1 time** using 300 µl of working strength wash buffer.
Automatic: Wash reagent wells **1 time** with 450 µl of working strength wash buffer (program setting: e.g. TECAN Columbus Washer “Overflow Modus”).

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer. Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

Urea incubation: (2. step)
Pipette 200 µl of urea solution into each of the microplate wells of the first microtiter strip and 200 µl of phosphate buffer into each of the microplate wells of the second microtiter strip. Incubate for **10 minutes** at room temperature (+18 °C to 25 °C).

Wash:
Empty the wells. Wash as described above, but wash **3 times** using working strength wash buffer for each wash.

Attention: Residual liquid (> 10 µl) in the reagent wells after washing can interfere with the substrate and lead to false low extinction values. Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or too short reaction times) can lead to false high extinction values.

Conjugate incubation: (3. step)
Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgG) into each of the microplate wells. Incubate for **30 minutes** at room temperature.

Wash:
Empty the wells. Wash as described above, but wash **3 times** using working strength wash buffer for each wash.

Substrate incubation: (4. step)
Pipette 100 µl of chromogen/substrate solution into each of the microplate wells. Incubate for **15 minutes** at room temperature (+18°C to 25°C) protect from direct sunlight.

Stopping the reaction:
Pipette 100 µl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:
Photometric measurement of the colour intensity should be made at a wavelength of 450 nm and a reference wavelength between 620 nm and 650 nm **within 30 minutes of adding the stop solution.** Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.

Pipetting protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	**pos HA**	**pos HA**	**P 7**	**P 7**	**P 15**	**P 15**						
B	**pos LA**	**pos LA**	**P 8**	**P 8**	**P 16**	**P 16**						
C	**P 1**	**P 1**	**P 9**	**P 9**	**P 17**	**P 17**						
D	**P 2**	**P 2**	**P 10**	**P 10**	**P 18**	**P 18**						
E	**P 3**	**P 3**	**P 11**	**P 11**								
F	**P 4**	**P 4**	**P 12**	**P 12**								
G	**P 5**	**P 5**	**P 13**	**P 13**								
H	**P 6**	**P 6**	**P 14**	**P 14**								

The above pipetting protocol is an example of the avidity determination of IgG antibodies in 18 patient samples (P 1 to P 18).

Controls (pos HA and pos LA) as well as the patient samples have been incubated in duplicate in one well each of two different microtiter strips. The reagent wells of the microtiter strips 1, 3, 5 etc. are treated with urea solution after the incubation with patients samples, the reagent wells of the microtiter strips 2, 4, 6 etc. are treated with phosphate buffer.
The wells can be broken off individually from the strips. This makes it possible to adjust the number of test substrates used to the number of samples to be examined and minimizes reagent wastage.
Both positive controls with high-avidity and low-avidity antibodies serve as internal controls for the reliability of the test procedure. They should be assayed with each test run.

Calculation of results

The presence of low avidity antibodies in a patient's serum has been proved if the ELISA extinction value is considerably reduced by urea treatment. For an objective interpretation the relative avidity index (RAI) is calculated and expressed in percent using the extinction values with and without urea treatment.

$$\frac{\textbf{Extinction of the sample with urea treatment x 100}}{\textbf{Extinction of the sample without urea treatment}} = \textbf{relative avidity index (RAI) in \%}$$

The upper limit of the range of low-avidity antibodies (**cut-off value**) recommended by EUROIMMUN is 40% RAI. Values below the indicated cut-off are to be considered as an indication of low-avidity antibodies, values between 40% and 60% RAI as equivocal, values above 60% as an indication of high-avidity antibodies. If a result is classified as equivocal, it is recommended to collect a second sample not less than 7 days later and to test it together with the first sample.

