*In vitro* diagnostic kit

Ustekinumab ELISA

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# Ustekinumab ELISA

IVD

REF 710601

The apDia Ustekinumab ELISA is an enzyme linked immunosorbent assay intended for the quantitative determination of ustekinumab (UST, Stelara®) in human serum and plasma.

## 1. BACKGROUND AND DIAGNOSTIC VALUE

Ustekinumab (UST) is a fully human monoclonal antibody that binds to the p40 subunit common to IL-12 and IL-23 thereby preventing the interaction with the cytokine receptors on T cells, natural killer cells and antigen-presenting cells [1]. UST has been approved for treatment of moderate to severe Crohn's disease (CD), plaque psoriasis and psoriatic arthritis [2,3,4].

A drug can only exert its pharmacological effect when adequate concentrations are achieved in the circulation. The serum concentration of biologicals just before the next administration, defined as trough concentration, has been used for therapeutic drug monitoring (TDM). Recent data have shown a positive relationship between UST serum concentration, either measured at trough or at an intermediate time point, and clinical outcomes in patients with Crohn's disease and plaque psoriasis, respectively. TDM may therefore be very instrumental to optimize treatment.

The apDia Ustekinumab ELISA uses highly specific monoclonal antibodies developed at the University of Leuven, Belgium (KU Leuven). Anti-TNF drugs (like infliximab, adalimumab, golimumab) or anti-integrin α4β7 drugs (like vedolizumab) do not interfere with the measurement.

As an example of TDM, the use of UST concentration measurements in plaque psoriasis and CD is described.

**Crohn's Disease**
UST is administered intravenously (IV) at week 0 and thereafter every 8 weeks subcutaneously (SC). The UNITI-1 and -2 induction trials demonstrated that 33.7% and 55.5% of patients had a clinical response at week 6, respectively. During maintenance therapy with SC ustekinumab every 8 weeks, 53.1% of patients were in remission at week 44 in the IM-UNITI trial [2].
Several studies have demonstrated the relationship between ustekinumab trough concentration and clinical, biological and endoscopic response, indicating the usefulness of therapeutic drug monitoring to guide clinical decision-making [5,6,7].

**Plaque psoriasis**
UST is weight-based administered subcutaneously at week 0, at week 4 and thereafter every 12 weeks. The PHOENIX 1 and 2 trials indicate that treatment with ustekinumab results in rapid, significant improvements in patients with moderate-to-severe psoriasis [3,4].
A recent study demonstrated a concentration-response relationship at week 4 upon injection for ustekinumab-treated psoriasis patients, indicating that monitoring 4-week post injection ustekinumab concentrations could timely identify underexposed patients who might benefit from treatment optimization [8].

**Immunogenicity**
It has been shown that the immunogenicity of ustekinumab is very low [8,9].

## 2. PRINCIPLE OF THE USTEKINUMAB ELISA

The apDia Ustekinumab ELISA uses two highly specific monoclonal antibodies – clones 56C1H12 and 56A2D11, both developed at the KU Leuven – that only detect ustekinumab (Stelara®).

Microtiterstrips coated with anti-ustekinumab monoclonal antibody clone 56C1H12 are incubated with calibrators, controls and diluted patient samples. During this incubation step ustekinumab binds specifically to the antibodies 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 anti-ustekinumab monoclonal antibody clone 56A2D11 directed to ustekinumab.
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 0.5 M $H_2SO_4$ 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 ustekinumab in patient samples is determined by interpolation from the calibration curve.

## 3. REAGENTS

| Component | Name + Symbol |
|---|---|
| 1 coated microtiter plate (12 x 8 strips)<br>Strips coated with anti-ustekinumab monoclonal antibodies, clone 56C1H12. | Precoated Strips<br>MTP |
| 6 vials, 1300 µl, ready-to-use<br>Each vial contains a ready-to-use calibrator solution, N having following values:<br>CAL 0: 0 ng/ml; CAL 2,5: 2,5 ng/ml;<br>CAL 10: 10 ng/ml; CAL 20: 20 ng/ml;<br>CAL 60: 60 ng/ml; CAL 120: 120 ng/ml.<br>Contain 0,09 % $NaN_3$. | Calibrator<br>CAL N |
| 1 vial, 1300 µl, ready-to-use<br>Positive Control for ustekinumab, level 1; contains 8 ng/ml ustekinumab.<br>Contains 0.09% $NaN_3$. | Positive Control 1<br>CTL1 |
| 1 vial, 1300 µl, ready-to-use<br>Positive Control for ustekinumab, level 2; contains 70 ng/ml ustekinumab.<br>Contains 0.09% $NaN_3$. | Positive Control 2<br>CTL2 |
| 1 bottle, 100 ml, ready-to-use<br>Sample dilution buffer.<br>Contains 0.09% $NaN_3$ and an inert orange dye. | Sample Diluent<br>DILSAM |
| 1 bottle, 12 ml, ready-to-use<br>Contains peroxidase-conjugated monoclonal anti-ustekinumab antibodies, clone 56A2D11.<br>Contains antimicrobial agents and an inert red dye. | Conjugate<br>CONJ |
| 1 vial, 12 ml, ready-to-use<br>Contains a solution of substrate ($H_2O_2$) and chromogen (tetramethylbenzidin). | Chromogen Solution<br>CHROM |
| 1 bottle, 50 ml, 20x concentrated<br>Contains detergent in phosphate buffered solution and antimicrobial agents. | Wash Solution<br>WASH 20x |
| 1 bottle, 6 ml, ready-to-use<br>Consists of 0.5 M $H_2SO_4$. | Stop Solution<br>STOP |
| 2 plate covers | - |

