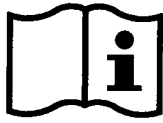


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Instruction for use

ANCA Profile ELISA

Enzyme Immunoassay for Qualitative Screening for IgG ANCA (anti-neutrophil cytoplasmic antibodies) in human serum or plasma



DE7040



96 Tests

PRINCIPLE OF THE TEST

Purified antigens PR3, MPO, BPI, Elastase, Cathepsin G, Lysozyme and Lactoferrin are coated on to individual rows A to H of the microwell plate.

Antibodies against the coated antigen, if present in diluted patient sample, bind to the respective antigen. Washing of the microwells removes unbound unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human antibodies immunologically detect the bound patient antibodies forming a conjugate/antibody/antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue colour. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow colour is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of antibodies present in the original sample.

SUMMARY AND EXPLANATION OF THE TEST

Anti-neutrophil cytoplasmic antibodies (ANCA) represent a group of autoantibodies directed towards cytoplasmic components of the neutrophil granulocytes and monocytes. The classical methods for the determination of ANCA are immunofluorescence tests. With these indirect immunofluorescence (IF) techniques two main patterns are distinguished: a cytoplasmic (cANCA) and a perinuclear (pANCA) type. The target antigen for 80-90 % of cANCA is proteinase 3 (PR3), a serine proteinase present in primary granules; 10-20 % of cANCA are directed to other proteins, such as bactericidal permeability-increasing protein (BPI). In rare cases, antibodies to elastase (4 %), lysozym (2 %) or cathepsin G (2 %) may show a cANCA-pattern. cANCA have also been detected in different non-rheumatic diseases. Approximately 90 % of pANCA positive sera contain autoantibodies directed to myeloperoxidase (MPO), which is located in the granules of neutrophil granulocytes. Antibodies to other antigens e.g. Lactoferrin, Elastase, Cathepsin-G and also Lysozyme often result in a similar pANCA pattern. These atypical pANCA occur in collagenosis and related inflammatory rheumatic diseases. Besides, different untypical variants of pANCA IF patterns – granulocyte specific antinuclear antibodies (GS-ANA) – are indistinguishable from pANCA. Therefore, a distinct interpretation and classification of the IF patterns is difficult and every positive IF-ANCA finding should be differentiated by ELISA techniques using the purified single antigens.

PR3 and MPO are well defined and reliable serologic markers for a definite group of primary systemic vasculitides (PSV), they are also called ANCA- associated vasculitides (AAV). The incidence is 1 in 1000 in the whole population and nearly 5 in 1000 in the elderly. The clinical appearance of AAV is characterised by manifestations in the kidneys, the lung and the respiratory tract.

PR3 is the most frequent component of cANCA and the landmark autoantigen in granulomatosis with polyangiitis (GPA, formerly named Wegener's granulomatosis) with a clinical specificity of more than 95% for the disease.

MPO, the main target antigen of pANCA, is present in 70% of patients with microscopic polyangiitis (MPA) and differentiates MPA from other autoimmune diseases.

Anti-PR3 and anti-MPO levels correlate with the clinical status; they are high in active disease. Antibody titres decrease under therapy and become undetectable after remission.

BPI (Bactericidal permeability-increasing protein) is located mainly in the primary granules of PMN but is also found on the surface of PMN and peripheral blood monocytes (PBM). BPI exhibits strong anti-microbial activity against Gram-negative bacteria and potent endotoxin-neutralizing activity, due to its high binding affinity for bacterial lipopolysaccharides. BPI is a target of ANCA in a variety of diseases of different aetiologies like cystic fibrosis, inflammatory bowel diseases (IBD), reactive arthritis, HIV, the peptide transporter complex associated with antigen presentation (TAP) deficiency and chronic obstructive pulmonary disease (COPD). Anti-BPI antibodies are detectable in Crohn's disease (23 %), ulcerative colitis (37 %) and primary sclerosing cholangitis (36 %). They constitute important markers for these diseases, but not for the ANCA-associated vasculitides.

