RIDASCREEN® Anti-IFX Antibodies

REF G09042

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1. Intended use

For *in-vitro* diagnostic use. The RIDASCREEN® Anti-IFX Antibodies is an enzyme linked immunoassay intended for the quantitative determination of antibodies to infliximab (ATI) in human serum and plasma.

2. Summary and explanation of the test

Therapeutic Drug Monitoring

Infliximab (IFX) is a chimeric therapeutic monoclonal antibody that targets the pro-inflammatory cytokine TNF-alpha. The introduction of infliximab has revolutionized the treatment of chronic inflammatory diseases like inflammatory bowel disease (IBD), rheumatoid arthritis (RA) and spondyloarthritis. It has been shown that infliximab can induce deep remission and improve the patient's quality of life [1]. Some patients do not respond to infliximab therapy upon induction (primary non-responders), while others lose response over time (secondary non-responders). [2]

Immunogenicity

Secondary loss of drug efficacy often occurs because of the immunogenic characteristics of the drug leading to the development of antibodies to infliximab (ATI). [3,4] ATI can develop in any patient undergoing infliximab therapy and are primarily neutralizing the activity of infliximab through immunocomplex formation. In addition, these immunocomplexes are rapidly cleared from the system. Analytically, they are responsible for subtherapeutic infliximab concentrations. Therefore, in the case of very low trough concentrations of infliximab (< 1 µg/ml), subsequent measurement of ATI may be helpful to determine the optimal treatment strategy.

Diagnostic Value

The diagnostic value of the RIDASCREEN® Anti-IFX Antibodies lies in its ability to stratify patients with subtherapeutic infliximab concentrations (< 1 µg/ml) into patients who need a dose increase and those who should be switched to another drug. Various studies have demonstrated that patients with low infliximab concentrations (< 1 µg/ml) and no or low ATI titers can benefit from an increase in infliximab dose. [5, 6] Importantly, the ATI titer in patients undergoing a dose increase must be monitored closely. Patients who have high ATI titers are preferably switched to a new drug, either from the same drug class or from another drug class.

Note: **RIDASCREEN® Anti-IFX Antibodies cannot detect ATI in the presence of high infliximab concentrations. It should only be used when < 1 µg/ml infliximab is quantified in the sample using RIDASCREEN® IFX Monitoring (G09041).**

3. Test principle

In the RIDASCREEN® Anti-IFX Antibodies, a highly specific monoclonal antibody (MA-IFX10F9), which was isolated and characterized at the University of Leuven (KU Leuven), is used in a bridging ELISA setup.[7] This antibody binds specifically to infliximab.

Infliximab molecules are applied to the surface of the well in the microwell plate. A dilution of the patients' serum or plasma sample which is to be tested is pipetted into the well of the microwell plate and incubated. During this incubation step anti-IFX antibodies bind specifically to the infliximab on the solid phase. After a washing step that removes the unbound serum proteins, the strips are incubated with biotin conjugated infliximab, binding directly to the antigen-antibody complex. After removal of the unbound biotin conjugate, the strips are incubated with peroxidase conjugated streptavidin. After addition of the substrate, the attached enzyme changes the color of the previously colorless solution in the wells of the microwell plate to blue if the test is positive. Upon addition of the stop reagent, the color changes from blue to yellow. The absorbance is proportional to the concentration of ATI present in the sample.

4. Reagents provided

One kit is sufficient for 96 determinations.

