RIDASCREEN® Leishmania IgG

Art. No. K7321

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1. Intended use

For *in vitro* diagnostic use. The RIDASCREEN® Leishmania IgG Test is an enzyme immunoassay for the qualitative determination of specific IgG antibodies against *Leishmania infantum* in human serum.

The test should be used for confirmation purposes when there is a suspected case of Leishmaniosis.

2. Summary and explanation of the test

After infection with Leishmania, specific antibodies are formed against the pathogen because of the response from the immune system. By using immunological methods, it is possible to determine the antibodies in the serum. The test method used and the choice of the pathogen-specific antigen both have a significant bearing on the meaningfulness of the test.

3. Test principle

Purified recombined antigen of *Leishmania infantum* is coated to a microwell plate. Antibodies in the patient samples bind to the antigen and are determined during the second incubation step by using enzyme-labelled anti-human antibodies (the conjugate). The enzyme converts the colourless substrate (urea peroxide/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 during 20 minutes after addition of stop solution.

4. Reagents provided

Tab. 1: Pack contents (there are enough reagents in a pack for 96 determinations)

			K7321 IgG
Plate	96 det.	Microwell plate, 12 microwell strips (can be divided) in strip holder; coated with recombinant antigens from *Leishmania infantum*	X
Diluent	100 ml	Sample buffer, phosphate-buffered NaCl solution, ready for use	X
SeroWP	100 ml	Wash buffer, 10-fold concentrate; tris-buffered NaCl solution	X
Control IgG | + *red lid*	1.2 ml	IgG positive control, human serum, ready for use	X
Control IgG | - *colourless lid*	1.2 ml	IgG negative control, human serum, ready for use	X
Control IgG | co *blue lid*	2.5 ml	IgG Cut-off control, human serum, ready for use	X
Conjugate *orange lid*	12 ml	anti-human IgG conjugate, ready for use; peroxidase conjugated anti-human IgG conjugate in stabilized protein solution	X
SeroSC	12 ml	Substrate H_2O_2/tetramethylbenzidine; ready for use	X
Stop	12 ml	Stop reagent 0.5 M sulphuric acid; ready for use	X

Details of hazardous substances according to labeling obligations. For more details: Safety Data Sheets (SDS) or product information look at www.r-biopharm.com.

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 or for 5 days when stored at room temperature (20 - 25 °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 stored in the aluminium bag. The reagents must not be allowed to become contaminated and the colourless substrate must be protected from exposure to direct light.

6. Materials required but not provided

6.1. Reagents

- distilled or deionised water

6.2. Accessories

- 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 % hypochlorite solution

7. Precautions for users

For *in vitro* diagnostic use.

This test must only be carried out by trained laboratory personnel. The guidelines for working in medical laboratories have to be followed. The instruction manual for the test procedure has to be followed. Do not pipet samples or reagents by mouth. Avoid contact with bruised skin or mucosal membranes. During handling reagents or samples, wear appropriate safety clothing (appropriate gloves, lab coat, safety goggles) and wash your hands after finishing the test procedure. Do not smoke, eat or drink in areas where samples or reagents are being used.

For more details see Safety Data Sheets (SDS) at www.r-biopharm.com.

All reagents and materials used have to be disposed properly after use. Please refer to the relevant national regulations for disposal.

The control sera (positive control, cut-off 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.

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8. Specimen collection and storage

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: 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

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 pour reagents back into vials as reagent contamination may occur. Do not pour reagents back into vials as this may lead to reagent 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 wash buffer, sample buffer, stop reagent and substrate are not test specific; they can also be used for other RIDASCREEN® ELISA for determining antibodies against parasites.

9.2. Preparing the wash buffer

1 part wash buffer concentrate SeroWP is mixed with 9 parts distilled water. In order to do this, place 100 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 before-hand by warming in a water bath at 37 °C. The diluted buffer can be used for a maximum of 4 weeks provided it is stored at 2 - 8 °C or for 5 days when stored at room temperature
(20 - 25 °C).

9.3. Preparing the samples

Dilute the serum samples to be tested with sample buffer Diluent 1:50 before starting the test.

e.g. 10 µl Serum +490 µl Diluent

Note:

The negative control, cut-off control and positive control are ready for use and must NOT be diluted.

9.4. First incubation

After inserting a sufficient number of wells in the frame, pipette 100 µl diluted sera and ready-to-use controls negative Control IgG | - , Cut-off Control IgG | co and positiv Control IgG | + into each of the corresponding wells and incubate at room temperature (20 - 25 °C) for 15 minutes. We recommend that you carry out the Cut-off control Control | co in duplicate.

9.5. Washing

The wells must be emptied into a waste container containing hypochlorite solution for disinfection. After this, knock out the plate onto absorbent paper in order to remove the residual moisture. After this, wash the plate 5 times using 300 µl 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, tap the plate upside down against clean absorbent paper in order to remove any residual liquid.

9.6. Second incubation

Add 100 µl of the anti-human IgG conjugate Conjugate to each well. Then incubate the plate at room temperature (20 - 25 °C) for 15 minutes.

9.7. Washing

Wash 5 times in accordance with Section 9.5.

9.8. Third incubation

Place 100 µl substrate SeroSC to each well. Then incubate the plate at room temperature (20 - 25 °C) for 15 minutes. After this, stop the reaction by adding 50 µl stop reagent Stop to each well and make your measurement at 450/620 nm during 20 minutes.

