RIDASCREEN® Chlamydia IgG/IgM

REF KGM3101

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CE 0123

1. Intended use

For *in vitro* diagnostic use. This RIDASCREEN® Chlamydia IgG/IgM test is an enzyme immunoassay for the determination of IgG or IgM antibodies against all Chlamydia (C.) species in human serum.
The tests should be used for confirmation purposes when there is a suspected case of infection with Chlamydia or for clarifying the immune status.

2. Summary and explanation of the test

In the group of the Chlamydia, there are three human pathogens: Chlamydia trachomatis, Chlamydophila psittaci and Chlamydophila pneumoniae.
Chlamydia trachomatis is the most frequently sexually transferred bacterial pathogen and is often the cause of a urethritis, cervicitis or salpingitis. The newborn children of infected mothers can develop conjunktivitis or pneumonia. Certain serovars give rise to venereal lymphogranuloma. Trachomatous conjunctivitis transferred by smear infection is caused by other serovars.
Until a few years ago, it was generally thought that Chlamydia pneumoniae was a subspecies of Chlamydia psittaci. The existence of this third Chlamydia species was established in 1989. More recent investigations led to the formation of the seperate genus Chlamydophila. Depending on factors specific to the host, Chlamydophila pneumoniae can give rise acute diseases of the upper respiratory system, bronchi and lung. Research results suggest that Chlamydophila pneumoniae plays a role in asthma, lung diseases and other chronic disorders. Chlamydophila psittaci is pathogen which is very common among birds and mammals. With humans, it occurs only rarely and usually causes respiratory disorders.
A tissue test (swab) or a serum antibody determination is necessary for the diagnosis of a chlamydia infection. Antibody determination is preferable where enrichment of the antigen is difficult or where the antigens only exist in undetectable quantities. By using chlamydia-specific LPS antigens (LPS = lipopolysaccharide), antibodies against all three species are detected simultaneously.

3. Test principle

A coating of purified LPS is applied to a microwell plate. Antibodies in the patient samples bind to the antigens and are determined during the second incubation step by using enzyme-labelled anti-human antibodies (the conjugate). The enzyme converts the colourless substrate (H_2O_2/TMB) to a blue end product. The enzyme reaction is stopped by adding sulphuric acid and the colour of the mixture switches from blue to yellow at the same time. The final measurement is carried out at 450 nm on a photometer using a reference wavelength 620 nm.

4. Reagents provided

Tab. 1: Reagents provided (The reagents in a kit are sufficient for 96 determinations)

			KGM3101 IgG/IgM
Plate	96 det..	Microwell plate; 12 microwell strips (can be divided) in strip holder; coated with Chlamydia LPS antigen	X
Diluent	25 ml	Sample buffer, ready for use; blue coloured phosphate-buffered; contains protein stabilisors, Neolone and Bronidox	X
Wash	50 ml	Wash buffer, (20 x concentrate); phosphate buffer, contains tween 20 and proclin	X
Control IgG | +	500 µl	Positive control IgG, human serum; contains Neolone and Bronidox	X
Control IgM | +	500 µl	Positive control IgM, human serum; contains Neolone and Bronidox	X
Control IgG | –	500 µl	Negative control IgG, human serum; contains Neolone and Bronidox	X
Control IgM | –	500 µl	Negative control IgM, human serum; contains Neolone and Bronidox	X
Cut Off IgG	500 µl	Cut-off control IgG, human serum; contains Neolone and Bronidox	X
Cut Off IgM	500 µl	Cut-off control IgM, human serum; contains Neolone and Bronidox	X
Conjugate IgG	15 ml	Anti-human IgG conjugate, ready for use; peroxidase conjugated antibodies in orange-coloured buffer solution, contains Neolone and Bronidox	X
Conjugate IgM	15 ml	Anti-human IgM conjugate, ready for use; peroxidase conjugated antibodies in orange-coloured buffer solution, contains Neolone and Bronidox	X
Substrate	15 ml	Substrate; H_2O_2/tetramethylbenzidine; ready for use	X
Stop	15 ml	Stop reagent 0.5 M sulphuric acid; ready for use	X

5. Storage instructions

- The test kit must be stored at 2 - 8 °C and can be used until the expiry date printed on the label.
- The diluted wash buffer can be used for a maximum of 4 weeks when stored at 2 - 8 °C.
- For all other reagents, the expiry date indicated on the packaging applies for storage at 2 -8 °C

- After the expiry date, the quality guarantee is no longer valid.
- The aluminium bag containing the microwell plate must be opened in such a way that the clip seal is not torn off. Any microwell strips which are not required must be returned to the aluminium bag immediately and stored between 2 and 8 °C.
- The reagents also must not be allowed to become contaminated and the colourless substrate must be protected from exposure to direct light.

