ADM-Fast

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

REF 9191TA

INTENDED PURPOSE

ADM-Fast is a lateral flow immunochromatographic assay for the quantitative detection of adalimumab (ADM, Humira®) in human serum and plasma.
For professional use only

SUMMARY AND EXPLANATION OF THE TEST

Therapeutic drug monitoring

Adalimumab (ADM) is a fully human monoclonal antibody that targets the pro-inflammatory cytokine TNFα. The drug is used to treat chronic inflammatory diseases such as inflammatory bowel disease, rheumatoid arthritis, spondyloarthritis and plaque psoriasis. Adalimumab has been shown to trigger long-term remission and improve the patient's quality of life. [1] In some patients, however, ADM treatment does not work (primary non-responders); in others, it loses its effectiveness over time (secondary non-responders). [2]
A drug can be effective only if there are adequate concentrations in the blood. The serum concentration of adalimumab just before the next injection, defined as the trough concentration, has been used for therapeutic drug monitoring (TDM). Current publications on TDM have shown that there is a relationship between good clinical efficacy and adequate trough concentration in inflammatory bowel disease [3] and rheumatoid arthritis patients. TDM may therefore be very instrumental to optimize treatment and to overcome secondary loss of response. [4]

The ADM-Fast kit uses a highly specific monoclonal antibody (MA-ADM40D8, isolated and characterized at the KU Leuven). [5] It detects only adalimumab; other anti-TNF drugs such as infliximab and golimumab do not interfere with the measurement.

Chronic inflammatory bowel diseases

Induction therapy phase: Induction therapy with adalimumab consists of a subcutaneous dose of 160 mg in week 0, followed by 80 mg in week 2, and 40 mg every other week from week 4 onwards. Upon good clinical response at week 12 - 14, treatment is continued (maintenance).

Maintenance treatment phase: It has been shown that patients receiving maintenance treatment with consistent trough concentrations are more likely to remain in remission than patients with non-detectable trough concentrations. [6,7,8] Regular monitoring of ADM trough concentrations during the maintenance treatment phase can be useful to check ADM treatment and adjust it, if necessary.
Patients with low or undetectable drug concentrations may benefit from a dose increase or interval shortening, while the interval in patients with very high ADM concentrations can be safely prolonged. [9,10] For adalimumab, a target therapeutic trough concentration window of 5 - 10 µg/ml has been recommended.[9]

Immunogenicity

Due to the immunogenic character of the drug, antibodies targeting the drug often develop. This is also the reason for a secondary loss of response. [6] In the case of undetectable trough concentrations, subsequent measurement of anti-drug antibodies may be helpful to determine a suitable treatment strategy.

TEST PRINCIPLE

ADM is detected via the formation of an antibody-antigen sandwich with MA-ADM40D8 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 ADM concentration calculated by using the standard curve which is stored in the instrument.

COMPONENTS

Materials provided

The reagents in the kit are sufficient for 25 determinations.

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 ADM-Fast can be ordered separately. ADM-Fast Control Set (cod. 9191AQ) contains two 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 ADM-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
- Test tubes for sample suspension (two per patient specimen)
- Micropipettes with disposable tips, 10 – 100 µl and 100 – 1000 µl
- Stopwatch
- Waste container with a 0.5% sodium-hypochlorite solution
- RIDA®QUICK SCAN II (cod. ZRQS2-KD)
- Vortex mixer

STORAGE BEFORE USE

The kit must be stored at 2 - 8°C and can be used until the printed date of expiration.
Do not allow reagents to remain at room temperature for any length of time.
After use, store them as quickly as possible at 2 - 8°C.
After the expiration date, the quality guarantee is no longer valid.
If the outer packaging is damaged, the usability of the test cassette cannot be guaranteed.

WARNINGS AND PRECAUTIONS

- The ADM-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 SAMPLE COLLECTION

In this assay, citrate plasma samples and serum samples can be used.

Following blood collection, the serum should be separated from the clot as quickly as possible to prevent hemolysis. Transfer the specimens into a clean storage tube.

STORAGE OF SAMPLE

Specimens can be stored at 2 - 8°C for three to four 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 band (labelled C on the test cassette) must appear each time the test is 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 band 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 must 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.
- RIDA®QUICK SCAN II must be switched on prior to starting the test. On initial use, the test method must be scanned in via a barcode reader and is then stored for further measurements on 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 ADM-Fast kit is 0.5 – 25 µg/ml.

1. First dilute 20 µl of the specimen in 980 µl of Diluent (1:50). Mix the reaction well.

2. In a separate reaction vial, mix 90 µl of Reagent A (blue fluid, bottle with blue lid) with 90 µl of Reagent B (yellow fluid, bottle with transparent lid). If multiple test strips are processed, the solution can also be used for several samples at the same time. The mixture of Reagent A (blue fluid) and Reagent B (yellow fluid) produces a green-colored solution.
3. Pipette 20 µl of the diluted specimen solution (point 1) into the 180 µl of reagents A and B mixture, which is equivalent to a further dilution of the sample of 1:10 (point 1). This produces a 1:500 final dilution of the starting sample.
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** using RIDA®QUICK SCAN II. The time needs to be strictly adhered to. Measurements taken 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.

The control band (labelled C on the test cassette) must appear each time the test is run. In case this band is missing, please follow the instructions according to the ‘Quality Control’ chapter.

