RIDASCREEN® Chlamydia IgG/IgM

Enzyme immunoassay for the detection of
antibodies (IgG, IgM) against Chlamydia species

Art. No.: K GM 3101

In vitro Test
Lagerung bei 2 - 8 °C
Storage at 2 - 8 °C

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1. Intended use

The RIDASCREEN® Chlamydia IgG/IgM test is an in vitro diagnosticum for specific detection of IgG and IgM antibodies against Chlamydia species in human serum.

2. General

The Chlamydiae are a group of obligate intracellular parasites with viral and bacterial characteristics. The order consists of three species, Chlamydia trachomatis, Chlamydia pneumoniae and Chlamydia psittaci.
C. trachomatis has been linked to urogenital infections (e.g. urethritis, epididymitis, cervicitis, PID), trachoma, infant pneumonia, and lymphogranuloma venereum. C. pneumoniae and C. psittaci are linked to pneumonia and respiratory diseases.
Identification of Chlamydia directly in specimen material by culture, monoclonal DIFA, ELISA and nucleic acid amplification techniques are the most often used methods for the aid in diagnosis of Chlamydia infections. Serology may be helpful as additional information, providing indirect evidence of exposure, but may not correlate with infected status. Chlamydia serologies are not recommended for diagnosis of active disease except in suspected cases of LGV, psittacosis, and infants with pneumonia (IgM only).
While direct antigen tests are ideal to detect infections of the lower genital tract, they fail in the case of silent chronic infections of the upper genital tract, which represent a frequent cause of female infertility. This is, by excellence, the field of investigation for serology. Due to sampling problems, they are of interest to investigate respiratory infection to one of the three Chlamydia species.

3. Test principle

This test is an enzyme immunoassay (EIA) for detection of antibodies. On the surface of the microtiter wells, purified Chlamydia-specific LPS antigens are bound. Diluted serum samples and controls are pipetted into the wells and incubated at 37 °C in a humid chamber. Present antibodies bind to the immobilized antigen. Unbound material is removed in a washing step. In a second step, a peroxidase-conjugated anti-human-antibody (anti-IgG or -IgM) is added. After incubation, unbound conjugate is removed by washing. Substrate (H_2O_2/TMB) is added to the wells and incubated at 37 °C in a humid chamber. The enzyme bound in the wells converts the colorless substrate to a blue color. Addition of stop solution converts the color from blue to yellow. The absorption is measured at 450 nm wavelength (reference wavelength ≥ 620 nm).

4. Reagents provided

The reagents in one package are sufficient for 96 determinations.
Each test kit contains:

1 x 12 Microwell Strips (breakable) with 8 wells each in a frame;
coated with Chlamydia LPS antigen;
in a resealable foil bag
1 x Sample Diluent (30 ml), dyed blue;
phosphate buffer, ready to use
1 x Washing Buffer (50 ml);
phosphate buffer, 20x conc.
1 x Positive Control IgG (250 µl);
human serum
1 x Negative Control IgG (250 µl);
human serum
1 x Cut off Control IgG (250 µl);
human serum
1 x Anti-human-IgG-Conjugate (15 ml);
peroxidase-conjugated antibody (goat);
ready to use, dyed red
1 x Positive Control IgM (250 µl);
human serum
1 x Negative Control IgM (250 µl);
human serum
1 x Cut off Control IgM (250 µl);
human serum
1 x Anti-human-IgM-Conjugate (15 ml);
peroxidase-conjugated antibody (goat);
ready to use, dyed red
1 x Substrate (15 ml);
H_2O_2/tetramethylbenzidine (TMB);
ready to use
1 x Stop Solution (15 ml);
1 N sulfuric acid
1 x Instructions for use

5. Reagents required but not provided

5.1. Reagents

- Distilled or deionized water

5.2. Accessories

- Vortex mixer
- Micropipets for 5 µl, 10 µl, 100 µl and 200 µl volumes
- Microplate washer or multichannel pipet
- Microplate reader (450 nm, reference wavelength ≥ 620 nm)
- Absorbent paper
- Measuring cylinder (1000 ml)
- Moist chamber at 37 °C
- Test tubes

6. Warnings and precautions for the users

The control sera (positive, negative and cut off control) have been tested for HIV- and HCV-Ab as well as for HBsAg and were found to be negative. However, they as well as the patient samples should be considered potentially contagious and be treated with the necessary safety precautions.

Hydrogen peroxide can cause cauterization. Handle with care!