6. Reagents required but not provided

6.1. Reagents

- distilled or deionised water
- Vircell ELISA SORBENT (Ref. Vircell S001) (see Section 9.3.)

6.2. Accessories

- Incubator or moist chamber at 37 °C
- Test tubes
- Vortex mixer
- Micropipettes for 10 - 100 µl and 100 - 1000 µl capacities
- Measuring cylinder (1000 ml)
- Stop clock
- Microplate washer or multichannel pipette
- Microplate reader (450 nm, reference wavelength 620 nm)
- Filter paper (laboratory towels)
- Waste container containing 0.5 % sodium hypochlorite solution

7. Warnings and precautions for the users

For *in vitro* diagnostic use only.

This test must only be carried out by trained laboratory personnel. The guidelines for working in medical laboratories must be followed and the instructions for carrying out the test must be strictly adhered to.

Samples or reagents must not be pipetted by mouth and contact with injured skin or mucous membranes must be prevented. When handling the samples, wear disposable gloves and when the test is finished, wash your hands. Do not smoke, eat or drink in areas where samples or test reagents are being used.

For further details, see safety data sheets (SDS) at www.r-biopharm.com.

The control sera (positive control, negative control and cut-off control) in the kit have been tested for HIV- and HCV-Ab as well as HbsAg with negative results. Nevertheless, they must be treated as potentially infectious in the same way as the patient samples and all other materials with which they come into contact and they must be handled in accordance with the relevant national safety regulations.

Hydrogen peroxide (substrate) can cause burns. Handle with care. If the skin is contaminated with the stop reagent, rinse it off with water.

The stop reagent contains 0.5 M sulphuric acid. Avoid contact with the skin and clothing. If the skin is contaminated with the stop reagent, rinse it off with water.

All reagents and materials coming into contact with potentially infectious samples must be treated with suitable disinfectants or autoclaved at 121 °C for at least 1 hour.

Note: To prevent the formation of poisonous gases, any liquid waste containing stop reagent must be neutralized before adding it to hypochlorite solution.

8. Collection and storage of specimens

The test has been developed for testing human serum samples. After blood collection, the blood should be separated from blood clots as soon as possible in order to prevent haemolysis. The samples must be stored cold or frozen until they are tested.
Repeated freezing and thawing of the samples and microbial contamination must be prevented at all costs. Using heat-inactivated, lipaemic, haemolytic, icteric or turbid samples can lead to false results.

Tab. 2: Specimen storage

undiluted Serum oder Plasma	
2 - 8 °C	-20 °C
1 week	> 1 week

9. Test procedure

9.1 General information

All reagents and the microwell plate must be brought to room temperature (20 - 25 °C) before use. The microwell strips must not be removed from the aluminium bag until they have reached room temperature. The reagents must be thoroughly mixed immediately before use. After use, the kit must be immediately returned to storage between 2 and 8 °C.
Take only the volume of reagents that is needed for test procedure. Do not return reagents back into vials as this may lead to contamination.
The microwell strips cannot be used more than once. The reagents and microwell strips must not be used if the packaging is damaged or the vials are leaking.
The sample buffer, wash buffer, substrate and stop reagent are not test specific; they can also be used for other RIDASCREEN® ELISA for determining antibodies against Chlamydia.

9.2 Preparing the wash buffer

1 part wash buffer concentrate Wash is mixed with 19 parts distilled water. In order to do this, place 50 ml of the concentrate in a 1000 ml measuring cylinder and make up

the solution to 1000 ml with distilled water. Any crystals present in the concentrate must be dissolved beforehand by warming in a water bath at 37 °C. The diluted wash buffer can be used for a maximum of 4 weeks when stored at 2 - 8 °C.