Plate	96 det.	Microtiter plate, 12 microwell strips (can be divided) in the strip holder; coated with infliximab
Standard | 1-6	1.3 ml	6 Standards; concentrations of the standards 1 to 6: 0 / 0.1 / 0.5 / 1 / 2.5 / 5 ng/ml anti-IFX MA-IFX10F9; contains 0.09 % NaN_3; ready for use
Low Control| +	1.3 ml	Low positive control for ATI; contains 0.375 ng/ml anti-IFX MA-IFX10F9 and 0.09 % NaN_3; ready for use
Control| +	1.3 ml	Positive control for ATI; contains 3 ng/ml anti-IFX MA-IFX10F9 and 0.09 % NaN_3; ready for use
Diluent	100 ml	Sample dilution buffer; contains 0.09 % NaN_3; ready for use; colored orange
Conjugate | 1	12 ml	Conjugate 1; biotin conjugated infliximab; ready for use; colored blue
Conjugate | 2	12 ml	Conjugate 2; peroxidase conjugated streptavidin; ready for use; colored red
Substrate	12 ml	Substrate; Hydrogen peroxide / tetramethylbenzidine (TMB); ready for use
Wash	50 ml	Wash buffer (20-fold conc.); phosphate-buffered NaCl solution; contains detergent and antimicrobial agents
Stop	6 ml	Stop reagent; 0.5 M H_2SO_4; ready for use
4 Plate covers		

Information on hazardous substances complies with the labeling requirement. For further details, see the safety data sheets (SDSs) at www.r-biopharm.com.

5. Storage instructions

All reagents must be stored at 2 - 8 °C and can be used until the expiry date printed on the label. Opened components (reagents, microwell strips) should be stored at 2 - 8 °C until next use and can be held for 6 months. The diluted wash buffer can be used for one month when stored at 2 - 8 °C. Microbial contamination must be prevented. After the expiry date, the quality guarantee is no longer valid.
The aluminum bag containing the microwell strips must be opened in such a way that the clip seal is not torn off. Any microwell strips which are not required must immediately be returned to the aluminum bag and stored at 2 - 8 °C. The colorless substrate must also be protected from direct light to prevent it from decomposing or turning blue due to auto-oxidation. Once the substrate has turned blue, it must not be used.

6. Reagents required but not provided

6.1. Reagents

- Distilled or deionized water

6.2. Accessories

- Precision micropipettes and standard laboratory pipettes
- Graduated cylinder (1000 ml)
- Clean glass or plastic tubes for the dilution of the samples
- Stopwatch
- Microplate washer or multichannel pipette (300 µl)
- Microplate reader (450 nm, reference filter 620 nm)
- Filter paper (laboratory towels)
- Waste container with 0.5 % hypochlorite solution
- 37 °C incubator

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.
Do not mix reagents or coated microtiter strips from kits with different lot numbers.
The control sera of the kit (Standard 1 - 6, Low positive control, Positive control) were tested for HIV- and HCV-Ab and HBs-Ag and found to be negative. Nevertheless, they should, be treated as potentially infectious and handled according to national safety regulations, just like the patient samples and all materials coming into contact with them.
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 stop reagent contains 0.5 M sulphuric acid. Avoid contact with the skin and clothing. If the skin is contaminated with the reagent, rinse it off with water.
The reagents contain NaN_3 as a preservative. This substance must not be allowed to come into contact with the skin or mucous membrane.
The substrate contains Hydrogen peroxide.

8. Collection and storage of specimens

In this assay, EDTA-plasma samples, citrate plasma samples and serum samples may be used. Following collection, the serum should be separated from the clot as quickly as possible to avoid hemolysis. Transfer the serum to a clean storage tube. Samples can be stored at 2 - 8 °C for 3 - 4 days, or at - 20 °C for at least one year.

Repeated freezing and thawing should be avoided. Samples must be diluted in sample diluent (see 9.3.1.).

Diluted samples may be stored for at least 8 hours at room temperature.

9. Test procedure

9.1. General information

All reagents and the Microtiter plate Plate must be brought to room temperature (20 - 25 °C) before use. The microwell strips must not be removed from the aluminum bag until they have reached room temperature. The reagents must be thoroughly mixed immediately before use. Once opened, unused microwell strips (in sealed bags) and the reagents must be stored at 2 - 8 °C. Once used, the microwell strips must not be used again. The reagents and microwell strips must not be used if the packaging is damaged or the vials are leaking. In order to prevent cross contamination, the samples must be prevented from coming into direct contact with the kit components. The test must not be carried out in direct sunlight. While incubating, we recommend covering the microwell plate or placing a film on it to prevent evaporation loss.

