Anti-Infliximab ELISA

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Anti-Infliximab ELISA IVD

REF 710101

The apDia Anti-Infliximab ELISA is an enzyme linked immunosorbent assay intended for the quantitative determination of antibodies to infliximab (ATI) in human serum and plasma.

1. BACKGROUND AND DIAGNOSTIC VALUE

Infliximab

Infliximab (IFX) is a chimeric antibody that targets the pro-inflammatory cytokine TNF-alpha. The introduction of infliximab has revolutionized the treatment of chronic inflammatory diseases like inflammatory bowel disease (IBD), rheumatoid arthritis (RA) and spondyloarthritis. It has been shown that infliximab can induce deep remission and improve the patient's quality of life[1]. Some patients do not respond to infliximab therapy upon induction (primary non-responders), while others lose response over time (secondary non-responders) [2].

Immunogenicity

Secondary loss of response is often due to the development of anti-infliximab antibodies (ATI), because of the immunogenic character of the drug[3]. ATI can develop in any patient undergoing infliximab therapy and are primarily neutralizing the activity of infliximab through immunocomplex formation. In addition, these immunocomplexes are rapidly cleared from the system. Analytically, they are responsible for subtherapeutic infliximab concentrations. Therefore, in the case of very low trough concentrations of infliximab (< 1 µg/mL), subsequent measurement of ATI may be helpful to determine the optimal treatment strategy.

Diagnostic Value

The diagnostic value of the Anti-Infliximab ELISA lies in its ability to stratify patients with subtherapeutic infliximab concentrations (< 1 µg/mL) in patients who need dose intensification or a drug (class) switch. Patients with low infliximab concentrations (< 1 µg/mL) and low ATI titers can benefit from infliximab dose intensification, as shown in several studies[4, 5]. However, the ATI titer of patients with low ATI titers undergoing a dose intensified treatment regimen must be adequally monitored. Patients that have high ATI titers are preferably switched to another drug, both within class or out of class. The course of ATI titers, based on the measurement of antibodies in at least two consecutive samples, is an important basis for the evaluation of the anti-drug antibody titer of the patient, rather than the absolute value of the titer.

Note: The Anti-Infliximab ELISA is not capable of measuring ATI in the presence of high concentrations of infliximab. It should only be used when < 1 µg/mL infliximab is quantified in the sample using the apDia Infliximab ELISA.

2. PRINCIPLE OF THE ANTI-INFLIXIMAB ELISA

The apDia anti-Infliximab ELISA uses for the calibrator and the controls a highly specific monoclonal antibody – clone 10F9, developed at the KU Leuven - that only bridges infliximab (Remicade®).[6]

Microtiter strips coated with infliximab (Remicade®) are incubated with calibrators, controls and diluted patient samples. During this incubation step ATI binds specifically to the infliximab on the solid phase. After removal of the unbound serum proteins by a washing procedure, the strips are incubated with biotin conjugated infliximab (Remicade®), binding directly to the antigen-antibody complex. After removal of the unbound biotin conjugate, the strips are incubated with peroxidase conjugated streptavidin. After removal of the unbound peroxidase 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 ATI 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 infliximab.	Precoated Strips MTP
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 0,1: 0,1 ng/ml; CAL 0,5: 0,5 ng/ml; CAL 1: 1 ng/ml; CAL 2,5: 2,5 ng/ml; CAL 5: 5 ng/ml anti-IFX clone 10F9. Contains 0,09 % NaN_3.	Calibrator CAL N
1 vial, 1300 µl, ready-to-use Control for ATI, level 1; contains 0,375 ng/ml anti-IFX clone 10F9. Contains 0.09% NaN_3.	Control 1 CTL1
1 vial, 1300 µl, ready-to-use Control for ATI, level 2; contains 3 ng/ml anti-IFX clone 10F9. Contains 0.09% NaN_3.	Control 2 CTL2
1 bottle, 100 ml, ready-to-use Sample dilution buffer Contains 0.09% NaN_3 and an inert orange dye.	Sample Diluent DILSAM
1 bottle, 12 ml, ready-to-use Contains biotin conjugated infliximab. Contains antimicrobial agents and an inert blue dye.	Conjugate 1 CONJ 1
1 bottle, 12 ml, ready-to-use Contains peroxidase conjugated streptavidin. Contains antimicrobial agents and an inert red dye.	Conjugate 2 CONJ 2
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
1 bottle, 6 ml, ready-to-use Consists of 0.5 M H_2SO_4.	Stop Solution STOP
4 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 microplate 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 microtiter strips from kits with different lot numbers.
3. 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.
4. Chromogen Solution contains the hazardous ingredient N-Methyl-2-pyrrolidone at a concentration > 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
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 microtiter strips 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 apDia Anti-Infliximab 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 pipetting instruments, please contact apDia.