## 4. MATERIALS REQUIRED BUT NOT SUPPLIED

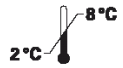
1. Precision micropipettes and standard laboratory pipettes.
2. Clean standard laboratory volumetric glassware.
3. Clean glass or plastic tubes for the dilution of the samples.
4. A microtiterplate reader capable of measuring absorbance at 450 nm with reference filter at 600-650 nm.

## 5. WARNINGS AND PRECAUTIONS FOR USERS

1. For *in vitro* diagnostic use only.
2. The kit has to be used by properly trained personnel in an appropriate laboratory setting.
3. Do not mix reagents or coated microtiterstrips from kits with different lot numbers.
4. 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.
5. Wear disposable gloves and lab clothing when performing a test run.
6. Although it might be advised to run calibrators and controls in duplicate, reliable results are equally obtained by doing the analysis in singlicate.
7. 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.
8. 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.
9. Stop Solution is a 0,5 M $H_2SO_4$ solution which is irritant. In case of contact with eyes or skin, rinse with plenty of water.
10. Inform the manufacturer in case the kit is supplied with any damaged or improperly closed component.

## 6. STORAGE CONDITIONS




1. Store the microtiterstrips in their original package with the desiccant until all the strips have been used. Do not freeze.
2. Opened components should be stored at 2-8°C until next use and can be maintained for 5 months.
3. 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 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 Ustekinumab 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 instruments). For instructions on how to perform the assay with ELISA 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 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 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 injection) during maintenance phase, dilute samples 1:100.
Example: add 10 µl patient sample to 990 µl Sample Diluent

For measuring concentrations during (re)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 ustekinumab concentration in the samples by multiplying the measured concentration by the dilution factor. For calculating the ustekinumab 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 30 ng/ml. The corresponding ustekinumab concentration in the undiluted sample is then 3 µg/ml.
Example: the outcome of 1:200 diluted sample, obtained by interpolation from the calibration curve is 40 ng/ml. The corresponding ustekinumab concentration in the undiluted sample is then 8 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 ustekinumab value and the best calibration curve (e.g. polygon) is constructed.
Use the average absorbance of each patient sample obtained in the Ustekinumab 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 | 0.009 |
| CAL 2,5 | 0,085 |
| CAL 10 | 0,306 |
| CAL 20 | 0,608 |
| CAL 60 | 1.637 |
| CAL 120 | 2.660 |

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

| | Level 1 | Level 2 | Level 3 | Level 4 |
|---|---|---|---|---|
| Mean (ng/ml) | 6.1 | 16.5 | 30.7 | 83.7 |
| SD | 0.4 | 0.8 | 1.8 | 7.6 |
| **% CV** | **5.9** | **5.0** | **6.0** | **9.0** |

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

| | Level 1 | Level 2 | Level 3 | Level 4 |
|---|---|---|---|---|
| Mean (ng/ml) | 5.8 | 16.2 | 30.1 | 86.5 |
| SD | 0.3 | 0.8 | 1.5 | 7.3 |
| **% CV** | **4.4** | **4.9** | **4.8** | **8.4** |

**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 UST, resulting in a specificity of 100 %.

**Specificity – interference**

A panel of 25 potentially interfering samples consisting of HAMA positive, high cholesterol, hemolyzed, lipemic and 1st semester pregnant women samples was tested. No interaction with the investigated factors was observed.

**Specificity – cross-reactivity**

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

**Diagnostic sensitivity**

A clinical sample panel of 15 specimens was analysed using the apDia Ustekinumab ELISA and results were compared with data obtained using the Ustekinumab ELISA developed at the KU Leuven in Belgium which served as reference assay. Pearson r value as indicator for the correlation between both assays is 0.97. All samples having measurable UST levels according to the reference assay were detected positive (14 specimens) resulting in a diagnostic sensitivity of 100%.

**Analytical sensitivity – Measuring range**

The limit of detection of the Ustekinumab ELISA is 0.12 ng/ml. Taking into account a dilution factor of 1:100 this corresponds to 0.01 µg/ml.

The limit of quantification is 0,36 ng/ml. Taking into account a dilution factor of 1:100, this corresponds to 0.04 µg/ml.
By diluting the samples 1:100, ustekinumab concentrations between 0,04 and 12 µg/ml can be determined. By diluting the samples 1:200, ustekinumab concentrations between 0,08 and 24 µg/ml can be determined.

**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: 0,8 µg/ml, range 0.55 – 1.10 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: 1.6 µg/ml, range 1.10 – 2.20 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).

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