DRG® ANCA combi (EIA-3595)

CE

RUO in the USA

Revised 29 Dec. 2009 rm (Vers. 2.0)

Please use only the valid version of the package insert provided with the kit.

NAME AND INTENDED USE

ANCA combi is an indirect solid phase enzyme immunoassay (ELISA) for the qualitative determination of anti-neutrophil cytoplasmic antibodies (ANCA) directed against PR3, MPO, BPI, Elastase, Cathepsin G, Lysozyme and Lactoferrin in human serum or plasma. The assay is intended for in vitro use only as an aid in the determination of certain autoimmune vasculitides.

SUMMARY AND EXPLANATION OF THE TEST

The acronym ANCA (Antineutrophil Cytoplasmic Autoantibodies) is defined by an accumulation of autoantibodies with specificity against different granulocytic, monozytic and probably endothelial cytoplasmic antigens.PR3 and MPO are well defined as reliable serological markers for a definite group of primary systemic vasculitides (PSV),which were also named ANCA associated vasculitides (AAV).The incidence of AAV is 1.5 per 1000 and in the group of older persons nearly 5 per 1000. The clinical appearance of the AAV is characterized trough manifestations in lung, kidney and respiratory tract. Up to now, ANCA screening has been done with immunofluorescence techniques, but often there have been difficulties in the evaluation and in clinical findings. Therefore, the results have to be scrutinized with counter examinations on other cells or in other test systems like ELISA. Moreover it was not possible to differentiate the single cANCA and pANCA antigens.

Proteinase 3:
The major antigen for the cANCA reactivity is the 29 kD neutral serin protease 3 (synonyms:p29,AGP7,Wegener autoantigen),which belongs to the Trypsin/Chymotrypsin family.PR3was already described in 1973 by Ohlsson and Olsson under the name neutrophile collagenase. In the meantime it seams certain, that autoantibodies against PR3 are highly specific as serological marker for the diagnosis of Wegener's granulomatosis (specificity: initial phase 50%, generalization phase > 90%).Moreover there is a correlation between the concentration of the autoantibodies and the disease activity.

Myeloperoxidase:
In nearly 60% of the pANCA findings MPO is the major antigen. The occurrence of autoantibodies against MPO is classified as relevant marker for the rapid progressive nephritis. Moreover these antibodies occur in 70-90% in all patients with serious kidney injury. Over and about they have also been detected at the Churg-Strauß-Syndrom (CSS),Microscopic Polyangiitis(MPA) and other vasculitis diseases. The concentration of the autoantibodies correlates well with the disease activity of MPA.MPA is also characterized by clinical manifestations of lung, kidney and respiratory tract, but these manifestations are, in contrast to WG, not granulomatous. However, these antibodies have, in contrast to the high specificity of PR3 antibodies for WG, a minor specificity of 60% in the diagnosis of MPA. The absence of autoantibodies against MPO and PR3, by simultaneous detection of ANA can be used as a tool for differential diagnosis between AAV and SLE induced vasculitis.

BPI:
Bactericidal permeability-increasing protein, BPI is a membrane-located 55 kD protein and is classed as an ANCA-antigen of polymorph-nuclear granulocytes and monocytes that bind endotoxin. Its autoantibodies are now classified as

cANCA. Due to BPIs high affinity to lipopolysaccharides its anti-microbial effect against Gram-negative bacteria is significant. Autoantibodies against BPI are above all detected in chronically infectious intestinal diseases such as Morbus Crohn or colitis ulcerosa. In contrast to anti-MPO and anti-PR3 autoantibodies, those against BPI seem not to have any association with vasculitis.

Elastase:
Elastase is a serine protease with a sequence homology of 54% to that of proteinase 3.Itoccurs mainly in polymorph-nuclear neutrophilic granulocytes (PMN),in macrophages and endothelial cells. Autoantibodies against this antigen are generally associated with inflammatory rheumatic disorders, e.g. rheumatoid arthritis and vasculitis.

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. The autoantibodies against Cathepsin G occur mainly in collagenosis and other related inflammatory rheumatic diseases, e.g. SLE, Sjögren syndrome and Felty syndrome.

Lysozyme:
LZ is a 14.6 kD glykosidase ,which decomposes the glycosidic bond between C-1 of MNAc andC-4 of GlcNAc. Lysozyme is localized in the azurophilic as well as in the specific granules of neutrophiles and in extracellular liquid compartments like tears and salivary, where it spreads out his antimicrobial activities against invading bacteria. LZ belongs also to the pANCA and autoantibodies against Lysozyme occur in higher frequency in rheumatoid vasculitis and inflammatory bowel disease like colitis ulcerosa.

