IFX-Fast

Rapid immunochromatographic assay for quantitative detection of Infliximab from human serum and plasma

REF 9191TI

INTENDED PURPOSE

IFX-Fast kit is a lateral flow immunochromatographic assay for the quantitative detection of infliximab (IFX, Remicade®) in human serum and plasma.
For professional use only.

SUMMARY AND EXPLANATION

Therapeutic Drug Monitoring

Infliximab (IFX) is a chimeric therapeutic monoclonal antibody that targets the pro-inflammatory cytokine TNFα. 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.

IFX-Fast kit uses a highly specific monoclonal antibody (MA-IFX6B7), which was isolated and characterized at the KU Leuven. [6] It detects only
infliximab (Remicade®) as well as the biosimilars Remsima®, Inflectra® and Flixabi®. [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 IFX-Fast, 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.

TEST PRINCIPLE

IFX is detected through the formation of an antibody-antigen-sandwich of MA-IFX6B7 and TNFα. This is made visible by the usage of marked colloidal gold nanoparticles. The generated signal is read out with the RIDA®QUICK SCAN II and the IFX concentration calculated by using the standard curve which is stored in the instrument.

COMPONENTS

Materials provided

Each kit contains sufficient reagents for 25 tests.

CASSETTE	1 pc.	25 test cassettes
DILUENT	25 ml	Sample dilution buffer; contains 0.09 % NaN_3; ready for use
REAGENT | A	2.5 ml	Reagent A; contains 0.09 % NaN_3; ready for use
REAGENT | B	2.5 ml	Reagent B; contains 0.09 % NaN_3; ready for use

All Reagents are not classified as hazardous pursuant to the provisions set forth in EC Regulation 1272/2008 (CLP) (and subsequent amendments and supplements).

Additional materials

Controls for IFX-Fast can be ordered separately. IFX-Fast Control Set (cod. 9191TI) contains 2 controls. They are used in the same way as patient samples and can be used to check the test reagents and test procedure.
Content of IFX-Fast Control Set

HIGH CONTROL	1.2 ml	Batch specific, high positive control
LOW CONTROL	1.2 ml	Batch specific, low positive control

Additional materials required but not provided

- Reaction tube
- Sample tube for sample suspension (two for each patient sample)
- Micropipettes with disposable tips 10 - 100 µl und 100 - 1000 µl
- Stopwatch
- Waste container with 0.5 % hypochlorite solution
- RIDA®QUICK SCAN II (available at R-Biopharm AG, Art. No.: ZRQS2-KD)
- Vortex mixer

STORAGE BEFORE USE

Store the kit at 2 - 8°C.
Kit contents are stable until the expiration date printed on the product label.
The reagents should only shortly be left at room temperature.
After usage, they should directly be stored at 2 - 8°C.
The quality of the product cannot be guaranteed after the expiration date. Likewise, the usability of the cassettes can no longer be guaranteed if the cassette packaging is damaged.

WARNINGS AND PRECAUTIONS

- The IFX-Fast kit is 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 from kits with different lot numbers.
- 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 reagents contain NaN_3 as a preservative. This substance must not be allowed to come into contact with the skin or mucous membrane.

PROCEDURE FOR SAMPLES COLLECTION

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.

STORAGE OF SAMPLE

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.

QUALITY CONTROL

The test can only be evaluated, if the test cassette is unharmed and there are no color changes or lines present before applying the sample suspension.
The control line (marked with C on the test cassette) has to show up in every test run. In case this band is missing, the following should be checked before repeating the test:

- Expiry date of the reagents and test cassette used
- Correct test procedure
- Contamination of reagents

If the control line is still not visible after repeating the test with a different test cassette contact the manufacturer or your local distributor.

ASSAY PROCEDURE

General information

- The samples, diluent, reagents A and B, and the test strips must be brought to room temperature (20 - 25°C) before use.

- Once used, the test strips may not be re-used.
- The test must not be carried out in direct sunlight.
- Excess reagents must not be returned to the vessels because this can result in contamination.
- The RIDA®QUICK SCAN II must be switched on prior to the start of the test. The test method must be scanned on first use using the barcode reader and is then saved for further measurements using the RIDA®QUICK SCAN II. The lot-specific parameters must also be scanned once for each lot prior to the start of the test. The QR codes for the test method and for the lot-specific parameters can be found on the analysis certificate included with the kit.

