

RIDASCREEN® IFX Monitoring

REF G09041



1. Intended use

For *in vitro* diagnostic use. RIDASCREEN® IFX Monitoring is an enzyme linked immunoassay intended for the quantitative determination of infliximab (IFX, Remicade®) 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 α . 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 IFX therapy upon induction (primary non-responders), while others lose response over time (secondary non-responders). [2]

A drug can only exert its pharmacologic effect when adequate concentrations are achieved in the circulation. The serum concentration of infliximab just before the next infusion, defined as the trough concentration, has been used for therapeutic drug monitoring (TDM). Recent data on TDM have shown that a good clinical response is associated with adequate trough concentrations in IBD [3] and RA [4, 5] patients. TDM may therefore be very instrumental to optimize treatment and to overcome secondary loss of response.

RIDASCREEN® IFX Monitoring uses a highly specific monoclonal antibody (MA-IFX6B7), which was isolated and characterized at the KU Leuven. [6] It detects only infliximab; it was shown that other anti-TNF drugs such as adalimumab or golimumab do not interfere with the measurement. [6]

Biosimilars of infliximab (Remsima®, Inflectra® and Flixabi®) are equally well quantified in the RIDASCREEN® IFX Monitoring. [7]

Inflammatory bowel disease

The diagnostic value of TDM in IBD patients is described below for both the induction therapy and maintenance therapy phase.

Induction therapy phase: It has been shown that IFX trough concentrations during (post-) induction treatment are associated with a sustained clinical response. [8, 9] The measurement of infliximab trough concentrations during or shortly after the induction therapy phase can help to identify underexposed patients and to optimize the individual dose. [10]

Maintenance therapy phase: It has been shown that patients with sustained infliximab concentrations during the maintenance therapy phase are more likely to stay in remission than patients with undetectable trough concentrations. [11] Regular monitoring of the trough concentration during the maintenance therapy phase is useful to optimize the dosing regimen and improve treatment outcomes. [12]

For the RIDASCREEN® IFX Monitoring, a target therapeutic trough concentration window of 3 - 7 µg/ml is recommended, following the TAXIT algorithm. ^[12]

In addition, it was shown that for patients who no longer responded to IFX, it is more useful to adjust the treatment individually based on IFX serum concentration measurements, than an empirical strategy that makes use of other treatment options. ^[13]

Patient samples withdrawn during the induction therapy phase (usually at week 2 and week 6) typically have higher trough concentrations than patient samples withdrawn during the maintenance therapy phase (week 12 - 14 onwards). Therefore, use of a higher dilution for patient samples withdrawn during the induction therapy phase is advised.

Immunogenicity

Secondary loss of response is often due to the development of anti-drug antibodies, because of the immunogenic character of the drug. ^[14] In the case of undetectable trough concentrations, subsequent measurement of anti-drug antibodies may be helpful to determine the optimal treatment strategy.

RIDASCREEN® Anti-IFX Antibodies (G09042) ELISA can be used for this analysis.

3. Test principle

In RIDASCREEN® IFX Monitoring, a highly specific monoclonal antibody against IFX (MA-IFX6B7, isolated and characterized at KU Leuven) is used in a sandwich-type method. TNFα molecules are applied to the surface of the well in the microwell plate. A dilution of the patients' serum or plasma sample is pipetted into the well of the microwell plate and incubated. During this incubation step, IFX binds specifically to the TNFα on the plate. After washing, a second incubation step follows with MA-IFX6B7, which is conjugated with horseradish peroxidase. In the presence of IFX, a sandwich complex is formed between immobilized TNFα, IFX and conjugated antibodies. Unattached enzyme-labelled antibodies are removed during a subsequent washing step. After addition of the substrate, the colorless solution in the microwells will turn blue in case of a positive test result. Upon addition of the stop reagent, the color changes from blue to yellow. The absorbance is proportional to the concentration of IFX 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 human TNF α
Standard 1-6	1300 μ l	6 Standards; concentrations of the standards 1 to 6: 0 / 5 / 10 / 20 / 60 / 120 ng/ml; contains 0.09 % NaN ₃ ; ready for use; Calibrated against the WHO International Standard for Infliximab 16/170
Low Control +	1300 μ l	Low positive control; contains 30 ng/ml IFX and 0.09 % NaN ₃ ; ready for use
Control +	1300 μ l	Positive control; contains 70 ng/ml IFX 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	12 ml	Conjugate; peroxidase conjugated monoclonal antibody (MA-IFX6B7); ready for use; colored red
Substrate	12 ml	Substrate; Hydrogen peroxide / tetramethylbenzidine (TMB); ready for use
Wash 20x	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 ₂ SO ₄ ; ready for use
2 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 sulfuric acid. Avoid contact with the skin and clothing. If the skin is contaminated with the reagent, rinse it off with water.