Depending on the adalimumab concentration in the samples, the signal band (labelled T on the test cassette) may appear after different durations and in differing 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 bands may change colors during the total test time and after drying. 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 ADM-Fast kit detects the free, functionally active proportion of ADM and not the proportion of ADM that is bound to anti-adalimumab antibodies, because of immunogenicity.

Individual adalimumab concentrations, measured using the ADM-Fast kit, 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.

ANALYTICAL PERFORMANCE CHARACTERISTICS

Analytical sensitivity

To determine analytical sensitivity, four control samples in three replicates each were tested over three days.
The limit of quantification (LoQ) is 0.32 µg/ml ADM.

Analytical Specificity

Interference

The presence of bilirubin (50 mg/L), cholesterol (2.5 g/L), triglycerides (5 g/L), and hemoglobin (200 mg/L) in the displayed concentrations in human serum specimens had no effect on the test results.

Cross-reactions

To identify antibodies that are potentially cross-reacting with the ADM-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
Infliximab (24 µg/ml)	< 0.5	NO
Golimumab (24 µg/ml)	< 0.5	NO

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

Precision

Intra-assay precision

Intra-assay precision was tested using 5 references in 20 replicates each. The ADM concentrations were determined using RIDA®QUICK SCAN II, from which the mean values (MV), the standard deviations (SD), and the coefficients of variation (CV) of the measurements were calculated for each sample.

The results are listed in the following table.

Reference	1	2	3	4	5
MV (µg/ml)	2.32	5.72	8.88	14.32	25.72
SD	0.39	0.70	0.76	1.50	3.03
CV (%)	**16.8**	**12.2**	**8.5**	**10.4**	**11.8**

Inter-assay precision

Inter-assay precision was tested using 5 references in 40 replicates each. The tests were conducted by three different operators on 10 different test days in two runs per day (morning and afternoon). The ADM concentrations were determined using RIDA®QUICK SCAN II, from which the mean values (MV), the standard deviations (SD), and the coefficients of variation (CV) of the measurements were calculated for each specimen.
The results are listed in the following table.

Reference	1	2	3	4	5
MV (µg/ml)	2.28	5.58	8.78	13.37	24.73
SD	0.27	0.93	0.95	1.98	2.99
CV (%)	**11.8**	**16.6**	**10.8**	**14.8**	**12.1**

Detection rate

Detection rate for Humira®

Three samples were mixed with each of the four different Humira® quantities and the ADM concentrations were determined using the RIDA®QUICK SCAN II.
The mean detection rate is 101%.

The results are listed in the following table:

Specimen	[µg/ml]	Added ADM [µg/ml]	Measured value [µg/ml]	Target value [µg/ml]	Recovery (%)
1	2.54	15.20	16.41	17.75	92
		3.80	7.06	6.34	111
		13.30	16.17	15.85	102
		9.50	11.92	12.04	99
Mean value					**101**
2	2.67	13.22	15.96	15.88	100
		11.33	13.95	13.99	100
		1.89	5.02	4.55	110
		9.44	12.22	12.11	101
Mean value					**103**
3	2.62	17.03	18.38	19.65	94
		5.68	8.81	8.30	106
		9.46	12.38	12.08	102
		7.57	10.44	10.19	102
Mean value					**101**

Correlation with the reference assay

Seventy-nine ADM-positive patient specimens ranging in concentrations between 0.5 µg/ml and 25µg/ml were measured using RIDASCREEN® ADM Monitoring as predicate and ADM-Fast kit, and the concentration was determined.
The calculated correlation coefficient was $R^2 = 0.95$. The results are shown in figure 1.

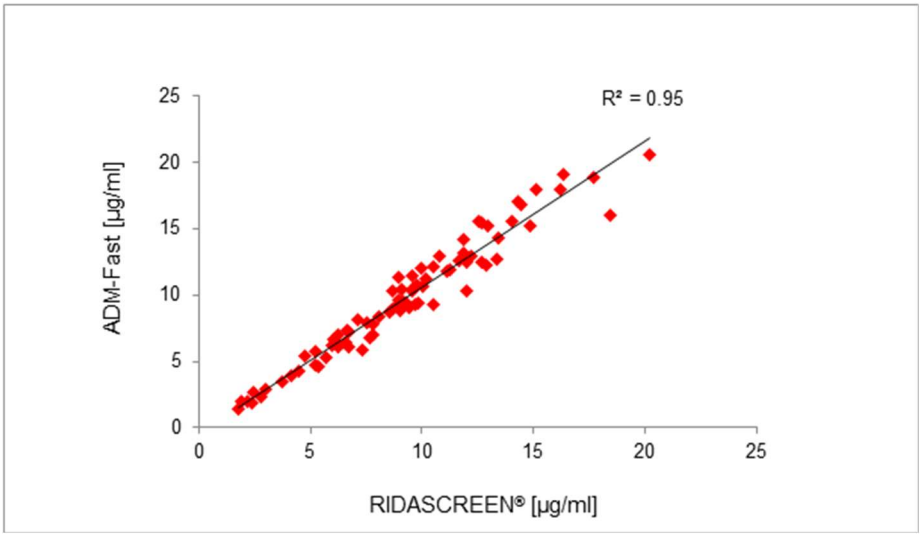

Figure 1. ADM-Fast Kit shows a very good correlation ($R^2 = 0.95$) with RIDASCREEN® ADM Monitoring (n = 79).

LITERATURE

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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