Preparation of the samples and reagents

The measurement range of IFX-Fast is between 0.5 – 10 µg/ml with use of the standard dilution (maintenance therapy phase).
The measurement range can be extended to 2 – 40 µg/ml through an additional dilution (induction phase).

a) Measuring the trough concentration during the maintenance therapy phase

To measure the trough concentration (drug concentration just before next dose administration) during the maintenance phase of treatment (from week 12 -14 and following), dilute the sample to 1:50.

1. a) Dilute 20 µl of the sample in 980 µl of diluent (1:50).

b) Measuring the trough concentration during the induction therapy phase

To measure the trough concentration during induction therapy (typically week 2 and 6), or to measure intermediate drug concentrations, or concentrations > 10 µg/ml, dilute the sample to 1:200.

1. b) dilute 20 µl of the sample in 980 µl of diluent (1:50). The 1:50 dilution from the maintenance therapy phase can also be used for this step. Next dilute 100 µl of this solution in 300 µl of diluent (1:4) so that, overall, the dilution of the initial sample is 1:200.
2. In a separate reaction vessel, mix 90 µl of Reagent A (blue liquid, bottle with blue lid) and 90 µl of Reagent B (yellow liquid, bottle with transparent lid). If multiple test strips are processed, the solution can also be used for several samples at the same time. Mixture of Reagent A (blue liquid) and Reagent B (yellow liquid) will create a green-colored solution.
3. Pipette 20 µl of the diluted sample (point 1a and/or 1b) into the 180 µl of the mixture of reagent A and B, which is equivalent to a further dilution of the sample of 1:10 (point 1a and/or 1b) In this way, the final dilution of the initial sample will be 1:500 (maintenance therapy phase) or 1:2.000 (induction therapy phase).
4. Mix the solutions thoroughly by inversion or vortexing to homogenize the sample mixture.

5. Remove the test cassette from the packaging and place it on a flat surface.

Incubation and test reading

1. Pipette 100 µl of the sample preparation from the reaction tube of step 3 into the sample well of the test cassette.
2. Incubate the reaction mixture at room temperature for exactly **5 minutes**.
3. The test result always has to be read after **15 (+ max. 2) minutes** via the RIDA®QUICK SCAN II. The time needs to be strictly adhered to. Measurement before or after completion of the **15 (+ max. 2) minutes** incubation time can lead to wrong results.
4. Color development of the lines can change during the entire development time and after drying. The color of the lines can vary from red to blue-violet/grey as the strip dries.

EVALUATION AND INTERPRETATION OF THE RESULTS

The read out is performed on the RIDA®QUICK SCAN II (also see RIDA®QUICK SCAN II-manual).

If the sample has previously been diluted by a factor of 4 (final dilution 1:2.000), the result of the RIDA®QUICK SCAN II must be multiplied by four in order to obtain the actual IFX concentration (in µg/ml) in the blood.

The control line (marked with C on the test cassette) has to show up in each run. In case this band is missing, please follow the instructions according to the ‘Quality Control’ chapter.
The test line (marked with T on the test cassette) shows up depending on the infliximab concentration of the sample after different incubation times and with different intensities.

Only after the total run time of 15 (+ max. 2) minutes the final test result can be determined by using the RIDA®QUICK SCAN II. The incubation time and time point for the read out must be strictly adhered to.

The bands can change during the total incubation time and may also change after drying. The color of the band can vary from red to blue-violet/grey.

LIMITATIONS OF THE METHOD

The IFX-Fast kit 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 RIDA®QUICK 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. 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]

ANALYTICAL PERFORMANCE CHARACTERISTICS

Analytical sensitivity

For the determination of the analytical sensitivity, three control samples were tested with one dilution series each in two different batches and the IFX concentrations were determined using the RIDA®QUICK SCAN II.
The detection limit is less than 0.5 µg/ml IFX.

Analytical Specificity

Interference

Bilirubin (50 mg/l), cholesterol (2.5 g/l), triglycerides (5 g/l) and hemoglobin (200 mg/l) did not have any effect on the test results when they are present in human serum samples at the indicated concentrations.

Cross-reactions

To identify antibodies that are potentially cross-reacting with the IFX-Fast kit, the reactivity of a collection of antibodies measured in duplicate has been evaluated (Table below):

Prospect candidate for cross-reactivity	Concentration [µg/ml]	Cross reactivity
Adalimumab (14 µg/ml)	< 0.5	NO
Golimumab (14 µg/ml)	< 0.5	NO
HAMA (15 µg/ml)	< 0.5	NO
HAMA (0.6 µg/ml)	< 0.5	NO
HAMA (0.3 µg/ml)	< 0.5	NO

None of the prospect candidates for cross-reactivity tested showed a positive result with IFX-Fast kit.

