Human Vasculitis ELISA Kit

Cat. No.:DEIA10419
Pkg.Size:96T

Intended use

The Human Vasculitis ELISA Kit is a solid phase enzyme immunoassay for the combined quantitative detection of antibodies against PR3 and MPO in human serum. Each well is coated with native human proteinase 3 (PR3) plus native myeloperoxidase (MPO), both highly puried from human peripheral blood polymorphnuclear cells. Anti-PR3 and anti-MPO antibodies recognize specicially conformational epitopes only accessible on the native target antigen. The assay is an aid in the differential diagnosis of autoimmune vasculitis.

General Description

Antibodies against myeloperoxidase (MPO) and proteinase 3 (PR3) belong to the group of anti-neutro-phil cytoplasmic antibodies (ANCA) which are directed against cytoplasmic components of neutrophilic granulocytes and monocytes. Indirect immunofluorescence test on ethanol-fixed neutrophils has been the established method for the detection of ANCAs. It became apparent that some ANCAs create a cytoplasmic fluorescence pattern (thus called cANCA) while others create a perinuclear pattern (the pANCA). As both patterns may cover multiple antigens, immunofluorescence is not suitable for a satisfying differential diagnosis of vasculitis; thus each IFT should be veried with specic ELISA tests. While proteinase 3 is the main antigen specific for cANCA the main antigen for pANCA has been identied as MPO but other cellular components (lactoferrin, cathepsin G, elastase e.g.) may cause perinuclear staining.
MPO is an enzyme from the primary granules of neutrophils with a molecular weight of approximately 140 kDa. Its highly negativ charge may be relevant for the location at positivly charged structures such as the nuclear membrane and DNA thus responsible for the perinuclear staining pattern of anti-MPO antibodies in patients`sera in IFT using ethanol-fixed neutrophils.
PR3 is a serine protease from the azurophile granules (lysosomes) of neutrophils with a molecular weight of 29 kDa. The cationic protein has a proteolytic activity to elastin, hemoglobin and collagen VII. In addition, PR3 promotes the activation of platelets by cathepsin G and inactivates the C1-inhibitor. ANCA are important markers for the differential diagnosis of autoimmune vasculitis. Anti-PR3 are a specic serological marker for Wegener`s granulomatosis (WG) and may play an active role in the pathogenesis of WG. Anti-PR3 titers are strongly associated with disease activity and inhibit the proteolytic activity of PR3. Anti-MPO are correlated with idiopathic or vasculitis associated necrotizing crescentic glomerulonephritis and are found frequently in 70% of patients with microscopic polyangiitis,and 5-50% of patients with Churg-Strauss syndrome.

Principle Of The Test

Serum samples diluted 1:101 are incubated in the microplates coated with the specific antigen. Patient's antibodies, if present in the specimen, bind to the antigen. The unbound fraction is washed off in the following step. Afterwards anti-human immunoglobulins conjugated to horseradish peroxidase (conjugate) are incubated and react with the antigen-antibody complex of the samples in the microplates. Unbound conjugate is washed off in the following step. Addition of TMB-substrate generates an enzymatic colorimetric (blue) reaction, which is stopped by diluted acid (color changes to yellow). The rate of color formation from the chromogen is a function of the amount of conjugate bound to the antigen-antibody complex and this is proportional to the initial concentration of the respective antibodies in the patient sample.

Reagents And Materials Provided

To be reconstituted:

1. 5x Sample Buffer: 1 vial, 20 ml-5x concentrated (capped white: yellow solution)

45-16 Ramsey Road Shirley, NY 11967, USA
Tel: 631-624-4882 ·Fax:631-614-7828
E-mail: info@creative-diagnostics.com
www.creative-diagnostics.com

Containing: Tris, NaCl, BSA, sodium azide (preservative)
2. 50x Wash Buffer: 1 vial, 20 ml-50x concentrated (capped white: green solution)
Containing: Tris, NaCl, Tween, sodium azide (preservative)
Ready to use:
1. Negative Control: 1 vial, 1.5 ml (capped green: yellow solution)
Containing: Human serum (diluted), sodium azide (preservative)
2. Positive Control: 1 vial, 1.5 ml (capped red: yellow solution)
Containing: Human serum (diluted), sodium azide (preservative)
3. Calibrators: 6 vials, 1.5 ml each 0, 3, 10, 30, 100, 300 U/ml (color increasing with concentration: yellow solution)
Containing: Human serum (diluted), sodium azide (preservative)
4. Conjugate: 1 vial,15 ml IgG (capped blue: blue solution)
Containing: Anti-human immunoglobulins conjugated to horseradish peroxidase
5. TMB Substrate: 1 vial, 15 ml (capped black)
Containing: Stabilized TMB/H2O2
6. Stop Solution: 1 vial, 15 ml (capped white: colorless solution)
Containing: 1M Hydrochloric Acid
7. Microtiterplate: 12 x 8 well strips with breakaway microwells

