In vitro diagnostic kit

Adalimumab ELISA

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REF 710201 IVD

The Adalimumab ELISA is an enzyme linked immunosorbent assay intended for the quantitative determination of adalimumab (ADM, Humira®, anti-TNF-alpha) in human serum and plasma.

1. BACKGROUND AND DIAGNOSTIC VALUE

Therapeutic Drug Monitoring

Adalimumab (ADM) is a fully human antibody that targets the pro-inflammatory cytokine TNF-alpha 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. Some patients do not respond to ADM therapy upon induction (primary non-responders), while others lose response over time (secondary non-responders).

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 and rheumatoid arthritis patients. TDM may therefore be very instrumental to optimize treatment and to overcome secondary loss of response.

The Adalimumab ELISA uses a highly specific monoclonal antibody – Clone 40D8, developed at the K.U. Leuven – that only detects adalimumab). Other anti-TNF drugs (for example infliximab and golimumab) do no interfere with the measurement. Biosimilars of Humira® (Amgevita®, Imraldi®) are equally well quantified in the Adalimumab ELISA of Advanced Practical Diagnostics BV.

As an example of TDM, the use of adalimumab trough concentration measurements in inflammatory bowel disease patients is described.

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 phase: It has been shown that patients on maintenance therapy having sustained trough concentrations, are more likely to remain in remission than patients with undetectable trough concentrations. 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.

Due to the dosing regimen, trough concentrations during induction at w2 and w4 are higher and serum samples need to be diluted more compared to the maintenance phase in which trough concentrations between 0.5-12 µg/ml are common.

Immunogenicity

Secondary loss of response is often due to the development of anti- drug antibodies, which have been observed despite of the fully human character of the drug. In case of undetectable trough concentrations, subsequent measurement of anti-drug antibodies may be helpful to determine the optimal treatment strategy.

2. PRINCIPLE OF THE ADALIMUMAB ELISA

The Adalimumab ELISA uses a highly specific monoclonal antibody – clone 40D8, developed at the KU Leuven - that only detects adalimumab (Humira®). Other anti-TNF drugs (like infliximab, golimumab) do not interfere with the measurement.

Microtiterstrips coated with TNF-alpha are incubated with calibrators, controls and diluted patient samples. During this incubation step ADM 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 40D8, developed at the KU Leuven) directed to ADM.

After removal of the unbound conjugate, the strips are incubated with a chromogenic solution containing tetramethylbenzidin 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_2SO_4 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 ADM in patient samples is determined by interpolation from the calibration curve.

3. REAGENTS

Component	Name + Symbol
1 coated microtiter plate (12 x 8 strips) Strips coated with human TNF-alpha.	Precoated Strips MTPI
6 vials, 1300 µl, ready-to-use 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. Contains 0,09 % NaN_3. Calibrated against the WHO International Standard for Adalimumab 17/236.	Calibrator CAL NI
1 vial, 1300 µl, ready-to-use Positive Control for ADM, level 1; contains 30 ng/ml ADM. Contains 0.09% NaN_3.	Positive Control 1 CTL1I
1 vial, 1300 µl, ready-to-use Positive Control for ADM, level 2; contains 70 ng/ml ADM. Contains 0.09% NaN_3.	Positive Control 2 CTL2I
1 bottle, 100 ml, ready-to-use Sample dilution buffer Contains 0.09% NaN_3 and an inert orange dye.	Sample Diluent DILSAMI
1 bottle, 12 ml, ready-to-use Contains peroxidase conjugated monoclonal anti-ADM clone 40D8 antibodies. Contains antimicrobial agents and an inert red dye.	Conjugate CONJI
1 vial, 12 ml, ready-to-use Contains a solution of substrate (H_2O_2) and chromogen (tetramethylbenzidin).	Chromogen Solution CHROM
1 bottle, 50 ml, 20x concentrated Contains detergent in phosphate buffered solution and antimicrobial agents.	Wash solution WASH 20x I
1 bottle, 6 ml, ready-to-use Consists of 0.5 M H_2SO_4.	Stop Solution STOPI
2 plate covers	-

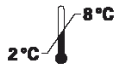
4. MATERIALS REQUIRED BUT NOT SUPPLIED

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 absorbances at 450 nm.