Precision

Intra-assay precision

The intra-assay precision was tested using five references with 20 replications each. The IFX concentrations were determined using the RIDA®QUICK SCAN II and the resulting mean values (MV), the standard deviations (SD) and the coefficient of variation (CV) of the readings were calculated for each sample.
The results are listed in the following table.

Reference	1	2	3	4	5
MV (µg/ml)	0.93	3.12	5.23	7.09	8.76
SD	0.10	0.51	0.82	0.74	0.89
CV (%)	**11.2**	**16.5**	**15.6**	**10.4**	**10.2**

Inter-assay precision

The inter-assay precision was tested using five references with 40 replications each. The tests were carried out by three different operators on ten different test days in two runs each day (morning and afternoon). The IFX concentrations were determined using the RIDA®QUICK SCAN II and the resulting mean values (MV), the standard deviations (SD) and the coefficient of variation (CV) of the readings were calculated for each sample.
The results are listed in the following table:

Reference	1	2	3	4	5
MV (µg/ml)	0.95	2.77	4.52	6.28	8.31
SD	0.13	0.41	0.74	0.82	1.30
CV (%)	**13.7**	**14.7**	**16.3**	**13.0**	**15.6**

Detection rate

Detection rate for Remicade®

Three samples were mixed with each of the four different Remicade® quantities and the IFX concentrations were determined using the RIDA®QUICK SCAN II.
The mean detection rate is 100%.

The results are listed in the following table:

Sample	(µg/ml)	Addition of IFX (µg/ml)	Measured value (µg/ml)	Target value (µg/ml)	Detection rate (%)
1	1.07	6.24	7.61	7.31	104
		1.56	2.47	2.63	94
		5.46	6.56	6.53	100
		3.90	5.32	4.97	107
Mean value					**101**
2	1.14	5.42	6.12	6.55	93
		4.64	5.88	5.78	102
		0.77	1.84	1.91	96
		3.87	5.30	5.01	106
Mean value					**99**
3	1.07	7.02	7.73	8.09	96
		2.34	3.45	3.41	101
		3.90	5.43	4.97	109
		3.12	4.16	4.19	99
Mean value					**101**

Detection rate for biosimilars

a) Detection rate for Remsima®

Three samples were mixed with each of the four different Remsima® quantities and the IFX concentrations were determined using the RIDA®QUICK SCAN II.

The mean detection rate is 106%.

The results are listed in the following tables:

Sample	(µg/ml)	Addition of IFX (µg/ml)	Measured value (µg/ml)	Target value (µg/ml)	Detection rate (%)
1	1.29	6.96	8.59	8.25	104
		1.74	2.93	3.03	97
		6.09	7.55	7.38	102
		4.35	6.18	5.64	110
Mean value					**103**
2	1.31	6.08	8.05	7.39	109
		5.21	6.89	6.52	106
		0.87	2.13	2.18	98
		4.34	6.27	5.65	111
Mean value					**106**

Sample	(µg/ml)	Addition of IFX (µg/ml)	Measured value (µg/ml)	Target value (µg/ml)	Detection rate (%)
3	1.30	7.82	9.43	9.12	103
		2.61	4.21	3.91	108
		4.35	6.47	5.65	115
		3.48	5.20	4.78	109
Mean value					**109**

b) Detection rate for Inflectra®

Three samples were mixed with each of the four different Inflectra® quantities and the IFX concentrations were determined using the RIDA®QUICK SCAN II.

The mean detection rate is 103%.

The results are listed in the following table:

Sample	(µg/ml)	Addition of IFX (µg/ml)	Measured value (µg/ml)	Target value (µg/ml)	Detection rate (%)
1	0.76	4.95	6.14	5.71	108
		1.24	2.15	2.00	108
		4.33	4.87	5.09	96
		3.09	3.80	3.85	99
Mean value					**102**
2	0.76	4.33	5.00	5.09	98
		3.71	4.35	4.47	97
		0.62	1.49	1.38	108
		3.09	4.16	3.85	108
Mean value					**103**
3	0.80	5.53	6.80	6.33	107
		1.84	2.67	2.65	101
		3.07	4.01	3.88	103
		2.46	3.55	3.26	109
Mean value					**105**

c) Detection rate for Flixabi®

Three samples were mixed with each of the four different Flixabi® quantities and the IFX concentrations were determined using the RIDA®QUICK SCAN II.