Lactoferrin:
Lactoferrin (LF) is an iron-binding protein, with a molecular weight of 77-93 kDa, which occurs in high concentrations in secretions at mucosa surfaces, in tears and in milk. LF also resides in the specific granules of polymorphnuclear neutrophil leukocytes (PMN) and becomes exocytosed upon PMN activation. LF belongs to the pANCA, depending on the redistribution from the granules toward the nuclei, upon ethanol fixation. Autoantibodies against Lactoferrin occur in higher frequency in patients with rheumatoid vasculitis (RV),colitis ulcerosa (CU) andprimary sclerosing cholangitis (PSC).

PRINCIPLE OF THE TEST

PR3,MPO,BPI,Elastase,Cathepsin G, Lysozyme and Lactoferrin are bound separatedly to microwells. Antibodies against these antigens, if present in diluted serum or plasma, bind to the respective antigens. Washing of the microwells removes unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human IgG immunologically detects 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 color. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow color is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of IgG antibodies present in the original sample.

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WARNINGS AND PRECAUTIONS

1. All reagents of this kit are strictly intended for in vitro determinations only.
2. Do not interchange kit components from different lots.
3. 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.
4. Avoid contact with the TMB (3,3´,5,5´-Tetramethyl-benzidine). If TMB comes into contact with skin, wash thoroughly with water and soap.
5. Avoid contact with the Stop Solution which is acid. If it comes into contact with skin, wash thoroughly with water and seek medical attention.
6. Some kit components (i.e. Controls, Sample buffer and Buffered Wash Solution) contain Sodium Azide as preservative. Sodium Azide (NaN_3) is highly toxic and reactive in pure form. At the product concentrations (0.09%), though not hazardous. Despite the classification as non-hazardous, we strongly recommend using prudent laboratory practices (see 8., 9., 10.).
7. Some kit components contain Proclin 300 as preservative. When disposing reagents containing Proclin 300, flush drains with copious amounts of water to dilute the components below active levels.
8. Wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.
9. Do not pipette by mouth.
10. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled.
11. Avoid contact between the buffered Peroxide Solution and easily oxidized materials; extreme temperature may initiate spontaneous combustion.

Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera. During handling of all kit reagents, controls and serum samples observe the existing legal regulations.

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CONTENTS OF THE KIT

Package size 96 determinations

Qty.1	Divisible microplate consisting of 12 modules of 8 wells each, coated with highly purified antigens: PR3, MPO, BPI, Elastase, Cathepsin G, Lysozyme and Lactoferrin. Ready to use.
4 vials, 1.5 ml each	ANCA Controls in a serum/buffer matrix (PBS, BSA, NaN_3 <0.1%(w/w)) Negative Control (A), Cut-Off Control (B) and Positive Control (C) For the respective concentrations see the enclosed QC insert. Ready to use.
1 vial, 20 ml	Sample buffer (Tris, NaN_3 <0.1% (w/w)), yellow, concentrate (5x).
1 vial, 15 ml	Enzyme conjugate solution (PBS, Proclin 300 <0.5% (v/v)), (light red) containing polyclonal rabbit anti-human IgG; labelled with horseradish peroxidase. Ready to use.
1 vial, 15 ml	TMB substrate solution. Ready to use.
1 vial, 15 ml	Stop solution (contains acid). Ready to use.
1 vial, 20 ml	Wash solution (PBS, NaN_3 <0.1% (w/w)), concentrate (50x).

STORAGE AND STABILITY

1. Store the kit at 2-8 °C.
2. Keep microplate wells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light during storage and usage.
5. Diluted sample buffer and wash buffer are stable for at least 30 days when stored at 2-8 °C.

MATERIALS REQUIRED

Equipment

- Microplate reader capable of endpoint measurements at 450 nm
- Multi-Channel Dispenser or repeatable pipet for 100 µl
- Vortex mixer
- Pipets for 10 µl, 100 µl and 1000 µl
- Laboratory timing device
- Data reduction software

Preparation of reagents

- Distilled or deionized water
- Graduated cylinder for 100 and 1000 ml
- Plastic container for storage of the wash solution

SPECIMEN COLLECTION, STORAGE AND HANDLING

- Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
- Allow blood to clot and separate the serum by centrifugation.
- Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia is best 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 samples. This may result in variable loss of autoantibody activity.
- Testing of heat-inactivated sera is not recommended.

PROCEDURAL NOTES

1. Do not use kit components beyond their expiration dates.
2. Do not interchange kit components from different lots.
3. All materials must be at room temperature (20-28 °C).
4. Have all reagents and samples ready before start of the assay. Once started, the test must be performed without interruption to get the most reliable and consistent results.
5. Perform the assay steps only in the order indicated.
6. Always use fresh sample dilutions.
7. Pipette all reagents and samples into the bottom of the wells.
8. To avoid carryover contamination change the tip between samples and different kit controls.
9. It is important to wash microwells thoroughly and remove the last droplets of wash buffer to achieve best results.
10. All incubation steps must be accurately timed.
11. Control sera or pools should routinely be assayed as unknowns to check performance of the reagents and the assay.
12. Do not re-use microplate wells.

For all controls, the respective concentrations are provided on the labels of each vial. Using these concentrations a calibration curve may be calculated to read off the patient results semi-quantitatively.