The stop solution contains 1 N sulfuric acid. Avoid contact with skin and clothing!

All reagents and materials coming in contact with potential infectious specimens must be treated with disinfectants or autoclaved at 121 °C for at least one hour.

An exchange of reagents between kits of different lot numbers is not possible.

Microwell strips and reagents must not be used if pouch is damaged or vials are leaking.

7. Storage instructions

All reagents have to be stored at 2 - 8 °C and can be used up to the expiry date printed on the labels. Microbial contamination has to be avoided. A quality warranty cannot be given beyond the kit expiration date.
Allow reagents and microwell strips to get room temperature before use. To avoid moisture within the strips, do not take the strips out of the foil bag before having reached room temperature. The foil bag should be opened with a pair of scissors without detaching the fastener. Return any unused strips to the foil bag, reseal

and store them directly at 2 - 8 °C. If stored properly after first opening, the microwell strips are stable up to three months.
If stored at 2 - 8 °C, the diluted washing buffer can be used up to 4 months.
It is absolutely necessary to avoid contamination of the substrate with the conjugate solution, since this results in a coloration of the substrate. In the same way, the colorless substrate must be protected from exposure to direct light to avoid deterioration or coloration by autooxidation. If the substrate has turned blue, the reagent should be discarded.

8. Indication of instability or deterioration of reagents

If measured at 450/620 nm, the following criteria may indicate a reagent deterioration:

- an O.D. value of the positive control < 0.9
- an O.D. value of the negative control > 0.55
- an O.D. value of the cut off control > 0.7 x O.D. of the positive control

9. Specimen collection and storage

The RIDASCREEN® Chlamydia IgG/IgM EIA has been evaluated for the investigation of human serum samples. Repeated freezing and thawing of the samples as well as microbial contamination must be avoided. Heat treated samples as well as samples containing sodium azide cannot be used in the test. The application of lipemic, hemolytic, icteric or turbid samples can lead to wrong results.
The sample material can be stored for up to 1 week at 2 - 8 °C if the test cannot be carried out immediately. A prolonged storage of samples is possible at -20 °C.

10. Test procedure

10.1. Preliminary comments

The test has to be used only by experienced laboratory personnel. Please refer to guidelines for safety regulations in medical laboratories. The test protocol must be followed strictly.
Bring all reagents and the microwell strips to room temperature before use. Mix the reagents well before use. Reproducibility in any EIA depends on exact pipetting, the observance of incubation times and temperature and the consistency of wash sequences. During the washing steps, take care that all wells are filled with buffer and that the liquid is completely removed from the wells. Do not allow microwells to dry between steps.

Avoid direct sunlight during all incubations. Covering the microtiter plate is recommended.

10.2. Preparation of the washing buffer

Dilute 1 part of the concentrated washing buffer with 19 parts of distilled water. Add 50 ml of the concentrated washing buffer to a 1000 ml graduated cylinder. Bring the final volume to 1000 ml with distilled or deionized water. Crystals in the buffer concentrate can be dissolved in a waterbath at 37 °C. If stored at 2 - 8 °C, the diluted washing buffer can be used up to 4 months.

10.3. Preparation of the samples

Before starting the test, serum samples and the controls have to be diluted in test tubes 1:21 with the sample diluent.

Dilution of serum samples and controls in test tubes:

e.g. 200 µl sample diluent + 10 µl serum or control

Alternatively, samples and controls can be directly diluted in the microtiter plate. In this case, 100 µl of sample diluent are pipetted into each well and 5 µl of sample or control are added. For mixing the plate should be shortly shaken.

Dilution of serum samples and controls in the microtiter plate:

100 µl sample diluent + 5 µl serum or control

For IgM determination, sera should be treated with an IgG-absorbent (e.g. RIDA® G-Adsorbens, Art. No. Z 0101 / Z 0102) prior to the test, following that it should be brought to its final dilution with the sample diluent. The controls must not be absorbed.

10.4. First incubation

After insertion of a sufficient number of cavities into the microwell holder, 100 µl each of the diluted sera and controls are added to the corresponding wells. The cut off control should be done in double determination.
The controls that correspond to the determinations (IgG or IgM) are to be used.

A1	negative control
B1	cut off control
C1	cut off control
D1	positive control
E1, F1	patient serum 1, 2, etc.

The plate is covered and incubated in a humid chamber at 37 °C for 45 min. The bottom of the cavities should not be in touch with materials that conduct temperature well (metal or wet paper).

