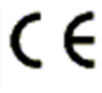




*In vitro* diagnostic kit

Golimumab ELISA



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# Golimumab ELISA

REF 710401

IVD

The Golimumab ELISA is an enzyme linked immunosorbent assay intended for the quantitative determination of Golimumab ( Simponi<sup>®</sup>, anti- TNF $\alpha$ ) in human serum and plasma.

## 1. BACKGROUND AND DIAGNOSTIC VALUE

Golimumab (GLM) is a human monoclonal antibody, derived from TNF- $\alpha$  immunized transgenic mice engineered to express human IgGs.<sup>1</sup> 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.<sup>2</sup> 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<sup>3</sup> and UC<sup>4</sup>. TDM may therefore be very instrumental to optimize treatment.

The Golimumab ELISA uses a highly specific monoclonal antibody – clone 171D8, developed at the KU Leuven – that only detects golimumab (Simponi<sup>®</sup>). Other anti-TNF drugs (like infliximab, adalimumab) do not interfere with the measurement. As an example of TDM, the use of GLM trough concentration measurements in UC is described.

### Ulcerative colitis

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 patient 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<sup>5-7</sup> reported clinical response rates of around 50% after golimumab induction therapy. An exposure-response relationship was observed, as patients with higher drug exposure were more likely to achieve improved outcomes.<sup>4,8</sup> 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.<sup>9</sup> 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.<sup>10</sup> However, in the case of undetectable trough concentrations, subsequent measurement of anti-drug antibodies may be helpful to determine the optimal treatment strategy.

## 2. PRINCIPLE OF THE GOLIMUMAB ELISA

Microtiterstrips coated with TNF- $\alpha$  are incubated with calibrators, controls and diluted patient samples. During this incubation step golimumab binds specifically to the TNF- $\alpha$  on the solid phase. After removal of the unbound serum proteins by a washing procedure, the antigen-antibody complex in each well is detected with specific peroxidase-conjugated monoclonal antibody (clone 171D8, developed at the KU Leuven) directed to golimumab.

After removal of the unbound conjugate, the strips are incubated with a chromogenic solution containing tetramethylbenzidine and hydrogen peroxide: a blue colour develops in proportion to the amount of immunocomplex bound to the wells of the strips. The enzymatic reaction is stopped by the addition of 0.5M H<sub>2</sub>SO<sub>4</sub> and the absorbance values at 450 nm are determined.

A standard curve is obtained by plotting the absorbance values versus the corresponding calibrator values. The concentration of golimumab in patient samples is determined by interpolation from the calibration curve.

## 3. REAGENTS

Component	Name + Symbol
1 coated microtiter plate (12 x 8 strips)	Precoated Strips
Strips coated with human TNF- $\alpha$ .	<b>MTP</b>
6 vials, 1300 $\mu$ l, ready-to-use	Calibrator
Each vial contains a ready-to-use calibrator solution, N having following values: CAL 0: 0 ng/ml; CAL 5: 5 ng/ml; CAL 10: 10 ng/ml; CAL 20: 20 ng/ml; CAL 60: 60 ng/ml; CAL 120: 120 ng/ml. Contain 0,09 % NaN <sub>3</sub> .	<b>CAL N</b>
1 vial, 1300 $\mu$ l, ready-to-use	Positive Control 1
Positive Control for golimumab, level 1; contains 30 ng/ml golimumab. Contains 0.09% NaN <sub>3</sub> .	<b>CTL1</b>
1 vial, 1300 $\mu$ l, ready-to-use	Positive Control 2
Positive Control for golimumab, level 2; contains 70 ng/ml golimumab. Contains 0.09% NaN <sub>3</sub> .	<b>CTL2</b>
1 bottle, 100 ml, ready-to-use	Sample Diluent
Sample dilution buffer Contains 0.09% NaN <sub>3</sub> and an inert orange dye.	<b>DILSAM</b>
1 bottle, 12 ml, ready-to-use	Conjugate
Contains peroxidase conjugated monoclonal anti-golimumab clone 171D8 antibodies. Contains antimicrobial agents and an inert red dye.	<b>CONJ</b>
1 vial, 12 ml, ready-to-use	Chromogen Solution
Contains a solution of substrate (H <sub>2</sub> O <sub>2</sub> ) and chromogen (tetramethylbenzidine).	<b>CHROM</b>
1 bottle, 50 ml, 20x concentrated	Wash Solution
Contains detergent in phosphate buffered solution and antimicrobial agents.	<b>WASH 20x</b>
1 bottle, 6 ml, ready-to-use	Stop Solution
Consists of 0.5 M H <sub>2</sub> SO <sub>4</sub> .	<b>STOP</b>
2 plate covers	-

## 4. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Precision micropipettes and standard laboratory pipettes.
2. Clean standard laboratory volumetric glassware.
3. Clean glass or plastic tubes for the dilution of the samples.
4. A microtiterplate reader capable of measuring absorbance at 450 nm with reference filter at 600-650 nm.

