RIDASCREEN® GLM Monitoring

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1. Intended use

For *in vitro* diagnostic use. RIDASCREEN® GLM Monitoring is an enzyme-linked immunosorbent assay (ELISA) for the quantitative determination of golimumab (GLM, Simponi®) in human serum and plasma.

2. Summary and explanation of the test

Therapeutic Drug Monitoring

Golimumab (GLM) is a human monoclonal antibody, derived from TNF-alpha immunized transgenic mice engineered to express human IgGs.[1] Golimumab binds to both the soluble and transmembrane bioactive forms of human TNF-alpha, giving rise to stable high-affinity complexes thereby preventing the binding of TNF-alpha to its receptors.[2] Golimumab has been approved for the treatment of various chronic immune-mediated inflammatory disorders in which TNF-alpha plays an important role, including rheumatoid arthritis (RA), psoriatic arthritis, ankylosing spondylitis and ulcerative colitis (UC).
A drug can only exert its pharmacologic effect when adequate concentrations are achieved in the circulation. The serum concentration of golimumab just before the next infusion, defined as the trough concentration, has been used for therapeutic drug monitoring (TDM). Recent data on TDM have shown a positive relation between GLM trough serum concentrations and clinical outcomes in patients with RA[3] and UC[4]. TDM could, therefore, be an important option for optimizing treatment.
RIDASCREEN® GLM Monitoring uses a highly specific monoclonal antibody (MA-GOM171D8), which was isolated and characterized at the KU Leuven. It detects only golimumab; other anti-TNF drugs (like infliximab, adalimumab) do not interfere with the measurement.

Ulcerative colitis

The diagnostic value of TDM in UC patients is described below.

In most European countries, patients receive the same induction treatment in daily clinical practice (200 mg at week 0 and 100 mg at week 2) followed by a body weight-based dose stratification during maintenance, i.e. 50 mg every four weeks for patients with a body weight of less than 80 kg and 100 mg golimumab every four weeks for patients with a body weight of at least 80 kg. Results from PURSUIT and real-world observational studies reported clinical response rates of around 50 % after golimumab induction therapy.[5-7] An exposure-response relationship was observed, as patients with higher drug exposure were more likely to achieve improved outcomes.[4,8] GLM trough concentration measurements during or shortly after induction may thus be used to identify undertreated patients. In PURSUIT-M, week 6 non-responders had lower serum drug levels compared to responders at week 6.

These early non-responders received 100 mg golimumab maintenance, and their drug exposure was increased to levels comparable with that of responders by week 14.[9]
Since July 2018, the posology has therefore changed and patients with an inadequate response to induction can be dose increased to 100 mg at week 6 and every four weeks thereafter. Regularly checking GLM trough concentrations during induction or maintenance therapy may thus be useful to evaluate the GLM treatment schedule.

Immunogenicity

Currently, it is unclear if loss of response to GLM is due to formation of anti-drug antibodies since studies have reported a low rate of immunogenicity.[10] However, in the case of undetectable trough concentrations, subsequent measurement of anti-drug antibodies may be helpful to determine the optimal treatment strategy.

3. Test principle

In RIDASCREEN® GLM Monitoring, a highly specific monoclonal antibody against GLM (MA-GOM171D8, 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, GLM binds specifically to the TNFα on the plate. After washing, a second incubation step follows with MA-GOM171D8, which is conjugated with horseradish peroxidase. In the presence of GLM, a sandwich complex is formed between immobilized TNFα, GLM 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 GLM present in the sample.

4. Reagents provided

One kit is sufficient for 96 determinations (Table 1).

Table 1: Reagents provided

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 GLM; contains 0.09 % NaN_3; ready for use
Low Control| +	1300 µl	Low positive control; contains 30 ng/ml GLM and 0.09 % NaN_3; ready for use
Control| +	1300 µl	Positive control; contains 70 ng/ml GLM 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-GOM171D8); 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 maintained for 1 month. 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 with the desccant and stored at

2 – 8 °C until all the strips have been used. 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.
Some ingredients of the standards and positive control mixtures are derived from materials of biological origin. No known tests can guarantee that such materials are completely free from infectious agents. Therefore, calibrators and positive control mixtures, as well as patient samples and all materials coming into contact with them, should be treated as potentially infectious.
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 which is irritant. Avoid contact with the skin and clothing. In case of contact with eyes or skin, rinse with plenty of water and seek medical advice.
The reagents contain NaN_3 as a preservative. This substance must not be allowed to come into contact with the skin or mucous membrane. In order to prevent the formation of potentially explosive metal azides in laboratory plumbing, flush drains thoroughly after disposal of these solutions.

The substrate contains hydrogen peroxide.

For further details, see the safety data sheets (SDSs) at www.r-biopharm.com.

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 six months. 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 six months (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, 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, GLM 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 or to measure intermediate drug concentrations, or concentrations > 12.0 µg/mL, the samples are diluted 1:200:
10 µl of the sample is diluted in 1990 µl sample dilution buffer Diluent (1:200).
100 µl of this final diluted sample is then used in the test.