The reagents contain NaN₃ 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 at least 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 losses.

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 | 20x** 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

a) Measurement of trough concentrations during therapy maintenance phase

To measure the trough concentration (drug concentration just before next dose administration) during the maintenance phase of the treatment (from week 12 - 14 and following), the samples are diluted 1:100:

10 µl of the sample is diluted in 990 µl sample dilution buffer **Diluent** (1:100).

100 µl of this final diluted sample is then used in the test.

If the sample is diluted 1:100, IFX concentrations between 0.5 and 12 µg/ml can be determined.

b) Measurement of trough concentrations during therapy induction phase

To measure trough concentrations during induction therapy (typically at week 2 and week 6) or to measure intermediate drug concentrations, or concentrations > 12.0 µg/mL, the samples are diluted 1:400:

10 µl of the sample is diluted in 390 µl sample dilution buffer [Diluent] (1:40), subsequently 100 µl of this solution is diluted in 900 µl [Diluent] (1:10).
100 µl of this final diluted sample is then used in the test.

If the sample is diluted 1:400, IFX concentrations between 2.0 and 48 µg/ml can be determined.

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 | +), 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 microtiter 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 conjugate [Conjugate] into each well. Then incubate the covered microtiter 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 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 | +** an 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 procedural.

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

a) If the dilution factor of 1:100 is used (maintenance therapy phase):

Concentration for the Low positive control **Low Control | +**:

3 µg/ml, range 2 - 4 µg/ml

Concentration for the Positive control **Control | +**:

7 µg/ml, range 5 - 10 µg/ml

b) If the dilution factor of 1:400 is used (induction therapy phase):

Concentration for the Low positive control **Low Control | +**:

12 µg/ml, range 8 - 16 µg/ml

Concentration for the Positive control **Control | +**:

28 µg/ml, range 20 - 40 µg/ml

For calculating the IFX concentration in the controls, the same multiplicity factor must be used as for the samples (see **Chapter 11. Evaluation and Interpretation**). Concentration is then expressed in µg/ml.

Calculation example for dilution factor 1:100:

60 ng/ml x 100 (dilution factor) = 6 µg/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.

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

Evaluation of RIDASCREEN® IFX Monitoring 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 IFX concentration in patient samples by multiplying the measured concentration by the dilution factor.

Example: The outcome of 1:100 diluted sample, obtained by interpolation from the calibration curve is 60 ng/ml. The corresponding IFX concentration in the undiluted sample is then 6 µg/ml.

Example: The outcome of 1:400 diluted sample, obtained by interpolation from the calibration curve is 60 ng/ml. The corresponding IFX concentration in the undiluted sample is then 24 µg/ml.

If using the RIDA®SOFT Win.net software the dilution factor is automatically applied when using the appropriate method:

For dilution 1:100 select: RIDA®SOFT Win.net method IFX100.met.

For dilution 1:400 select: RIDA®SOFT Win.net method IFX400.met.

The concentration is reported in µg/ml.

12. Limitations of the method

The RIDASCREEN® IFX Monitoring test detects the free, functionally active proportion of IFX and not the proportion of IFX that is bound by anti-infliximab antibodies, because of immunogenicity.

Individual infliximab concentrations, measured using the RIDASCREEN® IFX Monitoring, 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.

During the maintenance phase of therapy, a target therapeutic trough concentration window of 3 - 7 µg/ml is recommended. ^[12] However, threshold concentrations that associate with remission may vary among different patients because of intra- and inter-individual variability in pharmacokinetics and pharmacodynamics. In addition, higher trough concentrations have been suggested to be associated with response and remission in patients with specific disease phenotypes, such as patients with perianal disease, or when targeting endoscopic healing. ^[15,16]

13. Performance characteristics

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

Standard	O.D.
1	0.007
2	0.104
3	0.212
4	0.453
5	1.357
6	2.508

13.2. Precision

13.2.1. Intra-Assay-Precision

The intra-assay-precision was determined in a single run using 4 references in 21 replicates each. From the OD-values of these measurements IFX concentrations have been determined. The mean value, 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	4
Mean (µg/ml)	0.61	1.12	3.2	7.67
SD	0.04	0.05	0.15	0.51
% CV	6.6	4.6	4.7	6.8

13.2.2. Inter-Assay-Precision

The inter-assay-precision was determined in 5 runs using four references. From the OD-values of these measurements IFX concentrations have been determined. The mean value, 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	4
Mean (µg/ml)	0.77	1.58	4.17	9.82
SD	0.04	0.09	0.16	0.94
% CV	5.4	5.4	3.7	9.6

13.3. Specificity

13.3.1. Normal human serum/plasma

Specificity was determined by testing 72 donor samples of untreated persons of Belgian origin. None of the samples showed a detectable concentration of IFX, resulting in a specificity of 100 %.