For instructions on how to perform the assay with ELISA instruments, please contact R-Biopharm AG or your local distributor.

9.2. Preparing the wash buffer

Mix 1 part Wash buffer concentrate Wash with 19 parts distilled water (1:20). Pour 50 ml concentrate in a 1000 ml measuring cylinder and stock up with distilled water to 1000 ml. Reconstituted solution can be stored at least 1 month at 2 - 8 °C. At higher temperatures, the concentrated washing solution may appear cloudy without affecting its performance. Upon dilution, the solution will be clear.

9.3. Preparing the samples

Serum or plasma samples can be stored at 2 - 8 °C for 3 - 4 days, or at - 20 °C for at least one year (also see chapter 8). Repeated freezing and thawing should be avoided. Samples must be diluted in sample diluent (see 9.3.1.).
Diluted samples may be stored for at least 8 hours at room temperature.

9.3.1. Sample dilution

Prepare for each patient sample a dilution of 1:25 and 1:200.

a) 1:25 dilution

By diluting the samples 1:25, ATI concentrations between 2.5 and 125 ng/ml can be determined.
Example: add 25 µl patient sample to 600 µl sample dilution buffer Diluent.

If the obtained concentration is lower than 2.5 ng/ml, the result must not be extrapolated and is reported as < 2.5 ng/ml.
If the obtained concentration is higher than 125 ng/ml, the result must not be extrapolated and is reported as > 125 ng/ml.

b) 1:200 dilution

By diluting the samples 1:200, ATI concentrations between 20 and 1000 ng/ml can be determined.
Example: add 100 µl of dilution 1:25 to 700 µl sample dilution buffer Diluent.

If the obtained concentration is lower than 20 ng/ml, the result must not be extrapolated and is reported as < 20 ng/ml.
If the obtained concentration is higher than 1000 ng/ml, the result must not be extrapolated and is reported as > 1000 ng/ml.

If both 1:25 and 1:200 dilutions result in a measurable concentration value, the mean of both values is calculated and reported.

9.4. First incubation

After placing a sufficient number of wells in the holder, add 100 µl of Standards 1 - 6 Standard | 1 to Standard | 6, Positive control Control | +, the Low positive control Low control | + and samples to the relevant wells. Although it might be advised to run calibrators, controls and samples in duplicate, reliable results are equally obtained by doing the analysis in singlicate.
Then incubate the covered plate at 37 °C for 1 hour.

9.5. First washing

Careful washing is important in order to achieve the correct results and should therefore be carried out strictly in accordance with the instructions. The incubated substance in the wells must be emptied into a waste container containing hypochlorite solution for disinfection. Tap the plate upside down vigorously against absorbent paper to ensure complete removal of liquid from the microwells.
Then wash the plate 5 times using 300 µl diluted wash buffer each time (see 9.2).
Tap the plate upside down vigorously against absorbent paper to ensure complete removal of liquid from the microwells.
When using a microplate washer, make sure that the machine is correctly adjusted to the type of microwell plate being used. Also make sure that all of the liquid is sucked away during each washing phase. After washing for the last time, tap the plate upside down vigorously against absorbent paper to ensure complete removal of liquid from the microwells.

9.6. Second incubation

Add 100 µl conjugate1 Conjugate | 1 into each well. Then incubate the covered plate at 37 °C for 30 minutes.

9.7. Second washing

The incubated substance in the wells must be emptied into a waste container containing hypochlorite solution for disinfection. Tap the plate upside down vigorously against absorbent paper to ensure complete removal of liquid from the microwells. Then wash the plate 5 times using 300 µl diluted wash buffer each time. Tap the plate upside down vigorously against absorbent paper to ensure complete removal of liquid from the microwells.

9.8. Third incubation

Add 100 µl conjugate 2 Conjugate | 2 into each well. Then incubate the covered plate at 37 °C for 15 minutes.

9.9. Third Washing

The incubated substance in the wells must be emptied into a waste container containing hypochlorite solution for disinfection. Tap the plate upside down vigorously against absorbent paper to ensure complete removal of liquid from the microwells. Then wash the plate 5 times using 300 µl diluted wash buffer each time. Tap the plate upside down vigorously against absorbent paper to ensure complete removal of liquid from the microwells.