If the sample is diluted 1:200, GLM concentrations between 1.0 and 24 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 (all recommended in duplicate) 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 1:200 is used (induction therapy phase):

Concentration for the Low positive control Low Control | +:
6 µg/ml, range 4 - 8 µg/ml

Concentration for the Positive control Control | +:
14 µg/ml, range 10 - 20 µg/ml

For calculating the GLM 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® GLM 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 GLM 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 GLM concentration in the undiluted sample is then 6 µg/ml.

Example: The outcome of 1:200 diluted sample, obtained by interpolation from the calibration curve is 80 ng/ml. The corresponding GLM concentration in the undiluted sample is then 16 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 GLM100.met.
For dilution 1:200 select: RIDA®SOFT Win.net method GLM200.met.

The concentration is reported in µg/ml.

12. Limitations of the method

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

Individual golimumab concentrations, measured using the RIDASCREEN® GLM 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.

13. Performance characteristics

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

Table 2: Example of typical optical density values

Standard	O.D.
1	0.007
2	0.119
3	0.233
4	0.508
5	1.387
6	2.483

13.2. Precision

13.2.1. Intra-Assay Precision

The intra-assay-precision was determined in a single run using 4 references in 20 replicates each. From the OD-values of these measurements GLM 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 Table 3 below.

Table 3: Intra-assay precision

Reference	1	2	3	4
Mean (µg/ml)	0.64	1.33	3.35	9.29
SD	0.03	0.06	0.12	0.43
% CV	**4.8**	**4.2**	**3.6**	**4.6**

13.2.2. Inter-Assay Precision

The inter-assay-precision was determined in 5 runs using 4 references in 20 replicates each. From the OD-values of these measurements GLM 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 Table 4 below.

Table 4: Inter-assay precision

Reference	1	2	3	4
Mean (µg/ml)	0.66	1.32	3.21	8.20
SD	0.04	0.05	0.13	0.73
% CV	**6.3**	**4.1**	**3.9**	**8.9**

13.3. Specificity

13.3.1. Normal human serum/plasma

Specificity was determined by testing 100 healthy donor samples from Dutch and Belgian origin. None of the samples showed a detectable concentration of GLM, resulting in a specificity of 100 %.

13.3.2. Interference

A panel of 24 potentially interfering samples was tested consisting of HAMA positive, lipemic, high cholesterol, hemolyzed and first semester pregnant women samples. No interaction with the investigated factors was observed.
A panel of 27 rheumatoid factor positive clinical samples was tested and in 3 samples a detectable level of GLM, not higher than 0.2 µg/mL, was measured. Thus, rheumatoid factor might interfere in the RIDASCREEN® GLM Monitoring, but only to a small extent not exceeding the lower limit of quantification of the assay.

13.3.3. Cross-reactivity

No cross-reactivity has been observed for following biopharmaceuticals applied for treating auto-immune diseases: infliximab, adalimumab, vedolizumab and ustekinumab.

13.4. Analytical sensitivity

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

15 GLM-negative samples were spiked with varying GLM concentrations. Based on the OD values of this measurement, the GLM concentration was determined using the standard curve and the recovery calculated. The mean recovery is 93 % (Table 5).

Table 5: Recovery of 15 GLM-negative samples spiked with varying GLM concentrations

Nr.	GLM (µg/ml)	Recovery (%)
Reference value 1.71 µg/ml		
1	1.59	93 %
2	1.50	88 %
3	1.40	82 %
4	1.51	88 %
5	1.67	97 %
6	1.74	102 %
7	1.62	95 %
8	1.47	86 %
9	1.73	101 %
10	1.66	97 %
11	1.43	84 %
12	1.51	88 %
13	1.59	93 %
14	1.55	90 %
15	1.56	91 %
Reference value 3.46 µg/ml		
1	2.80	81 %
2	3.01	87 %
3	2.79	81 %
4	3.19	92 %
5	2.96	86 %
6	2.93	85 %
7	3.59	104 %
8	3.12	90 %
9	3.50	101 %
10	3.49	101 %
11	3.15	91 %
12	3.30	95 %
13	3.36	97 %
14	2.85	82 %
15	2.86	83 %

Nr.	GLM (µg/ml)	Recovery (%)
Reference value 10.01 µg/ml		
1	10.75	107 %
2	9.97	100 %
3	10.30	103 %
4	9.75	97 %
5	9.84	98 %
6	10.63	106 %
7	9.29	93 %
8	9.06	90 %
9	8.85	88 %
10	8.86	88 %
11	9.98	100 %
12	9.10	91 %
13	9.33	93 %
14	9.61	96 %
15	10.21	102 %
Mean		**93 %**

13.6. Correlation with reference assay and diagnostic sensitivity

A clinical sample panel of 18 specimens was analyzed using the RIDASCREEN® GLM Monitoring and the results were compared with those of the reference assay (GLM ELISA developed at KU Leuven). A correlation coefficient of 0.91 was determined.
All samples having measurable GLM levels according to the reference assay were detected positive (18 specimens) resulting in a diagnostic sensitivity of 100 %.

14. Version history

Table 6: Version history

Version number	Chapter and description
2019-12-10	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

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