Materials Required But Not Supplied

Microtiter plate reader 450 nm reading lter and optional 620 nm reference lter (600-690 nm). Glass ware, test tubes for dilutions. Distilled water. Vortex mixer, precision pipettes (10, 100, 200, 500, 1000 µl) or multipipette. Microplate washing device (multichannel pipette or automated system), adsorbent paper.

Storage

Store all reagents and the microplate at 2-8°C/35-46°F, in their original containers. Once prepared, reconstituted solutions are stable for 1 month at 4°C/39°F, at least. Reagents and the microplate shall be used within the expiry date indicated on each component, only. Avoid intense exposure of TMB solution to light. Store microplates in designated foil, including the desiccant, and seal tightly.

Specimen Collection And Handling

Use preferentially freshly collected serum samples. Do not use icteric, lipemic, hemolysed or bacterially contaminated samples. Sera with particles should be cleared by low speed centrifugation (<1000 x g). Blood samples should be collected in dry tubes. After separation, the serum samples should be used immediately, respectively stored at 2-8°C/35-46°F up to three days, or frozen at -20°C/64°F for longer periods.

Assay Steps

1. Preparations prior to pipetting
Dilute concentrated reagents:
Dilute the concentrated sample buffer 1:5 with distilled water (e.g. 20 ml plus 80 ml).
Dilute the concentrated wash buffer 1:50 with distilled water (e.g. 20 ml plus 980 ml).
Samples
Dilute serum samples 1:101 with sample buffer (1x)
e.g. 1000 µl sample buffer (1x) + 10 µl serum. Mix well!
Washing
Prepare 20 ml of diluted wash buffer (1x) per 8 wells or 200 ml for 96 wells.

45-16 Ramsey Road Shirley, NY 11967, USA
Tel: 631-624-4882 ·Fax:631-614-7828
E-mail: info@creative-diagnostics.com
www.creative-diagnostics.com

(e.g. 4 ml concentrate plus 196 ml distilled water).

Automated washing:

Consider excess volumes required for setting up the instrument and dead volume of robot pipette.

Manual washing:

Discard liquid from wells by inverting the plate. Knock the microwell frame with wells downside vigorously on clean adsorbent paper. Pipette 300 µl of diluted wash buffer into each well, wait for 20 seconds. Repeat the whole procedure twice again.

Microplates

Calculate the number of wells required for the test. Remove unused wells from the frame, replace and store in the provided plastic bag, together with desiccant, seal tightly (2-8°C/35-46°F).

2. Work flow

1. Pipette 100 µl of each patient's diluted serum into the designated microwells.
2. Pipette 100 µl calibrators OR cut-off control and negative and positive controls into the designated wells.
3. Incubate for 30 minutes at room temperature (20-26°C/64-78.8°F).
4. Wash 3x with 300 µl washing buffer (diluted 1:50).
5. Pipette 100 µl conjugate into each well.
6. Incubate for 15 minutes at room temperature (20-26°C/64-78.8°F).
7. Wash 3x with 300 µl washing buffer (diluted 1:50).
8. Pipette 100 µl TMB substrate into each well.
9. Incubate for 15 minutes at room temperature (20-26°C/64-78.8°F), in the dark.
10. Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.
11. Incubate 5 minutes minimum.
12. Agitate plate carefully for 5 sec. Read absorbance at 450 nm (optionally 450/620 nm) within 30 minutes.

Calculation

Due to the lack of international reference calibration this assay is calibrated in arbitrary units (U/ml).

Typical Standard Curve

We recommend pipetting calibrators in parallel for each run.

Table 1.

Calibrators IgG	*OD 450/620 nm*	*CV %*
0 U/ml	0.101	4.1
3 U/ml	0.225	1.9
10 U/ml	0.380	4.3
30 U/ml	0.732	0.9
100 U/ml	1.277	0.8
300 U/ml	2.150	0.5

Example of calculation

Table 2.