RAI < 40%:	Indication of low-avidity antibodies
RAI 40% - 60%:	Equivocal range
RAI > 60%:	Indication of high-avidity antibodies

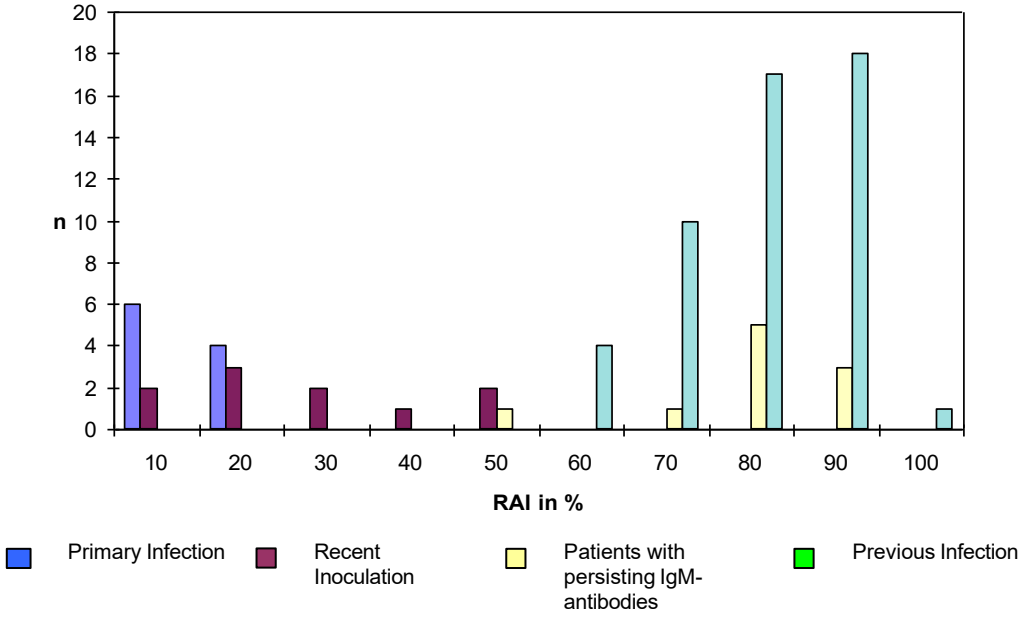
Reliable results in the the measurement of IgG antibody avidity can only be yielded if the patient sample contains a diagnostically significant concentration of specific antibodies. Generally, the determination of the relative avidity index is not helpful in samples which have an O.D. of <0.140 after incubation without urea treatment.

For diagnosis, the clinical symptoms of the patient should always be taken into account alongside the serological results.

Attention:
In some patients with an acute infection, very high titers of IgG antibodies can be found. Even though the specific IgG antibody population is in different maturation stages, both high-avidity and low-avidity, the vacant antigen epitopes are predominantly occupied by high-avidity antibodies in high titer samples. The determination of the avidity of the whole specific IgG antibody population can lead to false high RAI values in results.
False high RAI values were found in some cases of acute infections when the extinction value of the IgG measurement without urea treatment was >1.200.
It is recommended for samples with extinction values of >1.200 to repeat the avidity determination with a higher sample dilution (e. g. 1:401). If low avidity of IgG antibodies is already found at extinction values of >1.200, no further testing is necessary.

Clinical significance

1. In a study performed at EUROIMMUN 10 sera of patients with a recent infection (RAI 5% to 18%, mean 9.0%) as well as 10 blood donors with a recent inoculation (RAI 6% to 43%, mean 22.9%) showed a relative avidity index below 50%. In each of the 10 serum samples of patients with persisiting IgM antibodies (48% to 83%, mean 73.6%) as well as in 50 serum samples of patients with previous infection (55% to 95%, mean 75.4%) the RAI was significantly higher.



2. In an external study carried out by the Department of Medical Microbiology and Infectious Diseases at the University of Manitoba, Winnipeg and by the National Microbiology Laboratory at the Public Health Agency of Canada, the EUROIMMUN ELISA for the determination of the avidity of antibodies against Rubella virus was compared together with four other commercial tests with a well-established in-house reference test. A panel of n = 66 characterised serum samples was investigated. In this study, the present test showed an excellent correlation with the reference test and proved to be the best ELISA in comparison with the commercial tests of the other manufacturers.