## 5. WARNINGS AND PRECAUTIONS FOR USERS

1. For *in vitro* diagnostic use only. The kit has to be used by properly trained personnel in an appropriate laboratory setting.
2. Do not mix reagents or coated microtiterstrips from kits with different lot numbers.
3. Treat kit controls, calibrators and patient samples as if they are potentially infectious. Dispose of all materials used to perform this test according to appropriate safety regulations.
4. Wear disposable gloves and lab clothing when performing a test run.
5. Although it might be advised to run calibrators and controls in duplicate, reliable results are equally obtained by doing the analysis in singlicate.
6. Some kit components contain sodium azide as a preservative. In order to prevent the formation of potentially explosive metal azides in laboratory plumbing, flush drains thoroughly after disposal of these solutions.
7. Chromogen Solution contains the hazardous ingredient N-Methyl-2-pyrrolidone at a concentration of > 0,3 %. It is classified as a Reproductive Toxicant Category 1B.

Following hazard statements are applicable:

H360D: May damage the unborn child.

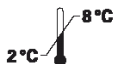
Following precautionary statements are applicable:

P280: Wear protective gloves/protective clothing/eye protection/face protection.

P308+P313: If exposed or concerned: Get medical advice/attention.

8. Stop Solution is a 0.5 M H<sub>2</sub>SO<sub>4</sub> solution which is irritant. In case of contact with eyes or skin, rinse with plenty of water.

9. Inform the manufacturer in case the kit is supplied with any damaged or improperly closed component.



## 6. STORAGE CONDITIONS

1. The kit and its components should be stored at 2-8 °C. Do not freeze.
2. Store the microtiter strips in their original package with the desiccant until all the strips have been used.
3. Opened components should be stored at 2-8°C until next use and can be maintained for 1 month.
4. Never use any kit components beyond the expiration date.

## 7. SPECIMEN COLLECTION AND PREPARATION

EDTA plasma, citrate plasma and serum samples may be used in this assay. Remove serum from clot as soon as possible to avoid haemolysis. Transfer the serum to a clean storage tube. Specimens may be stored at 2-8 °C for 3-4 days, or they can be stored frozen for at least 6 months. Avoid repeated freezing and thawing.

Samples must be diluted in sample diluent, see chapter 9.

## 8. ASSAY PROCEDURE

### 8.1 General Remarks

1. Use a separate disposable tip for each sample transfer to avoid cross-contamination.
2. All reagents must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
3. Once the assay has been started, all steps should be completed without interruption.
4. The use of an ELISA Washer is recommended, however depending on the apparatus it may be necessary to adapt the washing procedure for obtaining optimal results.
5. The Golimumab ELISA may be used on any open ELISA automate after validation. Depending on the reader capacity of the instrument, it might be required to reduce the incubation time for the Chromogen solution from 10 to 6 minutes (applicable for the Dynex DS2 and Dynex DSX instruments). For instructions on how to perform the assay with ELISA instruments, please contact the manufacturer.

### 8.2 Reconstitution of Reagents

**Wash Solution:** dilute 50 ml of concentrated Wash Solution to 1000 ml with distilled water. If stored at 2-8°C, the diluted Wash Solution can be used at least for 1 month. At higher temperatures, the concentrated Wash Solution may appear cloudy without affecting its performance. Upon dilution, the solution will be clear.

### 8.3 Assay Procedure

**Before starting the assay, dilute the patient samples according to the guidelines in chapter 9.**

1. Pipette 100 µl of the calibrators, controls and diluted samples into the wells.
2. Incubate the covered microtiterstrips for 60 ± 2 min at 37 °C (± 2 °C).
3. Empty the wells entirely by aspiration. Fill the wells completely with 350-400 µl of reconstituted washing solution, avoiding overflow of buffer from one well to another. Repeat the washing procedure two more times for a total of three washes. Finally, aspirate the content of the wells and remove any residual liquid by gently tapping the inverted wells on clean absorbent paper. Incomplete washing will adversely affect the test outcome.
4. Add 100 µl of Conjugate Solution and incubate the covered microtiterstrips for 30 ± 2 min at 37 °C (± 2 °C).
5. Repeat the washing procedure as described in 3.
6. Add 100 µl of Chromogen Solution to each well.
7. Incubate for 10 ± 1 min at 37 °C (± 2 °C). Avoid light exposure during this step.
8. Add 50 µl of Stop Solution to each well.
9. Determine the absorbance of each well at 450 nm with reference filter 600-650 nm within 30 min following the addition of Stop Solution.

