RIDASCREEN® ADM Monitoring

REF G09043

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1. Intended use

For *in vitro* diagnostic use. RIDASCREEN® ADM Monitoring is an enzyme immunoassay for the quantitative determination of adalimumab (ADM, Humira®) in human serum and plasma.

2. Summary and explanation of the test

Therapeutic Drug Monitoring

Adalimumab (ADM) is a fully human monoclonal antibody that targets the pro-inflammatory cytokine TNFα and is used to treat chronic inflammatory diseases like inflammatory bowel disease, rheumatoid arthritis, spondyloarthritis and plaque psoriasis. It has been shown that adalimumab can induce deep remission and improve the patient's quality of life. [1] Some patients do not respond to ADM 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 adalimumab just before the next injection, 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 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 RIDASCREEN® ADM Monitoring uses a highly specific monoclonal antibody (MA-ADM40D8, isolated and characterized at the KU Leuven). [5] It detects only adalimumab; it was shown that other anti-TNF drugs such as infliximab and golimumab do not interfere with the measurement. [5]
Biosimilars of adalimumab (Amgevita®, Imraldi®) are equally well quantified in the RIDASCREEN® ADM Monitoring.

Inflammatory bowel disease

Induction therapy of adalimumab consists of a subcutaneous dose of 160 mg at week 0, followed by 80 mg at 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 therapy phase: It has been shown that patients with sustained adalimumab concentrations during the maintenance therapy phase are more likely to stay in remission than patients with undetectable trough concentrations.[6,7,8] Thus, regularly checking ADM trough concentrations during maintenance therapy may be useful to evaluate the ADM treatment schedule and make adjustments when 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]

Patient samples withdrawn during the induction therapy phase (usually at week 2 and week 4) 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. [6] In the case of undetectable trough concentrations, subsequent measurement of anti-drug antibodies may be helpful to determine the optimal treatment strategy.
RIDASCREEN® Anti-ADM Antibodies (G09044) can be used for this analysis.

3. Test principle

In RIDASCREEN® ADM Monitoring, a highly specific monoclonal antibody against ADM (MA-ADM40D8, 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' sample which is to be tested is pipetted into the well of the microwell plate and incubated. During this incubation step, ADM binds specifically to the TNFα on the plate. After washing, a second incubation step follows with MA-ADM40D8, which is conjugated with horseradish peroxidase. In the presence of ADM, a sandwich complex is formed between immobilized TNFα, ADM 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 ADM 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 TNFα
Standard |1-6	1300 µl	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
Low Control| +	1300 µl	Low positive control; contains 30 ng/ml ADM and 0.09 % NaN_3; ready for use
Control| +	1300 µl	Positive control; contains 70 ng/ml ADM 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-ADM40D8); 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_2SO_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 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 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 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 | 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 concentration 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, ADM concentrations between 0.5 and 12 µg/ml can be determined.

b) Measurement of trough concentration during therapy induction phase

To measure trough concentrations during induction therapy (typically at week 2 and week 4) 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 add 100 µl of this solution to 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, ADM 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 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 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 reagent 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 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 ADM 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® ADM Monitoring is achieved by a 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 ADM 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 ADM 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 ADM 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 ADM100.met.
For dilution 1:400 select: RIDA®SOFT Win.net method ADM400.met.

The concentration is reported in µg/mL.

12. Limitations of the method

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

Individual adalimumab concentrations, measured using the RIDASCREEN® ADM 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 5 - 10 µg/mL is recommended. [9] 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 achieve mucosal healing and avoid future undetectable drug concentrations and ADA development. [9]

13. Performance characteristics

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

Standard	O.D.
1	0.014
2	0.078
3	0.151
4	0.345
5	1.055
6	2.101

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 ADM 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	4
Mean (µg/ml)	0.83	1.49	3.58	9.29
SD	0.06	0.15	0.30	0.92
% CV	**7.6**	**10.1**	**8.4**	**9.9**

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 ADM 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	4
Mean (µg/ml)	0.66	1.48	3.55	9.82
SD	0.09	0.11	0.37	1.10
% CV	**14.2**	**7.6**	**10.3**	**11.2**

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 ADM, resulting in a specificity of 100 %.

13.3.2. Interference

A panel of 35 potentially interfering samples was tested consisting of HAMA positive, lipemic, high bilirubin, high cholesterol, high total protein, 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: infliximab and golimumab.

13.4. Analytical sensitivity

The minimal detectable concentration of ADM is lower than 1 ng/ml. Taking into account a dilution factor of 1:100 this corresponds to 0.1 µg/ml.