10. Quality control – indications of instability or deterioration

For quality control purposes, the negative control, the cut-off control and the positive control must be used every time the test is carried out. The Cut-off control has to be used in duplicate and if the two individual measurements deviate from the average by

more than 25 %, the test must be repeated. The test has been carried out correctly if the average extinctions of the controls fulfill the following criteria:

Tab. 3: Criteria of quality controls

	OD
negative control	< 0.15
cut-off control	0.2 - 0.5
positive control	≥ Index 20

If the values differ from those required, if the substrate is turbid or 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. Calculating the sample 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 cut-off.

For example:

	Cut-off control 1	O.D. = 0.44
	Cut-off control 2	O.D. = 0.42
	Sample	O.D. = 1.591

$$\text{Mean value cut-off control} = \frac{OD\ Cut{-}off\ 1 + OD\ Cut{-}off\ 2}{2} = \frac{0.44+0.42}{2} = 0.43$$

$$\text{Sample index} = \left(\frac{OD\ sample}{OD\ mean\ value\ cut{-}off\ control}\right) * 10 = \left(\frac{1.591}{0.43}\right) * 10 = 37$$

11.2. Test result

Tab. 4: Evaluating the sample index

	negative	equivocal	positive
Sample index	< 9	9 - 11	> 11

12. Limitations of the method

The RIDASCREEN® Leishmania IgG enzyme immunoassay determines specific IgG antibodies against *Leishmania infantum* and should be carried out in cases of suspected Leishmaniosis. The results obtained must always be interpreted in combination with the clinical picture and other diagnostic findings.
Antibody signals are dependent on the localisation of the parasitosis and may vary from patient to patient.
Equivocal and weakly positive results may be produced in the following cases:

- persons who have been stung by infected phlebotomae (sand flies) in regions where the disease is endemic and produce low antibody titres without manifestation of the infection
- persons, who have recovered from or just recovered from an asymptomatic infection so that they are still producing low antibody titres
- persons, who have suffered from the illness in the past and still produce residual titers
- persons in the early stages of the disease
- factors which are non-identifiable and non-specific

These cases should be re-examined by testing another sample, by considering the clinical symptoms and by other diagnostic methods. In the absence of any other exact diagnosis, the test should be repeated after two to four weeks with a new sample.
Medium and high positive results can be produced with persons who have suffered from the acute illness or have just recently suffered from the illness. Even higher antibody titers may be produced after longer infections which have occurred in the past. In cases which are not clear, the result must be interpreted in the light of the case history, clinical symptoms and other diagnostic methods.
A negative result does not necessarily rule out Leishmaniosis. During the early stages of the infection, the number of antibodies may be so small that the test yields a negative result. If Leishmaniosis is suspected on the basis of the case history, another serum sample should be tested after four weeks.
A cross reaction with serums containing antibodies against *T. cruzi* (Chagas' disease) has been observed in some cases in South America. This must be taken into consideration when carrying out the test in areas of South America where Chagas' disease is endemic.

A positive result does not rule out the presence of another infectious pathogen.

13. Performance characteristics

Tab. 5: Inter-assay variation (n = 30)

Inter-assay variation	IgG	
	Index	CV
Serum 1	44.7	5.8 %
Serum 2	15.4	9.6 %
Serum 3	13.1	8.3 %
Serum 4	0.6	n/a

Tab. 6: Intra-assay variation (n = 23)

Intra-assay variation	IgG	
	Index	CV
Serum 1	39.1	3.0 %
Serum 2	15.7	3.6 %
Serum 3	12.3	2.0 %
Serum 4	0.2	n/a

Tab. 7: Sensitivity and specificity in comparison with three other commercial available ELISAs

	IgG
Sensitivity	100 %
Specificity	100 %

Tab. 8: Result from testing 200 blood-donor sera taken from a blood donor center in Germany

200 blood donor sera	IgG
negative	100 %
equivocal	0 %
positive	0 %

14. Version history

Version number	Chapter and designation
2017-12-15	Release version

15. Explanation of symbols

General symbols

	For in vitro diagnostic use
	Consult instructions for use
	Lot number
	Expiry
	Store at
	Article number
	Number of tests
	Date of manufacture
	Manufacturer

Testspecific symbols

Plate	Microtiterplate
Diluent	Sample buffer
SeroWP	Wash buffer 10x
Control IgG | +	Positive Control IgG
Control IgG | -	Negative Control IgG
Control IgG | co	Cut-off Control IgG
Conjugate	Anti-human IgG conjugate
SeroSC	TMB Substrate
Stop	Stop reagent

16. Bibliography

1. *Bray, R.S*. and *Lainson, R*., Trans. Roy. Soc. Trop. Med. Hyg., 60, 605-609 (1966).
2. *Bray, R.S*. and *Lainson, R*., Trans. Roy. Soc. Trop. Med. Hyg., 61, 490-505 (1967).
3. *Bray, R.S*., Ecol. Dis., 4, 257-267 (1982).
4. *Hommel, M., Peters, W., Ranque, J., Quilici, M. and Lanotte, G., Ann.* Trop. Med. Parasitol., 72, 213-218 (1978).
5. *Kager, P.A., Rees, P.H., Wellde, B.T., Hockmeyer, W.T.* and *Lyerly, W.H*., Trans. R. Soc. Trop. Med. Hyg., 75, 556-559 (1981).
6. *Lainson, R*., Trans. Roy. Soc. Trop. Med. Hyg., 77, 569-596 (1983).
7. *Low-A-Chee, R.M., Rose, P.* and *Ridley, D.S.*, Ann. Trop. Med. Parasitol., 77, 255-260 (1986).
8. *Ranque, J.* and *Quilici, M*., Journal of Parasitology, 56, 277-278 (1970).