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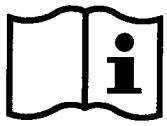

Instruction for use

Cathepsin G Ab ELISA

Enzyme Immunoassay for Quantitative Determination of IgG Auto-antibodies to Cathepsin G in human serum or plasma

CE

IVD

REF **DE7120**

Σ **96 Tests**

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JS
Updated 130417

PRINCIPLE OF THE TEST

Highly purified Cathepsin G is bound to microwells. 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.
Cathepsin G: The cathepsins belong to a group of intracellular proteases mainly found in lysosomes, especially of the spleen, the liver and the kidney. Cathepsin G is a serine protease and a further pANCA antigen. It participates to a great part in the destruction of osteoid tissue as of its hydrolytic properties. The autoantibodies against Cathepsin G occur mainly in collagenosis and other related inflammatory rheumatic diseases, e.g. SLE, Sjögren´s syndrome and Felty's syndrome.

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

Diseases	**IF pattern**	**Target antigen**
Systemic Vasculitis Syndromes		
Wegener's Granulomatosis	c-ANCA, rare p-ANCA	PR3, rare MPO
Microscopic Polyangitis	c-ANCA, p-ANCA	PR3, MPO
Churg-Strauss-Snydrome	p-ANCA	MPO
Polyarteritis nodosa	Rare ANCA	Rare PR3 and MPO
Unclassified Vasculitis	Rare	No PR3 and MPO
Collagen Diseases and other Rheumatic Disorders		
Rheumatoid arthritis	GS-ANA, p-ANCA, atypical ANCA	Unknown, ANA, rare MPO, Lactoferrin
SLE	p-ANCA	Rare MPO, Lactoferrin
Other Diseases		
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.

6x 1.5 ml Calibrator A-F (0, 6.3, 12.5, 25, 50, 100 U/ml), containing serum/buffer matrix (PBS, BSA, detergent, NaN_3 0.09%), yellow. Ready to use.

2x 1.5 ml Control positive (1) and negative (2), containing cathepsin G antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN_3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.

20 ml Sample Buffer P, containing PBS, BSA, detergent, preservative NaN_3 0.09%, yellow, 5x 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 NaN_3 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.

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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.
- Calibrators, Controls, sample buffer and Wash Solution contain sodium azide ($NaN_{3)}$ 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 nitrile 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.

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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
A	A	P1										
B	B	P2										
C	C	P3										
D	D											
E	E											
F	F											
G	C+											
H	C-											

P1, ... patient sample A-F calibrators C+, 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

For quantitative results plot the optical density of each calibrator versus the calibrator concentration to create a calibration curve. The concentration of patient samples may then be estimated from the calibration curve by interpolation.
Using data reduction software a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

PERFORMANCE CHARACTERISTICS

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

Measuring range
The calculation range of this ELISA assay is 0 - 100 U/ml

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 10 U/ml

Interpretation of results
Negative: < 10 U/ml
Positive: ≥ 10 U/ml

Linearity
Patient samples containing high levels of specific antibody were serially diluted in sample buffer to demonstrate the dynamic range of the assay and the upper / lower end of linearity. Activity for each dilution was calculated from the calibration curve using a 4-Parameter-Fit with lin-log coordinates.

Sample	Dilution	Observed U/ml	Expected U/ml	O/E [%]
1	1:100	85.4	85.4	100
.	1:200	43.1	42.7	101
.	1:400	21.8	21.4	102
.	1:800	10.3	10.7	96
.	1:1600	4.8	5.3	91
2	1:100	78.9	78.9	100
.	1:200	40.2	39.5	102
.	1:400	20.0	19.7	102
.	1:800	9.7	9.9	98
.	1:1600	4.5	4.9	92

Limit of detection
Functional sensitivity was determined to be: 0.5 U/ml

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, Heparin). 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 U/ml	CV %
1	11.1	5.7
2	25.8	3.1
3	60.4	4.2

Inter-Assay		
Sample	Mean U/ml	CV %
1	11.5	6.3
2	26.2	3.3
3	56.9	4.5

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Study results

Study population	IFA	n	n Pos	%
ANCA vasculitis	Pos	54	23	42.6
Other conditions	Pos	35	26	74.3
Non-ANCA vasculitis	Pos	13	8	61.5
Healthy controls	Neg	120	0	0.0
Non-rheumatological	Neg	72	16	22.2
Non-ANCA vasculitis	neg	42	13	31.0

Clinical Diagnosis

	Pos	Neg	
Pos	57	29	
Neg	45	205	
	102	234	336

Sensitivity:	55.9 %
Specificity:	87.6 %
Overall agreement:	78.0 %

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-Konfirmitätskennzeichnung	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

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