## 9. SAMPLE DILUTION FACTOR

For measuring trough concentrations (samples taken just before next infusion) during maintenance phase, dilute samples 1:100.

Example: add 10 µl patient sample to 990 µl Sample Diluent

For measuring concentrations during induction phase or for measuring intermediate concentrations, dilute samples 1:200.

Example: add 10 µl patient sample to 1990 µl Sample Diluent.

The dilution factor must be taken into account when calculating golimumab concentration in the samples by multiplying the measured concentration by the dilution factor. For calculating the golimumab concentration in the controls, the same multiplicity factor must be used as for the samples. Concentration is then expressed in µg/ml.

Example: the outcome of 1:100 diluted sample, obtained by interpolation from the calibration curve is 60 ng/ml. The corresponding golimumab 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 golimumab concentration in the undiluted sample is then 16 µg/ml.

By diluting the samples 1:100, golimumab concentrations between 0.5 and 12 µg/ml can be determined. By diluting the samples 1:200, golimumab concentrations between 1 and 24 µg/ml can be determined.

Diluted samples may be stored for at least 8 HR.

## 10. RESULTS

The average absorbance value of each calibrator is plotted against the corresponding golimumab value and the best calibration curve (e.g. polygon) is constructed.

Use the average absorbance of each patient sample obtained in the Golimumab ELISA to determine the corresponding value by interpolation from the curve. Multiply the obtained value by the dilution factor.

Depending on the experience and/or availability of software, other methods of data reduction may be used.

## 11. PERFORMANCE CHARACTERISTICS

**Example of typical optical density (OD) values:**

CALIBRATOR	OD
CAL 0	0,007
CAL 5	0,119
CAL 10	0,233
CAL 20	0,508
CAL 60	1,387
CAL 120	2,483

### Precision

*Intra-assay variation (n=20; 1 run)*

	Level 1	Level 2	Level 3	Level 4
Mean (ng/ml)	6.4	13.3	33.5	92.9
SD	0.3	0.6	1.2	4.3
% CV	<b>4.8</b>	<b>4.2</b>	<b>3.6</b>	<b>4.6</b>

*Inter-assay variation (n=20; 5 runs; 5 days; 3 operators)*

	Level 1	Level 2	Level 3	Level 4
Mean (ng/ml)	6.6	13.2	32.1	82.0
SD	0.4	0.5	1.3	7.3
% CV	<b>6.3</b>	<b>4.1</b>	<b>3.9</b>	<b>8.9</b>

### Specificity – normal human serum/plasma

Specificity has been evaluated 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 %.

### Specificity – interference

A panel of 24 potentially interfering samples consisting of HAMA positive, high cholesterol, hemolyzed, lipemic and 1<sup>st</sup> semester pregnant women samples was tested. 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 Golimumab ELISA, but only to a small extent not exceeding the lower limit of quantification of the assay.

### Specificity – cross-reactivity

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

## Diagnostic sensitivity

A clinical sample panel of 18 specimens was analysed using the Golimumab ELISA and results were compared with data obtained using the GLM ELISA developed at the KU Leuven which served as reference assay. Pearson r value as indicator for the correlation between both assays is 0.91. All samples having measurable GLM levels according to the reference assay were detected positive (18 specimens) resulting in a diagnostic sensitivity of 100%.

## Analytical sensitivity

The limit of detection of GLM is 1 ng/ml. Taking into account a dilution factor of 1:100 this corresponds to 0.1 µg/ml.

## Test validity

The following specifications must be met for each run to be valid:

OD value for the zero calibrator: < 0.080

OD value for the highest value calibrator: > 1.400

*If multiplicity factor of 1:100 is applicable:*

Concentration value for positive control CTL1: 3 µg/ml, range 2 – 4 µg/ml

Concentration value for positive control CTL2: 7 µg/ml, range 5 – 10 µg/ml

*If multiplicity factor of 1:200 is applicable:*

Concentration value for positive control CTL1: 6 µg/ml, range 4 – 8 µg/ml

Concentration value for positive control CTL2: 14 µg/ml, range 10 – 20 µg/ml

If one of the specifications is not met, the test run should be repeated.

## 12. TROUBLE SHOOTING

In case of high background signal (OD CAL0 > 0.08), the washing was insufficient. Repeat the test with more vigorous washing (increased number of cycles, soak time).

## REFERENCES

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GLM11-21

## Version history

Version number	Description
GLM04-19	Original version
GLM11-21	Chapter 5, Adding additional warning and precaution clauses. Chapter 8.2, storage of diluted Wash Solution. Writing in full the manufacturer name, according to its legal status: Advanced Practical Diagnostics BV instead of the abbreviation apDia.