Attention!
The ELISA plate should not be placed in a cold incubation container which is heated to 37 °C during the incubation. The container must already be adapted to 37 °C prior to the incubation.

10.5. Washing

Decant or aspirate all microwells into a waste container with a disinfectant. Ensure complete removal of the liquid from the microwells by tapping the inverted plate onto absorbent paper. Then wash all wells 5 times with 300 µl of prepared washing buffer. Be sure to remove residual washing solution by firmly tapping the inverted microwells on absorbent paper after single washing steps.

If a microplate washer is used, take care that the washer is adjusted to the used microplate type.

10.6. Second incubation

Put 100 µl of the anti-human-IgG- or anti-human-IgM-conjugate into the corresponding wells. Cover the plate and incubate for 30 min at 37 °C in a humid chamber.

10.7. Washing

Wash 5 times according to step 10.5.

10.8. Third incubation

Add 100 µl of substrate to each well. Cover the plate and incubate for 20 min at room temperature. Following incubation, reaction is stopped by adding 50 µl of the stop solution to each well. After careful mixing (soft tapping on the edge of the plate) the absorbance is measured in a microplate reader at 450 nm (reference wavelength $\geq$ 620 nm). Zero adjustment is done against air (without plate). Measurement should be done within one hour after addition of stop solution.

Remark:
To remove moisture, wipe the bottom of the microplate before measuring.

Summary of the test procedure

1. Bring all reagents to room temperature
2. Dilute the washing buffer (1:20)
3. Dilute the serum samples and controls (1:21)
4. Pipet 100 µl of the negative control, the cut off control, the positive control or the diluted samples into the microwells; 45 minutes incubation at 37 °C in a humid chamber
5. Discard the incubate and wash 5 times with 300 µl of washing buffer
6. Add 100 µl of conjugate; 30 minutes incubation at 37 °C in a humid chamber
7. Discard the incubate and wash 5 times with 300 µl of washing buffer
8. Add 100 µl of substrate; 20 minutes incubation at room temperature
9. Addition of 50 µl stop solution; after short mixing spectrophotometric determination at 450 nm (reference wavelength ≥ 620 nm)

11. Analysis

11.1. Quality control

For the quality control, positive control, negative control and cut off control must be carried along with each test procedure. The test was carried out correctly, if the absorbance value of the negative control is lower than 0.55 and the absorbance value of the positive control is higher than 0.9. The cut off control should be done in double determination. The single values must not differ more than 20 % from the mean absorbance value. The mean absorbance value of the cut off control must be lower than 70 % of the value of the positive control (< 0.7 x O.D. of positive control).

If the expected control values are not fulfilled, please check the following before repeating the test:

- expiration date of the reagents
- correct function of the used instruments (calibration etc.)
- exact test procedure
- visual examination of kit components for signs of contamination, deterioration or leakage; substrate solution must not be used if turned blue

If the control data are not fulfilled after repeating, please contact your local distributor of R-Biopharm.

11.2. Calculation of the sample index

1. Calculate the mean O.D. of the cut off control.
2. Calculate the sample index by dividing the sample O.D. through the value obtained in point 1).

e.g.: cut off control 1 O.D. = 0.821
cut off control 2 O.D. = 0.865
sample O.D. = 1.508

$$\text{sample index} = \frac{1.508}{0.843} = 1.79$$

11.3. Test result

Tab. 1: Valuation of sample index

	negative	equivocal	positive
sample index	< 0.9	0.9 – 1.1	> 1.1

12. Remarks about the test procedure and interpretation

The RIDASCREEN® Chlamydia IgG/IgM EIA detects antibodies against Chlamydia species. It should be carried out if a well-founded suspicion of a Chlamydia infection exists. A correlation between measured OD values and the existence or severity of clinical findings is not given. The results obtained should always be interpreted in connection with the clinical picture.

Negative antibody findings cannot exclude an infection. Due to a low antibody titer at the beginning of an infection, the test can show negative results. If a clinical suspicion subsists, after two weeks another patient sample should be tested.

A positive result does not exclude the presence of other infectious agents.

The RIDASCREEN® Chlamydia IgG/IgM EIA uses Chlamydia LPS antigen that cannot differentiate between the different species of Chlamydia. Patients may be infected or exposed and have a serological response to one or more species of Chlamydia. Due to the fact that chlamydial infections are common and antibody may persist after infection, chlamydial antibody is often detected in healthy individuals.

Appendix

Literature

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