5. WARNINGS AND PRECAUTIONS FOR USERS

1. For *in vitro* diagnostic use only.
2. Do not mix reagents or coated microtiterstrips from kits with different lot numbers.
3. Chromogen Solution contains the hazardous ingredient N-Methyl-2-pyrrolidone at a concentration > 0,3 %. It is classified as a Reproductive Toxicant Category B.
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.
4. 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.
5. Although it might be advised to run calibrators/controls and samples in duplicate, reliable results are equally obtained by doing the analysis in singlicate.

6. STORAGE CONDITIONS



1. Store the microtiterstrips in their original package with the desiccant until all the strips have been used.
2. Opened components should be stored at 2-8°C until next use and can be maintained for 6 months.
3. Never use any kit components beyond the expiration date.

7. SPECIMEN COLLECTION AND PREPARATION

Serum and plasma (EDTA, citrate) 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 one year. 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 Adalimumab 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

Washing Solution: dilute 50 ml of concentrated Washing Solution to 1000 ml with distilled water. Reconstituted solution can be stored at least 1 month, store 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.

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 Stopping Solution to each well.
9. Determine the absorbance of each well at 450 nm or at 450 nm with reference filter 600-650 nm within 30 min following the addition of acid.

9. SAMPLE DILUTION FACTOR

For measuring trough concentrations (samples taken just before next injection) during maintenance phase, dilute samples 1:100.
Example: add 10 µl patient sample to 990 µl Sample Diluent

For measuring trough concentrations during induction or for measuring intermediate concentrations, dilute samples 1:400.
Example: add 10 µl patient sample to 390 µl Sample Diluent = solution 1; subsequently add 100 µl of solution 1 to 900 µl Sample Diluent.

The dilution factor must be taken into account when calculating ADM concentration in the samples by multiplying the measured concentration by the dilution factor. For calculating the ADM 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 ADM concentration in the undiluted sample is then 6 µg/ml.

By diluting the samples 1:100, ADM concentrations between 0.5 and 12 µg/ml can be determined. By diluting the samples 1:400, ADM concentrations between 2 and 48 µg/ml can be determined.
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.
Diluted samples may be stored for at least 8 HR.

10. RESULTS

The average absorbance value of each calibrator is plotted against the corresponding ADM value and the best calibration curve (e.g. quadratic regression) is constructed. Use the average absorbance of each patient sample obtained in the ADM ELISA to determine the corresponding value by simple 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	0.014
CAL 5	0.078
CAL 10	0.151
CAL 20	0.345
CAL 60	1.055
CAL 120	2.101

Precision

Intra-assay variation (n=21; 1 run)

	Level 1	Level 2	Level 3	Level 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**

Inter-assay variation (n=15; 5 runs; 5 days; 3 operators)

	Level 1	Level 2	Level 3	Level 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**

Specificity – 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 %.

Specificity – interference
A panel of 35 potentially interfering samples was tested consisting of HAMA positive, lipemic, high bilirubin, high cholesterol, high total protein, haemolysed and 1st semester pregnant women samples. No interaction with the investigated factors was observed.

Diagnostic sensitivity
Two clinical sample panels of 21 and 20 specimens respectively were analysed using the Adalimumab ELISA 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 0.96 respectively while 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%.

Minimal detectable concentration
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.

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 1:400 is applicable:
Concentration value for positive control CTL1: 12 µg/ml, range 8 – 16 µg/ml
Concentration value for positive control CTL2: 28 µg/ml, range 20 – 40 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).

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Version history

Version number	Description
ADM10-20	Previous version
ADM11-21	Writing in full the manufacturer name, according to its legal status: Advanced Practical Diagnostics BV instead of the abbreviation apDia.