13.3.2. Interference

A panel of 30 potentially interfering samples was tested consisting of HAMA positive, lipemic, high bilirubin, high cholesterol, hemolyzed and first semester pregnant women samples. No interaction with the investigated factors was observed.

13.3.3. Cross-reactivity

No cross-reactivity has been observed for following biopharmaceuticals applied for treating auto-immune diseases: adalimumab and golimumab.

13.4. Analytical sensitivity

For the determination of the analytical sensitivity serial dilutions of Standard 2 (5 ng/ml) were prepared and tested along with two control samples. The detection limit was determined as < 1 ng/ml. Taking into account a dilution factor of 1:100 this corresponds to 0.1 µg/ml.

13.5. Recovery

17 IFX-negative samples were spiked with varying IFX concentrations. Based on the OD values of this measurement, the IFX concentration was determined using the standard curve and the recovery calculated. The mean recovery is 103.5 %.

Sample	Expected (µg/ml)	Observed (µg/ml)	Recovery %
1	0.25	0.285	114.0
2	0.5	0.5	100.0
3	1	0.95	95.0
4	2	1.95	97.5
5	3	3.18	106.0
6	3.5	3.53	100.9
7	4	3.88	97.0
8	4.5	4.74	105.3
9	5	5.34	106.8
10	5.5	6.07	110.4
11	6	6.57	109.5
12	7	7.73	110.4
13	8	8.42	105.3
14	9	8.86	98.4
15	10	10.29	102.9
16	11	11.26	102.4
17	12	11.68	97.3
Mean			103.5

13.6. Correlation with reference assay










Two clinical sample panels with a number of 102 and 30 samples were analyzed using the RIDASCREEN® IFX Monitoring and the results were compared with those of the reference assay (IFX ELISA developed at KU Leuven). Correlation coefficients of 0.95 to 0.97 were determined for the two sample panels.

14. Version history

Version number	Chapter and description
2019-11-14	4. Reagents provided 9.4 First incubation

15. Explanation of symbols

General symbols

	For <i>in vitro</i> diagnostic use
	Consult instructions for use
	Lot number
	Expiry
	Store at
	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	Conjugate
Substrate	Substrate
Wash 20x	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. Vermeire S, Gils A. Value of drug level testing and antibody assays in optimising biological therapy. *Frontline Gastroenterol* 2013;4:41-43.
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. Mulleman D, Meric JC, Paintaud G, et al. Infliximab concentration monitoring improves the control of disease activity in rheumatoid arthritis. *Arthritis Res Ther* 2009;11:R178.
6. Van Stappen T, Brouwers E, Tops S, et al. Generation of a highly specific monoclonal antibody standard for harmonization of TNF-coated infliximab assays. *Ther Drug Monit* 2015;37:479-485.
7. Gils A, Van Stappen T, Dreesen E, et al. Harmonization of Infliximab and Anti-Infliximab Assays Facilitates the Comparison Between Originators and Biosimilars in Clinical Samples. *Inflamm Bowel Dis*. 2016;22:969-975.

8. Cornillie F, Hanauer SB, Diamond RH, et al. Postinduction serum infliximab trough level and decrease of C-reactive protein level are associated with durable sustained response to infliximab: a retrospective analysis of the ACCENT I trial. *Gut* 2014;63:1721-1727.
9. Vande Casteele N, Ballet V, Van Assche G, et al. Early serial trough and antidrug antibody level measurements predict clinical outcome of infliximab and adalimumab treatment. *Gut* 2012;321; author reply 322.
10. Van Stappen T, Bollen L, Vande Casteele N, et al. Rapid test for infliximab drug concentration allows immediate dose adaptation. *Clin Transl Gastroenterol* 2016;7:e206.
11. Maser EA, Villela R, Silverberg MS, et al. Association of trough serum infliximab to clinical outcome after scheduled maintenance treatment for Crohn's disease. *Clin Gastroenterol Hepatol* 2006;4:1248-1254.
12. 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.
13. 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.
14. 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.
15. Yarur AJ, Kanagala V, Stein DJ, et al. Higher infliximab trough levels are associated with perianal fistula healing in patients with Crohn's disease. *Aliment Pharmacol Ther* 2017;45:933-940.
16. Ungar B, Levy I, Yavne Y, et al. Optimizing anti-TNF- α therapy: Serum levels of infliximab and adalimumab associate with mucosal healing in patients with inflammatory bowel diseases. *Clin Gastroenterol Hepatol* 2016;14:550–557.