45-16 Ramsey Road Shirley, NY 11967, USA
Tel: 631-624-4882 ·Fax:631-614-7828
E-mail: info@creative-diagnostics.com
www.creative-diagnostics.com

Patient	Replicate (OD)	Mean (OD)	Result (U/ml)
P 01	1.405/1.383	1.394	110.8
P 02	0.909/0.931	0.920	49.3

For lot specific data, see enclosed quality control leaflet. Medical laboratories might perform an in-house Quality Control by using own controls and/or internal pooled sera, as foreseen by EU regulations. Do not use this example for interpreting patients results!

Interpretation of Results

Establish the standard curve by plotting the optical density (OD) of each calibrator (y-axis) with respect to the corresponding concentration values in U/ml (x-axis). For best results we recommend log/lin coordinats and 4-Parameter Fit. From the OD of each sample, read the corresponding antibody concentrations expressed in U/ml.
Normal Range: < 15 U/ml
Positive Results: > 15 U/ml

Detection Range

0-300 U/ml

Sensitivity

The analytical sensitivity of this kit has been found at 1.0 U/ml.

Specificity

The microplates are coated with native human proteinase 3 and native human myeloperoxidase highly purified from human neutrophil granulocytes. No cross reactivities to other autoantigens have been found. The diagnostic specificity of PR3 antibodies for Wegener´s Granulomatosis is >95%. The diagnostic sensitivity of PR3 antibodies for active Wegener´s Granulomatosis is 90% and 75% in the absence of renal involvement.
Anti-MPO are found frequently in 70% of patients with microscopic polyangiitis,and 5-50% of patients with Churg-Strauss syndrome.

Linearity

Chosen sera have been tested with this kit and found to dilute linearly. However, due to the heterogeneous nature of human autoantibodies there might be samples that do not follow this rule.
Table 3.

45-16 Ramsey Road Shirley, NY 11967, USA
Tel: 631-624-4882 ·Fax:631-614-7828
E-mail: info@creative-diagnostics.com
www.creative-diagnostics.com

Sample No.	Dilution Factor	measured concentration (U/ml)	expected concentration (U/ml)	Recovery (%)
1	1 / 100	218.0	220.0	99.1
	1 / 200	105.0	110.0	95.5
	1 / 400	51.4	55.0	93.5
	1 / 800	25.3	27.5	92.0
2	1 / 100	112.6	115.0	97.9
	1 / 200	56.3	57.5	97.9
	1 / 400	27.1	28.8	94.1
	1 / 800	13.4	14.4	93.1

Reproducibility

INTRA-ASSAY (%CV): 1.5%-3.2%
INTER-ASSAY (%CV): 2.5%-3.4%

Precautions

1. Health hazard data

Staff trained and shall perform the kit. Although this product is not considered particularly toxic or dangerous in conditions of normal use, refer to the following for maximum safety:

Recommendations and precautions

This kit contains potentially hazardous components. Though kit reagents are not classied being irritant to eyes and skin we recommend to avoid contact with eyes and skin and wear disposable gloves. Do not smoke, eat or drink when manipulating the kit. Do not pipette by mouth. All human source material used for some reagents of this kit (controls, standards e.g.) has been tested by approved methods and found negative for HbsAg, Hepatitis C and HIV 1. However, no test can guarantee the absence of viral agents in such material completely. Thus handle kit controls, standards and patient samples as if capable of transmitting infectious diseases.

2. General directions for use

Do not mix or substitute reagents or microplates from different lot numbers. This may lead to variations in the results. Allow all components to reach room temperature (20-26°C/64-78.8°F) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test. Always pipet substrate solution with clean tips only. Protect this reagent from light. Never pipette conjugate with tips used with other reagents prior.

REFERENCES

1. Falk, RJ Jennette JC (1988). Antineutrophil cytoplasmic autoantibodies with specicity for myeloperoxidase in patients with systemic vasculitis and idiopathic necrotizing and crescentic glomerulonephritis. N Engl J Med 318: 1651-1657.
2. Lüdemann J, Utecht B, Gross WL (1990). Antineutrophil cytoplasm antibodies in Wegener`s granulomatosis recognize an elastinophil enzyme. J Exp Med 171: 375-362.
3. Csernok E, Muller A, Gross WL (1999). Immunopathology of ANCA-associated vasculitis. Intern Med 38: 759-765.

45-16 Ramsey Road Shirley, NY 11967, USA
Tel: 631-624-4882 ·Fax:631-614-7828
E-mail: info@creative-diagnostics.com
www.creative-diagnostics.com