9.10. Fourth incubation

Add 100 µl substrate Substrate to each well. Then incubate the plate at 37 °C in the dark for 10 minutes. After this, 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 filter 620 nm) in a plate reader.

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

For quality control purposes, each Standard 1 to Standard 6 Standard | 1 – Standard | 6 , Positive control Control | + and Low positive control Low control | + (each in duplicate recommended) must be used every time the test is carried out to ensure reagent stability and correct test execution.

The following specifications must be met during each run in order to be valid:
O. D. Value for Standard 1 Standard | 1 < 0.080
O. D. Value for Standard 6 Standard | 6 > 1.400
If one of the specifications is not met, the run should be repeated.

Concentration value for the Low positive control Low control | +:
0.375 ng/ml, range 0.25 - 0.50 ng/ml
Concentration value for the Positive control Control | +:
3 ng/ml, range 2 - 4 ng/ml

If the values differ from those required, if the substrate is turbid or has turned blue before adding to the wells, this 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.

In case of high background signal (OD Standard 1 > 0.08) the washing was insufficient. Repeat the test with more vigorous washing (increased number of cycles, soak time).

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

11. Evaluation and interpretation

For the analysis of the results RIDA®SOFT Win.net is required. The RIDA®SOFT Win.net or an update is available on request from R-Biopharm AG or your local R-Biopharm distributor.

Another evaluation software that provides the 4-parameter logistic-log-model can also be used as an alternative to RIDA®SOFT Win.net.

Evaluation of RIDASCREEN® Anti-IFX Antibodies is achieved by standard curve that must always be processed when running the test.

In final quality control, R-Biopharm AG has determined the target values and the allowed range of concentration for the positive control and low-positive control for each kit lot under optimal test conditions.

The dilution factor must be taken into account when calculating the ATI concentration in patient samples by multiplying the measured concentration by the dilution factor.

Example: The outcome of 1:25 diluted sample, obtained by interpolation from the calibration curve is 2 ng/ml. The corresponding ATI concentration in the undiluted sample is then 50 ng/ml.

Example: The outcome of 1:200 diluted sample, obtained by interpolation from the calibration curve is 2 ng/ml. The corresponding ATI concentration in the undiluted sample is then 400 ng/ml.

If both 1:25 and 1:200 dilutions result in a measurable concentration value, the mean of both values is calculated and reported.

If using the RIDA®SOFT Win.net Software this is automatically done when using the appropriate method:
For dilution 1:25 select: RIDA®SOFT Win.net method A-IFX25.met.
For dilution 1:200 select: RIDA®SOFT Win.net method A-IFX200.met.

The concentration is reported in ng/ml.

12. Limitations of the method

The RIDASCREEN® Anti-IFX Antibodies is a drug-sensitive assay and only detects the free, unbound anti-IFX antibodies. For optimal interpretation, it is advised to measure anti-IFX Antibodies in serum/plasma samples collected at trough, just before the next IFX administration.

Individual ATI concentrations, measured using the RIDASCREEN® Anti-IFX Antibodies, cannot be used as a sole indicator for making changes in treatment regimen and each patient should be thoroughly evaluated clinically before changes in treatment regimens are made.

13. Performance characteristics

13.1. Example of typical optical density (O.D.) values

Standard	O.D.
1	0.031
2	0.083
3	0.269
4	0.543
5	1.539
6	2.685

13.2. Precision

13.2.1. Intra-Assay-Precision

The intra-assay-precision was determined in a single run using 3 references in 21 replicates each. From the OD-values of these measurements ATI concentrations have been determined. The mean value (MV), standard deviation (SD) and coefficient of variation (CV) were calculated for each sample. The results are listed in the table below.