9.3 Specimen preparation

Dilute the serum samples and the controls with sample buffer Diluent 1:21 before starting the test. Carry out the dilution directly on the microwell plate. The cut-off control must be determined in duplicate. The controls which correspond to the determination (IgG or IgM) must be used.

A1 Negative control
B1 Cut off control
C1 Cut off control
D1 Positive control
E1, F1 Patient sample 1, 2, usw.

After placing a sufficient number of cavities for controls and the samples in the strip holder, pipette 100 µl sample buffer into each of the microwells in the plate for the IgG determination and then add 5 µl sample or control. The plate should be vibrated briefly for mixing:

IgG:
sample and controls -> 100 µl Diluent + 5 µl sample or control

For the IgM determinations, the samples in the plate should be subjected to IgG absorption (e.g with the Vircell ELISA SORBENT (Ref. Vircell S001)) and only then adjusted with the sample buffer to the dilution required in the test. When using the Vircell ELISA SORBENT, proceed as follows:

IgM:
controls -> 100 µl Diluent + 5 µl control
sample -> 25 µl Vircell ELISA SORBENT + 5 µl sample + 75 µl Diluent

Note: Controls must not be absorbed.

9.4 First incubation

Incubate the plate for 45 minutes at 37 °C in an incubator or moist chamber. During this process, the bottoms of the wells must not be in contact with heat-conductive materials (such as metals or moist paper). The microwell plate must be covered during incubation.

Note: The microwell plate must not be placed in a cold incubation container reaching 37 °C during incubation. The temperature of the container must be adjusted to 37 °C beforehand.

9.5 Washing

The wells must be emptied into a waste container containing hypochlorite solution for disinfection. Then knock out the plate onto absorbent paper in order to remove the residual moisture. After this, wash the plate 5 times using 300 µl diluted wash buffer each time. Make sure that the wells are emptied completely by knocking them out on an unused part of the absorbent paper after each wash.

When using a microplate washer, make sure that the machine is correctly adjusted to the type of plate being used. After washing, knock out the plate onto clean absorbent paper in order to remove any residual moisture.

9.6 Second incubation

Add 100 µl of the conjugate Conjugate IgG or Conjugate IgM to each of the corresponding wells. Next, cover the plate and incubate it at 37 °C for 30 minutes (see Section 9.4).

9.7 Washing

Wash 5 times in accordance with Section 9.5.

9.8 Third incubation

Add 100 µl substrate Substrate to each well. After this, cover the plate and incubate at room temperature for 20 minutes, protected from light. Then, stop the reaction by adding 50 µl stop reagent Stop to each well. After mixing carefully (by lightly tapping the side of the plate), measure the absorbance at 450 nm (reference wavelength 620 nm) in a plate photometer. Calibrate the zero against air. The measurement must be carried out within one hour after adding the stop reagent.

Note: When using a moist chamber, the underside of the microwell plate must be wiped off to remove condensation water before the measurement is carried out.

10. Quality control – indications of instability or expiration of reagents

For quality control purposes, positive, negative and cut-off controls must be used each time the test is carried out. The cut-off control is determined in duplicate and the average value calculated from the two measurements. The test has been carried out correctly when the extinction values (O.D.) satisfy the following criteria:

Tab. 3: Quality control criteria

	OD
Negative control	< 0.5
Cut off control	> 0.55 < 1.5
Positive control	> 0.9

If the values differ from those required, if the reagent is turbid or the substrate has turned blue before adding to the wells, it may indicate that the reagents have expired. If the stipulated values are not met, the following points must be checked before repeating the test:

- Expiry date of the reagents used
- Functionality of the equipment being used (e.g. calibration)
- Correct test procedure
- Visual inspection of the kit components for contamination or leaks – a substrate solution which has turned blue must not be used.

If the conditions are still not fulfilled after repeating the test, please contact your local R-Biopharm distributor.

11. Evaluation and interpretation

11.1 Calculation of the specimen index

1. The average absorbance is calculated for the cut-off control.
2. The sample index is obtained by dividing the absorbance for the sample by the calculated average value.