Rubella virus is an enveloped virus of the Matonaviridae family and consists of the structural proteins C, E1 and E2 as well as two non-structural proteins (p90 and p150) [1-5]. It causes mild illness which – prior to vaccine introduction – mainly affected children [3, 6, 7]. Rubella virus only occurs in humans [1-3, 6] and is mainly transmitted via aerosols [1-4, 6].

Up to 50% of rubella infections are asymptomatic or subclinical [1, 2, 8]. The incubation time is 14 to 21 days [1, 3, 4, 7]. Symptoms of rubella include swollen lymph nodes, mainly behind the ears and in the neck, as well as spotty rash that starts on the face and spreads to the rest of the body and usually lasts for 3 days [1-4, 8]. Infectiousness begins 7 days before the onset of the rash and lasts for approximately 10 days thereafter [1-4]. Arthralgia and arthritis are the most common complications of rubella and can last for up to a month [3, 4, 7, 8].

An infection in early pregnancy can result in miscarriage, stillbirth and premature labour as well as congenital rubella syndrome (CRS), which is characterised by defects of the heart (open ductus arteriosus), eyes (cataract) and ears (sensorineural hearing loss) in newborns [2-7]. Many other embryopathies can occur [2, 6]. A rubella virus infection in the first trimester of pregnancy carries a very high risk of CRS [2-5].

Reinfection cannot be excluded, but its frequency during pregnancy is unknown. A majority is inapparent and without risk to the embryo [1, 4, 7].

Rubella virus is endemic worldwide. In unvaccinated populations 80% to 90% of children get infected. In 2018, 15,000 cases of CRS were reported to the WHO, down from about 670,000 in 2000, most of which occur in African and South-East Asian WHO countries with low vaccination rates [2, 4].

Specific IgM against rubella virus is produced from rash onset and remains detectable for at least 6 to 8 weeks [2-4, 8]. Especially after vaccination, IgM can persist for more than 6 months and it also occurs in the course of reinfections [7, 8]. Specific IgG appears some weeks after the beginning of the infection and usually persists lifelong. Most of the neutralising antibodies target E1 [4-6].

Clinical diagnosis of rubella is very unreliable and laboratory tests should be performed in suspected cases of rubella infection [2-4, 7, 8]. Pathogen detection and serology are available. An acute infection is confirmed by a positive IgM result or by a fourfold increase in the IgG titer between two serum samples taken 2 to 4 weeks apart and determined in the same analysis [1-4, 8].

A positive IgM result in pregnancy should be verified using another test method in combination with a PCR test with throat swab, urine or blood as sample material or by determination of the avidity of specific IgG or detection of antibodies against the structural rubella virus protein E2 by immunoblot. These antibodies appear 3 to 5 months after infection [1, 2, 4, 8]. Low-avidity anti-rubella virus IgG usually persists for 2 to 3 months and indicates a recent primary infection [3, 4, 8]. High-avidity antibodies indicate previous vaccination, an infection that occurred at least 3 months prior, or a reinfection [1, 3, 7].

A positive IgG result is considered sufficient evidence of immune protection [2].

Similar exanthema to the rubella rash can occur in measles, scarlet fever, erythema infectiosum (fifth disease, parvovirus B19), exanthema subitum (human herpesvirus 6) or due to medication [2-4, 8].

Literature

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Liability

The test kit, including original accessories, must only be used in accordance with the intended use. EUROIMMUN does not assume liability for any other use (e.g. non-compliance with the instructions for use and improper use) and for resulting damages.

Technical support

In case of technical problems you can obtain assistance via the EUROIMMUN website (https://www.euroimmun.de/en/contact/).

Additional information

Regulatory information for customers in the European Union: Please observe the obligation to report any serious incidents occurring in connection with this test system to the competent authorities and to EUROIMMUN.