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 microtiter strips 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 1 and incubate the covered microtiter strips for 30 ± 2 min at 37 °C (± 2 °C).
5. Repeat the washing procedure as described in 3.
6. Add 100 µl of Conjugate 2 and incubate the covered microtiter strips for 15 ± 1 min at 37 °C (± 2 °C).
7. Repeat the washing procedure as described in 3.
8. Add 100 µl of Chromogen Solution to each well.
9. Incubate the covered micrtotiter strips for 10 ± 1 min at 37 °C (± 2 °C). Avoid light exposure during this step.
10. Add 50 µl of Stopping Solution to each well.
11. 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

Prepare for each patient sample a dilution of 1:25 and 1:200.

By diluting the samples 1:25, ATI concentrations between 2,5 and 125 ng/ml can be determined.
Example: add 25 µl patient sample to 600 µl Sample Diluent

By diluting the samples 1:200, ATI concentrations between 20 and 1000 ng/ml can be determined.
Example: add 100 µl of dilution 1:25 to 700 µl Sample Diluent

The dilution factor must be taken into account when calculating ATI concentration in the samples by multiplying the measured concentration by the dilution factor. Concentration is expressed in ng/ml.

10. RESULTS

The average absorbance value of each calibrator is plotted against the corresponding ATI concentration value and the best calibration curve (e.g. quadratic regression) is constructed.
Use the average absorbance of each patient sample obtained in the ATI ELISA to determine the corresponding concentration 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.

For sample dilution 1:25
Multiply the obtained concentration by factor 25.
If the obtained concentration is lower than 2,5 ng/ml, the results must not be extrapolated and is reported as < 2,5 ng/ml.
If the obtained concentration is higher than 125 ng/ml, the results must not be extrapolated and is reported as > 125 ng/ml.

For sample dilution 1:200
Multiply the obtained concentration by factor 200.
If the obtained concentration is lower than 20 ng/ml, the results must not be extrapolated and is reported as < 20 ng/ml.
If the obtained concentration is higher than 1000 ng/ml, the results must not be extrapolated and is reported as > 1000 ng/ml.

Only if both 1:25 and 1:200 dilution result in a measurable concentration value, the mean of both values is calculated and reported.

11. PERFORMANCE CHARACTERISTICS

Example of typical optical density (OD) values:

CALIBRATOR	OD
CAL 0	0.031
CAL 0,1	0.083
CAL 0,5	0.269
CAL 1	0.543
CAL 2,5	1.539
CAL 5	2.685

Precision

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

	Level 1	Level 2	Level 3
Mean (ng/ml)	0.67	1.25	2.44
SD	0.05	0.08	0.14
% CV	**7.5**	**6.7**	**5.9**

Inter-assay variation (n=12; 3 runs)

	Level 1	Level 2
Mean (ng/ml)	0.36	2.25
SD	0.05	0.27
% CV	**12.4**	**11.9**

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 ATI, 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, haemolysed, high total protein and 1st semester pregnant woman samples. No interaction with the investigated factors was observed.

Specificity – RF interference
The potential interference of rheumatoid factor (RF) in a clinical sample panel of patients suffering from auto-immune diseases and positive for RF, was evaluated in the apDia ATI ELISA. The results indicated that RF does not interfere in the assay.

Diagnostic sensitivity
Two clinical sample panels of 15 and 21 specimens respectively were analysed using the apDia Anti-Infliximab ELISA and results were compared with data obtained using the ATI ELISA developed at the KU Leuven which served as reference assay. All samples having measurable ATI levels according to the reference assay, were detected positive (8 samples for panel 1, 16 samples for panel 2) resulting in a diagnostic sensitivity of 100%.

Minimal detectable concentration

The minimal detectable concentration of ATI is 0.06 ng/ml.
Taking into account a dilution factor of 1:25 this corresponds to 1.5 ng/ml.
Taking into account a dilution factor of 1:200 this corresponds to 12 ng/ml.

For a 1:25 dilution a concentration lower than 2.5 ng/ml, corresponding to the lowest calibrator, should be reported as < 2.5 ng/ml.
For a 1:200 dilution a concentration lower than 20 ng/ml should be reported as < 20 ng/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 calibrator: > 1.400

Concentration value for positive control CTL1: 0.375 ng/ml, range 0.25-0.50 ng/ml
Concentration value for positive control CTL2: 3 ng/ml, range 2-4 ng/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 CAL 0 > 0.080), the washing was insufficient. Repeat the test with more vigorous washing (increased number of cycles, soak time).

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