



ORGENTEC Diagnostika GmbH
Carl-Zeiss-Straße 49
55129 Mainz
Tel.: 06131-9258-0
Fax: 06131-9258-58

ANCA-3-Line

ORG 789-08 8 strips

ORG 789-16 16 strips

**Membrane based enzyme immunoassay
for the semi-quantitative determination
of autoantibodies against PR3, MPO and
GBM.**

Instruction for use

CE

CONTENTS

<i>CONTENTS</i>	2
<i>NAME AND INTENDED USE</i>	3
<i>SUMMARY AND EXPLANATION OF THE TEST</i>	3
<i>PRINCIPLE OF THE TEST</i>	3
<i>WARNINGS AND PRECAUTIONS</i>	4
<i>CONTENTS OF THE KIT</i>	4
<i>STORAGE AND STABILITY</i>	5
<i>MATERIALS REQUIRED</i>	5
<i>SPECIMEN COLLECTION, STORAGE AND HANDLING</i>	5
<i>PROCEDURAL NOTES</i>	5
<i>PREPARATION OF REAGENTS</i>	6
<i>TEST PROCEDURE</i>	6
<i>INTERPRETATION OF RESULTS</i>	6
<i>PERFORMANCE CHARACTERISTICS</i>	7
<i>LIMITATIONS OF PROCEDURE</i>	7
<i>INTERFERING SUBSTANCES</i>	8
<i>REFERENCES</i>	8
<i>INCUBATION SCHEME</i>	9

NAME AND INTENDED USE

ANCA-3-Line is a membrane based enzyme immunoassay for the semi-quantitative determination of autoantibodies against PR3, MPO and GBM. The assay is intended for in vitro diagnostic use only as an aid in the diagnosis of vasculitis diseases.

SUMMARY AND EXPLANATION OF THE TEST

Anti-neutrophil cytoplasm antibodies (ANCA) represent a group of autoantibodies directed towards the cytoplasmic components of the neutrophil granulocytes and monocytes. The ANCA-3-Line provides a screening for autoantibodies against PR3, MPO and GBM. The determination of these antibodies is essential for the detection of systemic vasculitis and the differentiation of systemic diseases.

PR3 (Proteinase 3)

PR3-ANCA is the classical autoantigen in Wegener's granulomatosis with a clinical specificity of more than 95%. Wegener's granulomatosis is primarily a granulomatosis showing secondary a vasculitis. Normally the antibody titer correlates with the activity of the disease. Mostly in the active phase of the disease very high anti-PR3 titer can be found. During the therapy the antibody titer falls and becomes negative with the beginning of the remission.

MPO (Myeloperoxidase)

The target antigen myeloperoxidase (MPO) is mainly present (70%) in microscopic polyangiitis (MPA). Since the differentiation between MPA and other autoimmune manifestations with a pulmorenale syndrome (e.g. Goodpasture syndrome, systemic Lupus erythematosus, Wegener's granulomatosis) is often difficult, the detection of antibodies against MPO is, especially in early state of diagnosis, very important.

GBM (Glomerular basement membrane)

The reactivity of Goodpasture specific anti-GBM autoantibodies is directed against the 29 kDa NC1 domain of the α -3 chain of type IV collagen of GBM. Primarily, Goodpasture syndrome is an autoimmune disorder of the kidneys. The syndrome is considered as an autoimmune disorder consisting of the triad of glomerulonephritis, lung hemorrhage and antiglomerular basement antibodies formation.

The ANCA-3-Line combines the advantages of the immunoblot technique with a set of well selected antigens.

PRINCIPLE OF THE TEST

Highly purified antigens are bound to nitrocellulose membrane strips. Antibodies against these antigens, if present in diluted serum or plasma, bind to the respective antigens. Washing of the membrane strips removes unspecific serum and plasma components. Alkaline phosphatase conjugated anti-human IgG immunologically detects the bound patient antibodies forming a conjugate/antibody/antigen complex. Washing of the membrane strips removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form an insoluble blue-violet product. Washing of the membrane strips removes unhydrolyzed substrate. The amount of color is directly proportional to the concentration of IgG antibodies present in the original sample.