Elastase is a serine protease with sequence homology of 54 % to proteinase 3. It mainly occurs in polymorph nuclear neutrophil granulocytes (PMN), in macrophages and endothelial cells. Degradation of proteoglycans by neutrophils is mainly due to the proteolytic activity of elastase. Moreover, Elastase is significantly involved in tissue destruction related to emphysema and rheumatoid arthritis. Autoantibodies against elastase are generally associated with inflammatory rheumatic disorders, e.g. rheumatoid arthritis, systemic lupus erythematosus or vasculitis.

Lysozyme is a glycosidase, which cleaves the glycosidic bond between C-1 of MNAc and C-4 of GlcNAc. Lysozyme is localised in the azurophilic as well as in the specific granules of neutrophils and in extracellular liquid compartments like tears and salivary, where it spreads out its antimicrobial activities against invading bacteria. Lysozyme belongs also to the pANCA group. Autoantibodies against Lysozyme occur in higher frequency in rheumatoid vasculitis and inflammatory bowel disease like ulcerative colitis.

Lactoferrin is an iron-binding protein with physiological anti-microbial effect and it is a non-specific antiphlogistic defence factor at mucosal surfaces. Lactoferrin occurs in secretions such as milk, tears and saliva. Lactoferrin also resides in the specific granules of polymorph nuclear neutrophil leukocytes (PMN). During active inflammatory disease, raised serum levels of Lactoferrin can be measured. Autoantibodies against Lactoferrin belong to the pANCA group. They occur in higher frequency in patients with rheumatoid vasculitis (RV), ulcerative colitis (CU) and primary sclerosing cholangitis (PSC).

A survey of documented clinical indications, the corresponding immunofluorescence patterns and target antigens is given in the following table:

Diseases	IF patterns	Target antigen
<i>Systemic Vasculitis Syndromes</i>		
Wegener's Granulomatosis	c-ANCA, rare p-ANCA	PR3, rare MPO
Microscopic Polyangiitis	c-ANCA, p-ANCA	PR3, MPO
Churg-Strauss-Syndrome	p-ANCA	MPO
Polyarteritis nodosa	rare ANCA	rare PR3 and MPO
Unclassified Vasculitis	rare	no PR3 and MPO
<i>Collagen Diseases and other Rheumatic Disorders</i>		
Rheumatoid arthritis	GS-ANA, p-ANCA, atypical ANCA	unknown, ANA, rare MPO, Lactoferrin
SLE	p-ANCA	rare MPO, Lactoferrin
<i>Other Diseases</i>		
Ulcerative Colitis		Cathepsin-G, Lactoferrin

CONTENTS OF THE KIT

Sufficient for 96 determinations

1 One divisible microplate consisting of 12 modules of 8 wells each. Ready to use.

3x 1.5 ml Control A (negative), Control B (cut-off) and Control C (positive), containing ANCA antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN₃ 0.09%), yellow. Ready to use.

20 ml Sample Buffer P, containing PBS, BSA, detergent, preservative NaN₃ 0.09%, yellow, 5 x conc.

15 ml Enzyme Conjugate containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative ProClin 300 0.05%, light red. Ready to use.

15 ml TMB Substrate; containing 3,3', 5,5'- Tetramethylbenzidin, colorless. Ready to use.

15 ml Stop Solution; contains acid. Ready to use.

20 ml Wash Solution, containing Tris, detergent, preservative NaN₃ 0.09%; 50 x conc.

1 Instruction for Use

1 Certificate of Analysis

MATERIALS REQUIRED

- Microplate reader capable of endpoint measurements at 450 nm; optional: reference filter at 620 nm
- Data reduction software
- Multi-channel dispenser or repeatable pipette for 100 µl
- Vortex mixer
- Pipettes for 10 µl, 100 µl and 1000 µl
- Laboratory timing device
- Distilled or deionised water
- Measuring cylinder for 1000 ml and 100 ml
- Plastic container for storage of the wash solution

This ELISA assay is suitable for use on open automated ELISA processors. Each assay has to be validated on the respective automated system. Detailed information is provided upon request.

SPECIMEN COLLECTION, STORAGE AND HANDLING

- Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
- Allow blood to clot and separate the serum or plasma by centrifugation.
- Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia should be avoided, but does not interfere with this assay.
- Specimens may be refrigerated at 2-8°C for up to five days or stored at -20°C up to six months.
- Avoid repetitive freezing and thawing of serum or plasma samples. This may result in variable loss of antibody activity.
- Testing of heat-inactivated sera is not recommended.