   For example: Cut-off control 1 OD = 0.821
   Cut-off control 2 OD = 0.865
   Average value = 0.843
   Specimens OD = 1.508

$$\text{Specimen index} = \frac{1.508}{0.843} = 1.789$$

11.2 Interpretation of results

Tab. 4: Analysis of specimen index

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

12. Limitations of the method

RIDASCREEN® Chlamydia IgG/IgM ELISA detects IgG or IgM antibodies against all three human pathogenic Chlamydia species. The test cannot be used to derive a relationship between the determined extinction and occurrence of serious clinical symptoms. The test is not suitable for locating the site of infection. The results obtained must always be interpreted in combination with the clinical picture and other diagnostic methods (such as pathogen isolation).
A negative result does not rule out an infection, since the serum can be sampled too early for the antibodies to be detectable. In this case, where an infection is suspected for clinical reasons the test should be repeated on a second sample of the serum collected two to three weeks later.
Two consecutive samples of sera should always be collected from a patient and subjected to serological testing in parallel in order to improve the quality of the diagnosis. The progress of the titre is important for interpreting the findings.
Positive IgG findings with newborn children must be interpreted carefully since they can be caused by maternal antibodies. The detection of IgM antibodies is more helpful for children younger than six months.
IgG titres which are very high may lead to false negative results during IgM determinations. In addition to this, IgM determinations can lead to false positive results due to rheumatoid factors. This can be prevented by absorption of the serum before carrying out the test (see Section 9.3.).
Cross-reactions with anti-bodies against Chlamydophila psittaci have not been examined because of the low prevalence of this disease and lack of positive samples when using the available test.
The immune response for eyes infected with C. trachomatis is small.
The test has not been validated for its suitability for therapy control. The meaningfulness of the test for chronic diseases in children, patients with venereal lymphogranuloma (LGV) and chronic inflammations of the pelvis has not been investigated.
A positive result does not rule out the presence of another infectious pathogen as the cause of the disease.

13. Performance characteristics

Tab. 5: Inter-assay variance (n = 10)

Inter-assay variance	IgG	IgM
	CV	CV
Positive control	3,22 %	2,62 %
Negative control	10,99 %	9,24 %
Cut off control	5,00 %	7,36 %

Tab. 6: Intra-assay variance (n = 10)

Intra-assay variance	IgG	IgM
	CV	CV
Positive control	1,83 %	2,16 %
Negative control	6,98 %	8,00 %
Cut off control	6,55 %	7,79 %

Tab. 7: Sensitivity and specificity in comparison to the microimmuno-fluorescence test (MIF)

	IgG	IgM
Number of samples	101	62
Sensitivity	96 %	92 %
Specificity	98 %	100 %

The IgG test was also carried out on 11 samples from patients who were infected with *Rickettsia conorii* or other pathogens which give rise to similar clinical pictures to that of Chlamydia (HSV-2, *L. pneumophila, C. burnetii* and *M. pneumoniae*). Seven such samples were examined in the IgM test as well as two rheumatoid factor positive sera. No cross reactions or effects on the ELISA results were found for the sera examined.

14. Version history

Version number	Section and name
2015-11-01	Release revision
2018-08-15	General revision
2018-09-20	Adaptation CE symbol (Identification no. Notified Body)
2019-12-16	Revision 6.1. Reagents Revision 9.3.Specimen preparation

15. Explanation of symbols

General symbols

IVD	In-vitro diagnostics
	Observe the instruction for use
LOT	Lot number
	Use before
	Storage temperature
REF	Article number
Σ	Number of tests
	Date of manufacture
	Manufacturer

Test-specific symbols

Plate	Microtiter plate
Diluent	Sample dilution buffer
Wash	Wash buffer 20x
Control IgG | +	Positive control IgG
Control IgM | +	Positive control IgM
Control IgG | –	Negative control IgG
Control IgM | –	Negative control IgM
Cut Off IgG	Cut off control IgG
Cut Off IgM	Cut off control IgM
Conjugate IgG	Conjugate IgG
Conjugate IgM	Conjugate IgM
Substrate	Substrate
Stop	Stop solution

16. References

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