WARNINGS AND PRECAUTIONS

1. All reagents of this kit are strictly intended for in vitro diagnostic use 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 substrate solution BCIP/NBT (5-bromo-4-chloro-3-indolyl phosphate / p-nitro blue tetrazolium chloride). If BCIP/NBT comes into contact with skin, wash thoroughly with water and soap.
5. 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 7., 8., 9.).
6. 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.
7. Wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.
8. Do not pipette by mouth.
9. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled.

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.

CONTENTS OF THE KIT

Package size	8 or 16 determ.
Qty. 8 or 16	Nitrocellulose strips, loaded with highly purified native antigens. Ready to use.
1 vial, 20 ml	Sample buffer. Ready to use. This buffer is specifically adapted for the ANCA-3-Line and is not interchangeable with sample buffers of other immunoblots.
1 vial, 20 ml	Wash buffer, concentrate (50x).
1 vial, 20 ml	Enzyme conjugate solution (PBS, NaN_3 <0.1 % (w/w)), (pink) containing polyclonal rabbit anti-human IgG; labelled with alkaline phosphatase. Ready to use.
1 or 2 vials, 5 ml	Substrate solution (BCIP/NBT). Ready to use.
Qty. 1 or 2	Pre-developed nitrocellulose calibration strip (labelled CAL) for semiquantitative evaluation. Ready to use.
Qty. 1 or 2	Incubation tray.
Qty. 1 or 2	Documentation sheet. Ready to use.

STORAGE AND STABILITY

1. Store the kit at 2-8 °C.
2. Keep nitrocellulose strips dry; store together with dessicant and carefully sealed in the plastic tube.
3. Important: The calibration strip is very light-sensitive. Please store dark.
4. The reagents are stable until expiration of the kit.
5. Do not expose test reagents to heat, sun or strong light during storage and usage.
6. Wash buffer is stable for at least 30 days when stored at 2-8 °C.

MATERIALS REQUIRED

Equipment

- Pipets for 10 µl, 500 µl and 1000 µl
- Laboratory timing device
- Rocking platform
- Tweezers

Preparation of reagents

- Distilled or deionized water
- Graduated cylinder for 1000 ml

SPECIMEN COLLECTION, STORAGE AND HANDLING

1. Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
2. Allow blood to clot and separate the serum by centrifugation.
3. Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia is best avoided, but does not interfere with this assay.
4. Specimens may be refrigerated at 2-8 °C for up to five days or stored at -20 °C up to six months.
5. Avoid repetitive freezing and thawing of serum samples. This may result in variable loss of autoantibody activity.
6. 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. To avoid carryover contamination change the tip between samples and different kit controls.
8. Nitrocellulose strips must be handled with gloves or tweezers.
9. All incubation steps must be accurately timed.
10. Control sera or pools should routinely be assayed as unknowns to check performance of the reagents and the assay.

PREPARATION OF REAGENTS

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.

TEST PROCEDURE

1. Insert an ANCA-3-Line strip using tweezers then add 1.0 ml sample buffer to each chamber of the incubation tray. Allow to equilibrate for 5 minutes with gentle rocking.
2. Add 10 µl of patient serum directly to the chamber (effective dilution 1:101).
3. Incubate for 60 minutes with gentle rocking at room temperature.
4. Carefully remove the diluted serum completely from the strips.
5. Add 1.0 ml wash buffer, incubate for 5 minutes, then remove as in step 4. Repeat this procedure twice.
6. Add 1.0 ml enzyme conjugate to each chamber.
7. Incubate for 30 minutes with gentle rocking at room temperature.
8. Remove the diluted conjugate completely from the strips.
9. Add 1.0 ml wash buffer, incubate for 5 minutes, then remove as in step 4. Repeat this procedure twice.
10. Add 500 µl substrate to each strip.
11. Incubate for 10 minutes with gentle rocking at room temperature.
12. Remove the substrate and wash the strips with 1 ml distilled water three times 5 minutes each to stop the reaction.
13. Carefully blot the strips dry with a paper towel.
14. Allow strips to air dry before evaluating.

