In vitro diagnostic kit

Vedolizumab ELISA

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The Vedolizumab ELISA is an enzyme linked immunosorbent assay intended for the quantitative determination of Vedolizumab (VDZ, Entyvio®, anti-integrin $\alpha_4\beta_7$) in human serum and plasma.

1. BACKGROUND AND DIAGNOSTIC VALUE

Therapeutic drug monitoring
Vedolizumab (VDZ) is a humanised monoclonal antibody that binds exclusively to the lymphocyte integrin $\alpha_4\beta_7$. VDZ inhibits the interaction of $\alpha_4\beta_7$-expressing cells with mucosal addressin cell adhesion molecule-1 on endothelial cells, thereby hampering the infiltration of the $\alpha_4\beta_7$-expressing cells into the gastrointestinal mucosa and gut-associated lymphoid tissue. VDZ suppresses gut inflammation and has therefore been approved for the treatment of patients with moderate to severe ulcerative colitis (UC) [1] and Crohn's disease (CD) [2,3]. It has been shown that VDZ can induce clinical remission and improve the patient's quality of life.

A drug can only exert its pharmacologic effect when adequate concentrations are achieved in the circulation. The serum concentration of biologicals just before their next infusion, defined as trough concentration, has been used for therapeutic drug monitoring (TDM). Recent data on TDM have shown a positive relationship between VDZ trough serum concentrations and clinical outcomes in patients with UC and CD[4,5]. TDM may therefore be very instrumental to optimize treatment.

The Vedolizumab ELISA uses highly specific monoclonal antibodies developed at the KU Leuven. Anti-TNF drugs (like infliximab, adalimumab, golimumab) do not interfere with the measurement.

As an example of TDM, the use of VDZ trough concentration measurements in UC and CD is described.

Ulcerative colitis
VDZ is given at week 0, week 2 and week 6 (induction) and upon good clinical response at week 14, treatment is continued by infusions every 8 weeks (maintenance). The exposure-efficacy relationships of VDZ evaluated in GEMINI 1 revealed a positive exposure-response relationship for clinical remission, clinical response, and mucosal healing for VDZ induction therapy in UC[1]. VDZ trough concentration measurements during or shortly after induction may thus be used to identify undertreated patients.
It has been demonstrated that higher VDZ concentrations are associated with deep remission in patients with UC on maintenance therapy[5]. Thus, regularly checking VDZ trough concentrations during maintenance therapy may be useful to evaluate the VDZ treatment schedule.

Crohn's Disease
VDZ is given at week 0, week 2 and week 6 (induction) and upon good clinical response at week 14, treatment is continued by infusions every 8 weeks (maintenance). The exposure-efficacy relationships of VDZ evaluated in GEMINI 2 and 3 revealed a modest positive exposure-response relationship[2,3]. Clinical remission rates were higher at week 10 than at week 6 in both studies. The European Medicines Agency allows an additional dose at week 10 before assessment of an induction response at week 14.

Due to the dosing regimen, trough concentrations during induction at week 2, week 6, week 10 (CD) & 14 (CD) are higher compared to trough concentrations during maintenance when VDZ is given every 8 weeks.

Immunogenicity
Secondary loss of response is often due to the development of anti-drug antibodies. The immunogenicity rate during treatment with VDZ is very low (4%)[5].

2. PRINCIPLE OF THE VEDOLIZUMAB ELISA

The Vedolizumab ELISA uses highly specific monoclonal antibodies – clones 6F3 and 6E6, developed at the KU Leuven.

Microtiterstrips coated with anti-vedolizumab monoclonal antibody clone 6F3 are incubated with calibrators, controls and diluted patient samples. During this incubation step VDZ is captured specifically by 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 monoclonal antibody (clone 6E6) directed to VDZ.
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 VDZ 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 monoclonal antibody clone 6F3 to VDZ.	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 10: 10 ng/ml; CAL 30: 30 ng/ml; CAL 100: 100 ng/ml; CAL 200: 200 ng/ml; CAL 500: 500 ng/ml. Contain 0,09 % NaN_3.	Calibrator CAL N
1 vial, 1300 µl, ready-to-use Positive Control for VDZ, level 1; contains 120 ng/ml VDZ. Contains 0.09% NaN_3.	Positive Control 1 CTL1
1 vial, 1300 µl, ready-to-use Positive Control for VDZ, level 2; contains 250 ng/ml VDZ. Contains 0.09% NaN_3.	Positive 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 peroxidase conjugated monoclonal anti-VDZ clone 6E6 antibodies. Contains antimicrobial agents and an inert red dye.	Conjugate CONJ
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
2 plate covers	-

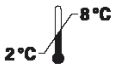
4. MATERIALS REQUIRED BUT NOT SUPPLIED

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. 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 singular.