13.5. Recovery

15 ADM-negative samples (5 negative serum samples, 5 negative EDTA plasma samples, 5 negative citrate plasma samples) were spiked with three different ADM-concentrations (8.0 µg/ml, 5.5 µg/ml and 1.5 µg/ml).
Based on the OD values of this measurement, the ADM concentration was determined using the standard curve and the recovery calculated. The mean recovery is 102.4 %.

Sample	Nr.	ADM (µg/ml)	Recovery (%)
Reference value 8.2 µg/ml			
Serum	1	9.3	113.9 %
	2	8.8	107.6 %
	3	7.4	89.8 %
	4	8.4	102.8 %
	5	7.3	89.0 %
EDTA Plasma	6	8.9	107.9 %
	7	9.0	110.1 %
	8	8.5	103.5 %
	9	8.0	97.7 %
	10	7.8	95.1 %
Citrate Plasma	11	8.3	101.2 %
	12	8.6	104.5 %
	13	9.1	111.0 %
	14	8.0	97.8 %
	15	7.6	92.6 %
Reference value 5.8 µg/ml			
Serum	1	4.7	81.4 %
	2	5.4	93.1 %
	3	6.0	102.8 %
	4	6.5	112.1 %
	5	5.1	88.3 %
Sample	**Nr.**	**ADM (µg/ml)**	**Recovery (%)**
EDTA Plasma	6	5.5	94.1 %
	7	4.9	84.5 %
	8	5.5	94.5 %
	9	5.3	90.7 %
	10	6.6	113.1 %
Citrate Plasma	**11**	5.7	97.8 %

	12	6.0	103.1 %
	13	5.4	92.6 %
	14	5.3	90.9 %
	15	6.2	107.2 %
Reference value 1.5 µg/ml			
Serum	1	1.5	100.7 %
	2	1.6	105.3 %
	3	1.5	102.0 %
	4	1.8	117.1 %
	5	1.7	111.5 %
EDTA Plasma	6	1.5	97.3 %
	7	1.7	114.0 %
	8	1.6	106.0 %
	9	1.7	114.4 %
	10	1.8	117.7 %
Citrate Plasma	11	1.7	115.3 %
	12	1.5	99.3 %
	13	1.8	117.9 %
	14	1.7	115.5 %
	15	1.6	104.9 %
Mean			**102.4 %**

13.6. Correlation with reference assay and diagnostic sensitivity

Two clinical sample panels of 20 and 21 specimens respectively were analyzed using the RIDASCREEN® ADM Monitoring and results were compared with data obtained using the ADM ELISA developed at the KU Leuven which served as reference assay. Pearson r values as indicator for the correlation between both assays were 0.99 and 0.97, respectively.
All samples having measurable ADM levels according to the reference assay, were detected positive (16 samples for panel 1, 19 samples for panel 2) resulting in a diagnostic sensitivity of 100 %.

14. Version history

Version number	Chapter and description
2019-11-04	2. Summary and Explanation of the test 9.4 First incubation

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
Σ	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. Restellini S, Chao CY, Lakatos PL, et al. Therapeutic drug monitoring guides the management of Crohn's patients with secondary loss of response to adalimumab. Inflamm Bowel Dis. 2018;24:1531-1538.
5. Bian S, Van Stappen T, Baert F, et al. Generation and characterization of a unique panel of anti-adalimumab specific antibodies and their application in therapeutic drug monitoring assays. J Pharm Biomed Anal 2016;125:62-67.
6. 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.
7. Baert F, Vande Castele N, Tops S, Noman M, Van Assche G, Rutgeerts P, Gils A, Vermeire S, FerranteM. Prior response to infliximab and early serum drug concentrations predict effects of adalimumab in ulcerative colitis. Aliment Pharmacol Ther. 2014 :40(11-12):1324-32.
8. Vande Casteele N, Gils A. Pharmacokinetics of anti-TNF monoclonal antibodies in inflammatory bowel disease: Adding value to current practice. J Clin Pharmacol. 2015;55:S39-S50.
9. Papamichael K, Cheifetz A. Use of anti-TNF drug levels to optimize patient management. Frontline Gastroenterol 2016;7:289-300.
10. Vande Casteele N, Feagan BG, Gils A, Vermeire S, Khanna R, Sandborn WJ, Levesque BG. Therapeutic drug monitoring in inflammatory bowel disease: current state and future perspectives. Curr Gastroenterol Rep 2014;16:378.