The mean detection rate is 93%.

The results are listed in the following table:

Sample	(µg/ml)	Addition of IFX (µg/ml)	Measured value (µg/ml)	Target value (µg/ml)	Detection rate (%)
1	1.02	7.57	7.04	8.58	82
		1.89	2.49	2.91	86
		6.62	6.72	7.64	88
		4.73	5.43	5.75	95
Mean value					**88**
2	1.14	6.53	7.06	7.67	92
		5.60	6.16	6.74	91
		0.93	2.18	2.07	105
		4.67	5.24	5.81	90
Mean value					**95**
3	1.14	8.40	9.62	9.54	101
		2.80	3.89	3.94	99
		4.67	5.36	5.81	92
		3.73	4.41	4.87	90
Mean value					**96**

Correlation with reference assay

The concentration of 20 IFX-positive samples in the concentration range of 1 µg/ml to 12 µg/ml was measured using the RIDASCREEN® IFX Monitoring as predicate and the IFX-Fast. The correlation coefficient was $R^2 = 0.98$ (Figure 1).

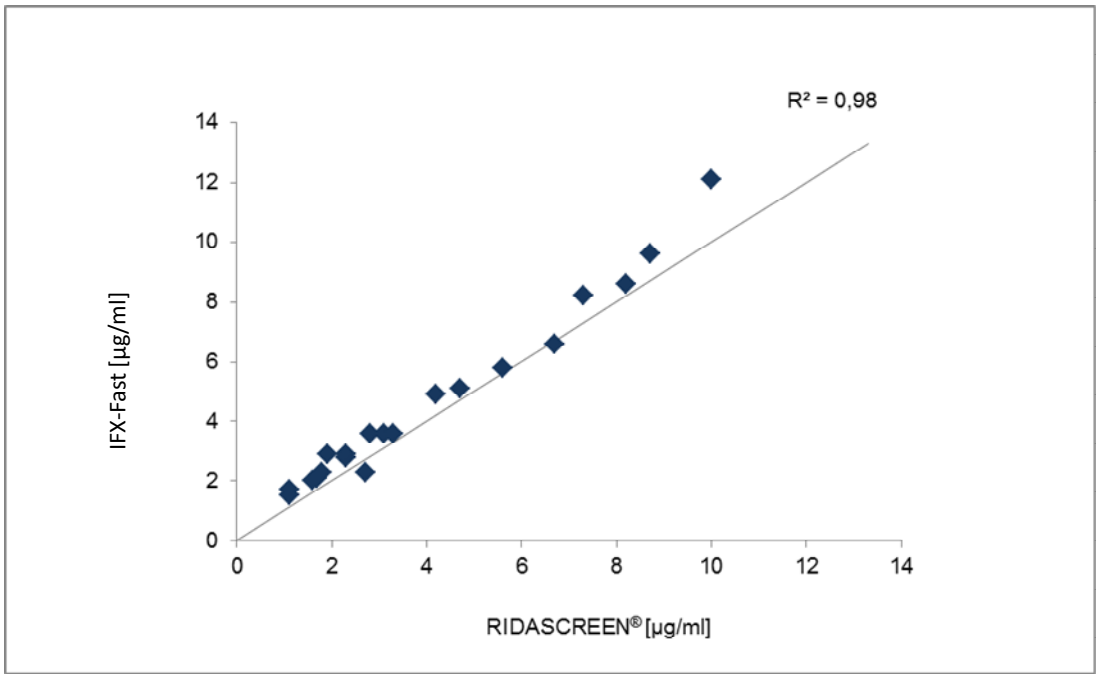

Figure 1. The IFX-Fast kit shows an excellent correlation ($R^2 = 0.98$) with the RIDASCREEN® IFX Monitoring (n=20).

LITERATURE

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.

IFX-Fast
25 tests, Ref. 9191TI

Revision date: 20.05.2022
Rev0

Eurospital SpA
34147 Trieste, via Flavia 122
Tel. +39 040 8997.1 - Fax +39 040 280944
www.eurospital.com - info@eurospital.it

LEGEND

IVD	In vitro diagnostic medical device
CE	European conformity
eIFU	Consult electronic instructions for use
LOT	Batch number
	Use by
	Temperature limitation
REF	Catalogue number
Σ	Sufficient for
	Manufacturer
	Disposable
CASSETTE	Test Cassette
DILUENT	Sample dilution buffer
REAGENT | A	Reagent A
REAGENT | B	Reagent B