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 2 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 Vedoluzimab 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 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 trough concentrations during induction phase 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 VDZ concentration in the samples by multiplying the measured concentration by the dilution factor. For calculating the VDZ 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 120 ng/ml. The corresponding VDZ concentration in the undiluted sample is then 12 µg/ml.
Example: the outcome of 1:400 diluted sample, obtained by interpolation from the calibration curve is 150 ng/ml. The corresponding VDZ concentration in the undiluted sample is then 60 µg/ml.

By diluting the samples 1:100, VDZ concentrations between 1 and 50 µg/ml can be determined. By diluting the samples 1:400, VDZ concentrations between 4 and 200 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 VDZ value and the best calibration curve (e.g. polygon, quadratic regression) is constructed.
Use the average absorbance of each patient sample obtained in the VDZ 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	0.011
CAL 10	0.091
CAL 30	0.232
CAL 100	0.740
CAL 200	1.323
CAL 500	2.499

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

	Level 1	Level 2	Level 3	Level 4
Mean (ng/ml)	26.4	71.1	138.7	323.5
SD	1.2	3.9	7.5	23.3
% CV	**4.6**	**5.5**	**5.4**	**7.2**

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

	Level 1	Level 2	Level 3	Level 4
Mean (ng/ml)	26.7	72.9	143.1	334.2
SD	1.5	4.9	9.3	40.5
% CV	**5.8**	**6.7**	**6.5**	**12.1**

Specificity – normal human serum/plasma

Specificity has been evaluated by testing 72 healthy donor samples from Dutch origin. None of the samples showed a detectable concentration of VDZ, resulting in a specificity of 100 %.

Specificity – interference

Specificity has been evaluated by testing 27 RF positive samples from patients suffering from autoimmune diseases, treated with medication other than Entyvio®. None of the samples showed a detectable concentration of VDZ, resulting in a specificity of 100%.

A panel of 25 potentially interfering samples consisting of HAMA positive, high cholesterol, haemolysed, 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 and golimumab.

Diagnostic sensitivity

A clinical sample panel of 17 specimens was analysed using the Vedolizumab ELISA and results were compared with data obtained using the VDZ ELISA developed at the KU Leuven which served as reference assay. Pearson r value as indicator for the correlation between both assays is 0.98. All samples having measurable VDZ levels according to the reference assay were detected positive (16 samples) resulting in a diagnostic sensitivity of 100%.

Analytical sensitivity

The limit of detection of VDZ is lower than 2.5 ng/ml. Taking into account a dilution factor of 1:100 this corresponds to 0.25 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: 12 µg/ml, range 8 – 16 µg/ml
Concentration value for positive control CTL2: 25 µg/ml, range 17 – 34 µg/ml
If multiplicity factor of 1:400 is applicable:
Concentration value for positive control CTL1: 48 µg/ml, range 32 – 64 µg/ml
Concentration value for positive control CTL2: 100 µg/ml, range 68 – 136 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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3. Sands BE, Feagan BG, Rutgeerts P, et al. Effects of vedolizumab induction therapy for patients with Crohn's disease in whom tumor necrosis factor antagonist treatment failed. Gastroenterology 2014: 147:618-627.
4. Rosario M, Dirks NL, Milch C, et al. A Review of the Clinical Pharmacokinetics, Pharmacodynamics, and Immunogenicity of Vedolizumab. Clin Pharmacokinet. 2017. doi: 10.1007/s40262-017-0546-0.
5. Rosario M, French JL, Dirks NL, et al. Exposure–efficacy Relationships for Vedolizumab Induction Therapy in Patients with Ulcerative Colitis or Crohn's Disease. JCC. 2017;1–9. doi: 10.1093/ecco-jcc/jjx021.

Version history

Version number	Description
VEDO11-18	Original version
VEDO11-21	Writing in full the manufacturer name, according to its legal status: Advanced Practical Diagnostics BV instead of the abbreviation apDia.