Reference	1	2	3
Mean (ng/ml)	0.67	1.25	2.44
SD	0.05	0.08	0.14
% CV	**7.5**	**6.7**	**5.9**

13.2.2. Inter-Assay-Precision

The inter-assay-precision was determined in 3 runs using 2 references. From the OD-values of these measurements ATI concentrations have been determined. The mean value (MV), standard deviation (SD) and coefficient of variation (CV) were calculated for each sample. The results are listed in the table below.

Reference	1	2
Mean (ng/ml)	0.36	2.25
SD	0.05	0.27
% CV	**12.4**	**11.9**

13.3. Specificity

13.3.1. Normal human serum/plasma

Specificity has been evaluated by testing 100 healthy donor samples from Dutch origin. None of the samples showed a detectable concentration of ATI, resulting in a specificity of 100 %.

13.3.2. Interference

The potential interference of rheumatoid factor (RF) in a clinical sample panel of patients suffering from auto-immune diseases and positive for RF was evaluated in the RIDASCREEN® Anti-IFX Antibodies. The results indicated that RF does not interfere in the assay.
A panel of 35 potentially interfering samples was tested consisting of HAMA positive, lipemic, high bilirubin, high cholesterol, haemolysed, high total protein and 1st semester pregnant woman samples. No interaction with the investigated factors was observed.

13.4. Analytical sensitivity

The minimal detectable concentration of ATI is 0.06 ng/ml.
Taking into account a dilution factor of 1:25, this corresponds to 1.5 ng/ml.
Taking into account a dilution factor of 1:200, this corresponds to 12 ng/ml.

For a 1:25 dilution a concentration lower than 2.5 ng/ml, corresponding to the lowest standard, should be reported as < 2.5 ng/ml.

For a 1:200 dilution a concentration lower than 20 ng/ml should be reported as < 20 ng/ml.

13.5. Diagnostic sensitivity

One clinical sample panel of 36 specimens was analysed using the RIDASCREEN Anti-IFX Antibodies and results were compared with data obtained using the ATI ELISA developed at the KU Leuven, which served as reference assay. All 24 samples having measurable ATI levels according to the reference assay, were detected positive, resulting in a diagnostic sensitivity of 100 %.

14. Version history

Version number	Chapter and description
2019-11-05	2. Summary and explanation of the test 9.4 First incubation 16. References

15. Explanation of symbols

General symbols

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

Test specific symbols

Plate	Microtiter plate
Standard | 1-6	Standard 1 - 6
Low Control | +	Low positive control
Control | +	Positive control
Diluent	Sample dilution buffer
Conjugate | 1	Conjugate 1
Conjugate | 2	Conjugate 2
Substrate	Substrate
Wash	Wash buffer (20-fold conc.)
Stop	Stop reagent

16. References

1. Vogelaar L, Spijker AV, van der Woude CJ. The impact of biologics on health-related quality of life in patients with inflammatory bowel disease. Clin Exp Gastroenterol 2009;2:101-109.

2. Yanai H, Hanauer SB. Assessing response and loss of response to biological therapies in IBD. Am J Gastroenterol 2011;106:685-698.

3. Baert F, Noman M, Vermeire S, et al. Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease. N Engl J Med 2003;348:601-608.

4. Ducourau E, Mulleman D, Paintaud G, et al. Antibodies toward infliximab are associated with low infliximab concentration at treatment initiation and poor infliximab maintenance in rheumatic diseases. Arthritis Res Ther 2011;13:R105.

5. Vande Casteele N, Ferrante M, Van Assche G, et al. Trough concentrations of infliximab guide dosing for patients with inflammatory bowel disease. Gastroenterology 2015;148:1320-1329.

6. Steenholdt C, Brynskov J, Thomsen OO, et al. Individualised therapy is more cost-effective than dose intensification in patients with Crohn's disease who lose response to anti-TNF treatment: a randomised, controlled trial. Gut 2014;63:919-927.

7. Van Stappen T, Billiet T, Vande Casteele N, et al. An Optimized Anti-infliximab Bridging Enzyme-linked Immunosorbent Assay for Harmonization of Anti-infliximab Antibody Titers in Patients with Inflammatory Bowel Diseases. Inflamm Bowel Dis 2015;21:2172-2177