RIDA®FLUOR Legionella IgG (3 Pools)

Art. No.: I8521
I8525
I8521C00
I8521C01

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1. Intended use

For *in vitro* diagnostic use. The RIDA® FLUOR Legionella IgG test is an indirect immunofluorescence assay (IFA) for the diagnosis of antibodies against Legionella pneumophila serogroups 1 - 14 and non-pneumophila species of Legionella in human serum.

2. General

Legionnaires' Disease, named after the outbreak in 1976 at the American Legion convention in Philadelphia, is caused by Legionella pneumophila and is characterized as an acute febrile respiratory illness ranging in severity from mild illness to fatal pneumonia. Known risk factors include immunosuppression, cigarette smoking, alcohol consumption and concomitant pulmonary disease. The young and the elderly are particularly susceptible.
Legionella are rod-like bacteria with flagella. Legionella pneumophila is responsible for 80 - 90 % of reported cases of Legionella infection with serogroup 1 accounting for more than 70 % of all legionellosis.

3. Test principle

Inactivated Legionella pneumophila and non - pneumophila species are fixed as different pools (pool I - III) in defined wells on the surface of test slides. Diluted serum samples are given to the wells. Present anti-Legionella-antibodies bind to cell surfaces and form antigen-antibody-complexes. After a washing step for removing unbound antibodies, the complexes become visible by adding FITC-conjugated anti-human IgG-immunoglobuline. After a further washing step for removing excess conjugate, the test can be analyzed by a fluorescence microscope with 400x magnification.

4. Reagents provided

Tab. 1: Contents of the kit

Slide	10 pieces	slides with 30 wells each; sealed separately; for coating see Tab. 2 The bacteria are suspended in 0.5% normal chicken yolk sac
Buffer	1 vial	Washing/Dilution Buffer (PBS salt, pH 7.2); for dilution in 1 L Aqua dest.
Control IgG | +	0.2 ml	Positive Control IgG; positive test serum, ready to use; contains NaN_3
Control IgG | –	0.2 ml	Negative Control IgG; negative test serum, ready to use; contains NaN_3
Conjugate IgG	2 x 1.1 ml	Anti-human IgG-FITC-Conjugate; FITC-conjugated antibody; ready to use, with Evans Blue for counterstaining; contains phosphate buffer, NaN_3 and a protein stabilizer
MF	3.0 ml	Mounting Fluid; contains buffered glycerol and NaN_3, ready to use

Additionally to the test kit RIDA®FLUOR Legionella IgG (3 Pools) (Art.No I8521) also the accessories testkit RIDA®FLUOR Legionella IgG Slides (3 Pools) (Art.No. I8525) is available. This accessories kit contains solely 10 slides with coating as described on table 2. The negative control (Art.No. I8521C00) and the positive control (Art.No. I8521C01) are also separately available.

Tab. 2: Coating of the slides

Coating
I8521/I8525 (3 Pools) upper row: L. p. Serogroup 1 - 6 (Pool I) middle row: L. p. Serogroup 7 - 14 (Pool II) lower row: L. boz (SG 1+2)-dum-gor-jord-longb(SG1+2)-mic (Pool III)

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 reconstituded wash buffer can be used for a maximum of 4 month when stored at 2 – 8 °C. After the expiry date, the quality guarantee is no longer valid.
Contamination must be avoided.

6. Materials required but not provided

6.1. Reagents

- Distilled or deionized water

6.2. Accessories

- Sample tubes
- Micropipettes for volumes of 10 - 100 µl and 100 - 1000 µl
- Moist chamber
- Washing trays for slides
- Coverslips (24 x 60 mm)
- Fluorescence microscope

7. Precautions for 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.

The control sera (positive control and negative 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.

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

Slides and reagents must not be used if their pouch is damaged or the vials are leaking.

Do not mix components from different lots or manufacturers.

Conjugate, mounting fluid and controls contain sodium azide (concentration <0.1%). Avoid contact with acids and heavy metals.

Mounting fluid contains glycerol. Avoid contact with acids and keep away from high temperature.

Evan´s blue (concentration <0.1%) is a carcinogen. Avoid contact with skin or eyes. In case of contact with this solution rinse thoroughly with water and seek medical attention.

The glass elements contained in this kit could cause physical damage in case of break. Handle with care.

8. Specimen collection and storage

The RIDA®FLUOR Legionella tests have been developed for the investigation of 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. 3: Sample storage

Undiluted serum		Diluted serum
2 – 8 °C	–20 °C	2 – 8 °C
1 week	> 1 week	7 hours

9. Test procedure

9.1. General information

Bring all reagents and the test slides to room temperature before use. Take the slides out of the aluminum foil after they have reached room temperature and mark them on the mask.
The reagents must be thoroughly mixed immediately before use. After use, the kit must be immediately returned to storage between 2 – 8 °C.
Take only the volume of reagents that is needed for test procedure. Do not pour reagents back into vials as reagent contamination may occur. Do not pour reagents back into vials as this may lead to reagent contamination.
Each slide can be used only once. Do not break it and do not reuse the unused wells.

9.2. Preparation of the washing / dilution buffer

Add the contents of the vial Buffer to 1 litre of distilled water and mix it until the complete dissolution. The diluted wash buffer can be used for a maximum of 4 weeks when stored at 2 – 8 °C or for 5 days when stored at room temperature (20 – 25 °C).

9.3. Preparation of the samples

Before starting the test, serum samples have to be diluted 1:64 and 1:128 with the washing/ dilution buffer.
e.g. 10 µl serum + 630 µl buffer for 1:64 - dilution.

When indicated high positive sera should be further titrated with the buffer.