STORAGE AND STABILITY

- Store test kit at 2-8°C in the dark.
- Do not expose reagents to heat, sun, or strong light during storage and usage.
- Store microplate sealed and desiccated in the clip bag provided.
- Unopened reagents are stable until expiration of the kit. See labels for individual batch.
- Diluted Wash Solution and Sample Buffer are stable for at least 30 days when stored at 2-8°C. We recommend consumption on the same day.

PROCEDURAL NOTES

- Do not use kit components beyond their expiration dates.
- Do not interchange kit components from different lots and products.
- All materials must be at room temperature (20-28°C) prior to use.
- Prepare all reagents and samples. Once started, perform the test without interruption.
- Double determinations may be done. By this means pipetting errors may become obvious.
- Perform the assay steps only in the order indicated.
- Always use fresh sample dilutions.
- Pipette all reagents and samples into the bottom of the wells.
- To avoid carryover or contamination, change the pipette tip between samples and different kit controls.
- Wash microwells thoroughly and remove the last droplets of Wash Solution.
- All incubation steps must be accurately timed.
- Do not re-use microplate wells.

WARNINGS AND PRECAUTIONS

- All reagents of this kit are intended for professional in vitro diagnostic use only.
 - Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
 - Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
 - Avoid contact with the substrate TMB (3,3',5,5'-Tetramethyl-benzidine).
 - Stop solution contains acid, classification is non-hazardous. Avoid contact with skin.
 - Controls, Calibrators, Sample Buffer and Wash Solution contain sodium azide (NaN₃) 0.09% as preservative. This concentration is classified as non-hazardous.
 - Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as non-hazardous.
 - During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:
 - First aid measures: In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into contact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
 - Personal precautions, protective equipment and emergency procedures:
 - Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.
 - Exposure controls / personal protection: Wear protective gloves of nitril rubber or natural latex. Wear protective glasses. Used according to intended use no dangerous reactions known.
 - Conditions to avoid: Since substrate solution is light-sensitive. Store in the dark.
 - For disposal of laboratory waste the national or regional legislation has to be observed.
- Observe the guidelines for performing quality control in medical laboratories by assaying control sera.

PREPARATION OF REAGENTS

Wash Solution

Dilute the contents of one vial of the buffered wash solution concentrate (50x) with distilled or deionised water to a final volume of 1000 ml prior to use.

Sample Buffer

Sample Buffer P: Prior to use dilute the contents (20 ml) of one vial of sample buffer 5x concentrate with distilled or deionised water to a final volume of 100 ml.

Preparation of samples

Dilute patient samples 1:100 before the assay: Put 990 µl of prediluted sample buffer in a polystyrene tube and add 10 µl of sample. Mix well. Note: Calibrators / Controls are ready to use and need not be diluted.

TEST PROCEDURE

Prepare enough microplate modules for all calibrators / controls and patient samples.

1. Pipette **100 µl** of calibrators, controls and prediluted patient samples into the wells.
2. Incubate for **30 minutes** at room temperature (20-28 °C).
3. Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
4. Dispense **100 µl** of enzyme conjugate into each well.
5. Incubate for **15 minutes** at room temperature.
6. Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
7. Dispense **100 µl** of TMB substrate solution into each well.
8. Incubate for **15 minutes** at room temperature
9. **Add 100 µl** of stop solution to each well of the modules
10. Incubate for **5 minutes** at room temperature.
11. Read the optical density at 450 nm (reference 600-690nm) and calculate the results. The developed colour is stable for at least 30 minutes. Read during this time.

Example for a pipetting scheme:

	1	2	3	4	5	6	7	8	9	10	11	12	Antigens coated in rows:
A	A	B	C										Reference
B	P1	P2	P3										PR3
C	P1	P2	P3										MPO
D	P1	P2	P3										BPI
E	P1	P2	P3										Elastase
F	P1	P2	P3										Cathepsin G
G	P1	P2	P3										Lysozym
H	P1	P2	P3										Lactoferrin

P1, ... patient sample A-C controls

VALIDATION

Test results are valid if the optical densities at 450 nm for calibrators / controls and the results for controls comply with the reference ranges indicated on the Certificate of Analysis enclosed in each test kit. If these quality control criteria are not met the assay run is invalid and should be repeated.