PREPARATION OF REAGENTS

Preparation of sample buffer

Dilute the contents of each vial of the sample buffer concentrate (5x) with distilled or deionized water to a final volume of 100 ml prior to use.
Store refrigerated: stable at 2-8 °C for at least 30 days after preparation or until the expiration date printed on the label.

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Preparation of wash solution

Dilute the contents of each vial of the buffered wash solution concentrate (50x) with distilled or deionized water to a final volume of 1000 ml prior to use.
Store refrigerated: stable at 2-8 °C for at least 30 days after preparation or until the expiration date printed on the label.

Sample preparation

Dilute all patient samples **1:100** with sample buffer before assay.
Therefore combine 10 µl of sample with 990 µl of sample buffer in a polystyrene tube. Mix well.

Controls are ready to use and need not be diluted.

TEST PROCEDURE

1. Prepare a sufficient number of microplate modules to accommodate controls and prediluted patient samples.
2. Pipet **100 µl** of controls and prediluted patient samples in duplicate into the wells.

		1	2	3	4	5	6
reference antigen	**a**	CA	CB	CC			
PR3	**b**	P1	P2	P3	P..		
MPO	**c**	P1	P2	P3	P..		
BPI	**d**	P1	P2	P3	P..		
Elastase	**e**	P1	P2	P3			
Cathepsin G	**f**	P1	P2	P3			
Lysosyme	**g**	P1	P2	P3			
Lactoferrin	**h**	P1	P2	P3			

CA - CC Controls A to C
P1, P2,.. Patient sample

3. Incubate for 30 minutes at room temperature (20-28 °C).
4. Discard the contents of the microwells and wash **3 times** with **300 µl** of wash solution.
5. Dispense **100 µl** of enzyme conjugate into each well.
6. Incubate for 15 minutes at room temperature.
7. Discard the contents of the microwells and wash **3 times** with **300 µl** of wash solution.
8. Dispense **100 µl** of TMB substrate solution into each well.
9. Incubate for 15 minutes at room temperature.
10. Add **100 µl** of stop solution to each well of the modules and incubate for 5 minutes at room temperature.
11. Read the optical density at 450 nm and calculate the results. Bi-chromatic measurement with a reference at 600-690 nm is recommended.

The developed colour is stable for at least 30 minutes. Read optical densities during this time.

INTERPRETATION OF RESULTS

Quality Control

This test is only valid if the optical density at 450 nm for Negative Control (A), Cut-Off Control (B), and Positive Control (C) complies with the respective range indicated on the Quality Control Certificate enclosed to each test kit ! If any of these criteria is not fulfilled, the results are invalid and the test should be repeated.

Calculation of results

Evaluation of the ANCA combi test is carried out by comparison of the optical densities of the patient sample with the optical density of the Cut-off Control. Previously, each patient`s OD must be corrected by a specific factor (please refer to the certificate).

For each patient sample 7 Index-values should be calculated according to the following equation:

$$\text{Index - value} = \frac{OD_{sample} \times \text{Factor}}{OD_{\text{Cut-Off control B}}}$$

Calculation example

The chart below shows typical results for ANCA combi. These data are intended for illustration only and should not be used to calculate results from another run.

Sample No.	Anti-	Row	OD_{Sample}	Factor	$OD_{Cut-off}$	OD-Quotient	Result
1	PR3	B	0,083	1,10		0,22	negative
1	MPO	C	0,920	1,00		2,19	positive
1	BPI	D	0,138	1,25		0,41	negative
1	Elastase	E	0,133	0,85	0,420	0,27	negative
1	Cath. G	F	0,505	0,75		0,90	negative
1	Lysozyme	G	0,420	1,00		1,00	positive
1	Lactoferrin	H	0,112	0,80		0,21	negative
2	PR3	B	2,143	1,10		5,61	high pos.
2	MPO	C	0,240	1,00	0,420	0,57	negative
2	BPI	D	0,079	1,25		0,24	negative
2	…	…	…	…		…	…

Interpretation of results

A sample is positive for one ANCA parameter if its OD-Quotient is ≥1.0:

	ANCA combi OD-Quotient
Negative:	< 1.0
Positive:	≥ 1.0

PERFORMANCE CHARACTERISTICS

Specificity

The microplate is coated with the antigens PR3, MPO, BPI, Elastase, Cathepsin G, Lysozyme and Lactoferrin, respectively. All antigen preparations are highly purified by affinity chromatography. The ANCA combi test is specific only for autoantibodies directed to these antigens. No cross reactivities have been observed.

Calibration

Since no international reference preparations for ANCA autoantibodies are available, the assay system is calibrated arbitrarily.

LIMITATIONS OF PROCEDURE

The ANCA combi ELISA 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.

INTERFERING SUBSTANCES

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

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Symbols used with DRG 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
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	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	Mindesthaltbarkeits-datum	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
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilsttrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	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..