Attention!
Positive and negative control are ready to use and must be used without dilution.

9.4. First incubation

The slides Slide are divided into single wells. Each row with ten wells is coated with another pool (I - III), which may show different compositions (see Table 2). Per patient 5 µl of the diluted serum samples (1:64 and 1:128) are pipetted to one well of each pool (see Fig 1). Hydrophobe slide coating between the wells protects mixing of the samples.

Put also 5 µl each of the positive control Control IgG | + and the negative control Control IgG | - to one well of each pool (see Fig 1).

Incubate the slides in a moist chamber at 37 °C for 30 minutes.

Fig. 1: Coat of slides and recommended placement of controls and patient samples

	1	2	3	4	5	6	7	8	9	10
	control	control	sample 1		sample 2		sample 3		sample 4	
	negative	positive	1:64	1:128	1:64	1:128	1:64	1:128	1:64	1:128
I	neg. ○	pos. ○	○	○	○	○	○	○	○	○
II	neg. ○	pos. ○	○	○	○	○	○	○	○	○
III	neg. ○	pos. ○	○	○	○	○	○	○	○	○

Pool I: Serogroup 1-6
Pool II: Serogroup 7-14
Pool III: L.b-d-g-j-l-m

9.5. Washing

Rinse the slide shortly with the washing buffer. Then put slide for 10 minutes into a container with fresh washing buffer. Rinse again slightly with distilled water and remove all liquid by tapping the slide vertically on an absorbent paper.
The fields must be dry for the following steps.

9.6. Second incubation

Add 5 µl of the anti-human IgG-FITC-conjugate Conjugate IgG to each well. Incubate the slides for 30 min at 37 °C in a moist chamber.

9.7. Washing

Wash according to step 9.5.

9.8. Microscopy

Add a small drop of mounting fluid MF to each well and carefully cover with a coverslip avoiding air bubbles. Remove access of mounting fluid by slightly pressing the coverslips on the slides. Read the result in a fluorescence microscope at 400x magnification.
In case of positive findings in both screening-dilutions further sera dilutions are recommended up to 1:2048 for determination the end titer.

10. Quality control – indications of instability or deterioration

For the quality control, positive control and negative control must be carried along with each test run. The test was carried out correctly if the positive control shows specific fluorescence and the negative control shows no reaction.

The specific fluorescence is a bright apple green coloration visible on the cell surfaces of the rod-shaped Legionella in a homogenous distribution within the well.

If the positive control does not show a reaction, which is manifested in just a weak fluorescence of the cell surface and a non-homogenous distribution of the fluorescence within the well, it may indicate that the reagents have expired.

If the conditions are not fulfilled, please check the following before repeating the test:

- kit expiry
- functional capability of the instruments used (pipettes, microscope)
- correct test execution
- visual examination of kit components for contamination, deterioration or leakage

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

11. Evaluation and interpretation

Specific fluorescence is decisive for positive evaluation of a sample. The specific fluorescence is a bright apple green coloration visible on the cell surfaces of the rod-shaped Legionella in a homogenous distribution within the well. Single or in some cases several serogroups can show fluorescence. Intensity of the fluorescence can vary from weak to very strong.

If no specific fluorescence is visible, the test is valuated negative.

12. Limitations of the method

The RIDA®FLUOR Legionella tests detect specific antibodies against Legionella. They should be used in reasonable suspected cases of Legionella infections. A relation between the fluorescence intensity and the clinical relevance is not given. The results obtained should always be interpreted in connection with the clinical picture.
This test will not indicate the site of infection. It is not intended to replace isolation.
Samples collected very early in the course of an infection may not have detectable levels of IgG. In such cases, it is recommended to perform an IgM assay or to obtain a second serum sample after 14 or 21 days later which could be tested in parallel with the original sample to determine seroconversion.
Results in IgG detection in neonates must be interpreted with caution since maternal IgG is transferred passively from the mother to the foetus before birth. Generally IgM assays are more useful indicators of infection in children below 6 months of age.
Some sera may contain antibodies reacting with egg antigens that give unspecific fluorescence with yolk sac, used to fix the antigen to the slide and to avoid bacterial aggregation. When this occurs, the sera should not be analyzed by IFA.

Serum samples with titers of 1:<128 are considered negative.

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

A titer of 1:128 is classified as equivocal. It can be a residual titer of a former infection or can be the beginning of antibody formation in case of a fresh infection. Therefore, a second serum sample should be investigated after two weeks.

Titers of 1:≥256 are considered **positive**. For the proof of an acute infection, titer development should be investigated with two successively taken serum samples. A four fold titer increase indicates an acute infection. To improve the diagnostic statement, two consecutive sera of a patient should be generally examined.

A positive result does not exclude the presence of other pathogens as cause for an illness.

13. Performance characteristics

Inter-assay variation (n = 3)

3 sera (2 positive and 1 negative) were individually pipetted on 5 different conditions in which the operator or the test day were different. Titer shifted of no more than one dilution.

Intra-assay variation (n = 3)

3 sera (2 positive and 1 negative) were individually pipette in groups of 5 in a single assay performed by the same operator in essentially unchanged conditions. Titer shifted of no more than one dilution.

Sensitivity and specificity in comparison to another IFA assay

n = 41	sensitivity	specificity
IgG	87.5%	93.9%

Sera with non-specific reactivity were excluded from final calculations.

Cross reactivity and interferences

12 samples known to be positive for other bacteria of the syndromic group (Mycoplasma pneumoniae, Chlamydophila pneumoniae, Coxiella burnetti) and antinuclear antibodies were tested. The negative results of the test demonstrate the specific reaction of the kit with no cross reaction or interferences with the referred specimens.

Literature

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