CALCULATION OF RESULTS

First for each antigen-coated row optical density (OD) of a sample is adjusted by a lot-specific factor stated in the certificate of analysis:

$$\text{OD sample adj.} = \text{OD sample} * \text{lot-specific factor}$$

For each antigen-coated row the OD sample adj. is expressed as Index value

$$\text{Index} = \text{OD sample adj.} / \text{OD Control B}$$

PERFORMANCE CHARACTERISTICS**Calibration**

This assay system is calibrated in relative arbitrary units, since no international reference preparation is available for this assay.

Measuring range

not applicable

Expected values

In a normal range study with samples from healthy blood donors the following ranges have been established with this ELISA assay: Cut-off Index 1.0

Interpretation of results

Negative: Index < 1.0

Positive: Index ≥ 1.0

Linearity

Patient samples containing high levels of specific antibody were serially diluted in sample buffer. Activity for each dilution step was calculated as Index- Value.

Sample	Dilution	Observed Index	Expected Index	O/E [%]
PR3	1:100	3.7	3.7	100
.	1:200	1.9	1.8	103
.	1:400	1.0	0.9	103
MPO	1:100	3.4	3.4	100
.	1:200	1.8	1.7	106
.	1:400	0.9	0.9	100
BPI	1:100	3.8	3.8	100
.	1:200	2.0	1.9	104
.	1:400	0.9	0.9	100
Elastase	1:100	3.0	3.0	100
.	1:200	1.6	1.5	105
.	1:400	0.7	0.7	100
Cathepsin G	1:100	2.8	2.8	100
.	1:200	1.3	1.4	91
.	1:400	0.6	0.7	83
Lysozyme	1:100	3.1	3.1	100
.	1:200	1.5	1.6	94
.	1:400	0.7	0.8	91
Lactoferrin	1:100	3.6	3.6	100
.	1:200	1.7	1.8	94
.	1:400	0.8	0.9	89

Limit of detection

not applicable

Interfering substances

No interference has been observed with haemolytic (up to 1000 mg/dl) or lipemic (up to 3 g/dl triglycerides) sera or plasma, or bilirubin (up to 40 mg/dl) containing sera or plasma. Nor have any interfering effects been observed with the use of anticoagulants (Citrate, EDTA, Heparine). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

Reproducibility

Intra-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 24 determinations in a single run. Results for precision-within-assay are shown in the table below.

Inter-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 6 determinations in 5 different runs. Results for run-to-run precision are shown in the table below.

Intra-Assay		
Sample	Mean Index	CV %
PR3	1.8	3.2
MPO	2.0	2.8
BPI	1.3	4.2
Elastase	1.3	4.6
Cathepsin G	1.4	5.1
Lysozyme	1.1	4.5
Lactoferrin	1.9	3.0

Inter-Assay		
Sample	Mean Index	CV %
PR3	1.7	2.8
MPO	1.9	2.0
BPI	1.3	2.8
Elastase	1.2	3.3
Cathepsin G	1.4	2.8
Lysozyme	1.0	3.7
Lactoferrin	1.8	2.4

Study results

Study population	IFA	n	n Pos	%
ANCA vasculitis/ non ANCA vasculitis or other conditions	pos	102	92	90.2
healthy controls or non-rheumatological or non-ANCA vasculitis	neg	234	32	13.7

Clinical Diagnosis

	Pos	Neg	
Pos	92	32	
Neg	10	202	
	102	234	336

Sensitivity:	90.2 %
Specificity:	86.3 %
Overall agreement:	87.5 %

LIMITATIONS OF THE PROCEDURE






This assay is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated concerning the entire clinical picture of the patient. Also every decision for therapy should be taken individually.

The above pathological and normal reference ranges for antibodies in patient samples should be regarded as recommendations only. Each laboratory should establish its own ranges according to ISO 15189 or other applicable laboratory guidelines.

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SYMBOLS USED WITH DEMEDITEC ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
	European Conformity	CE-Konformitätskennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeitsdatum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
				
	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilstrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevaringstemperatur	Förvaringstemperatur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..