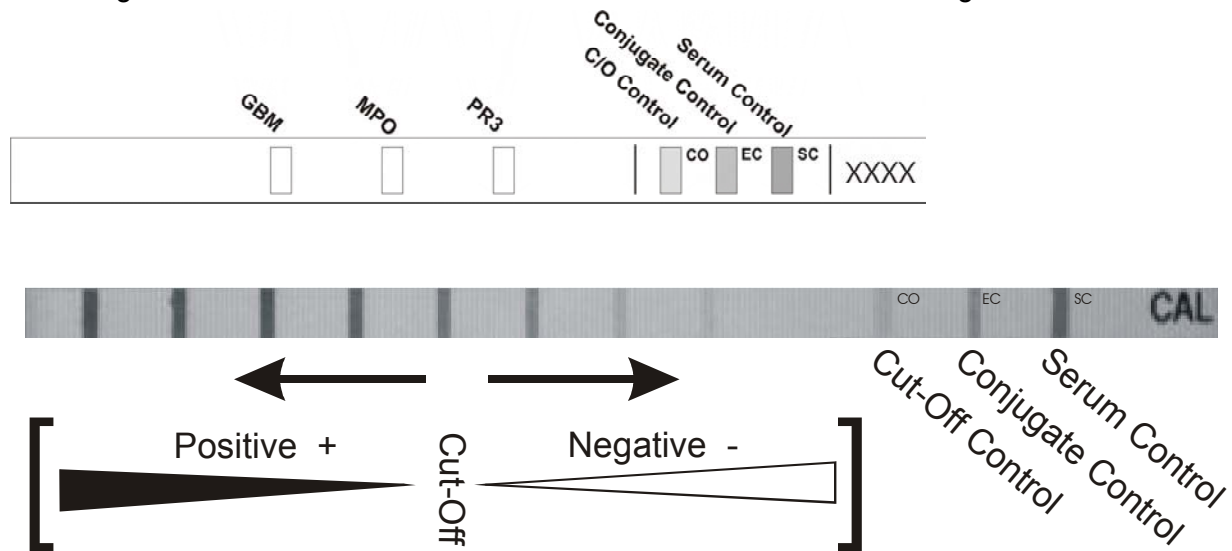
INTERPRETATION OF RESULTS

Quality Control

This test is only valid if the Serum Control (first line), Conjugate Control (second line) and Cut-Off Control (Third line) show a turn-over of substrate in terms of developed bands! If this criteria is not fulfilled, the result is invalid and the test should be repeated.

Interpretation of results

The antigens are coated on the membrane in the order illustrated in the figure below.



Notes to interpretation of patient results:

This is a semi-quantitative assay for the determination of the specificity of autoantibodies in patient serum, allowing a discrimination between negative, borderline, weak positive, positive, and strong positive. Borderline samples should be repeated or tested using an alternative procedure.

PERFORMANCE CHARACTERISTICS

Specificity

The ANCA-3-Line test was evaluated by testing patient sera of known specificity and blood donor sera using the general test procedure. All blood donors gave negative lines for all antigen specificities.

Calibration

The sensitivity, specificity and dose response of the ANCA-3-Line immunoblot was evaluated using clinically defined in house quality control sera containing varying relative amounts of sera with known specificity.

LIMITATIONS OF PROCEDURE

The ANCA-3-Line immunoblot 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.

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.

REFERENCES

1. Gross, W.L.; Csernok, E.; Helmchen, U. Antineutrophil cytoplasmic autoantibodies, autoantigens, and systemic vasculitis. *APMIS*, Vol. 103, 81-97, 1995
2. Hagen, E.C.; Ballieux, B.E.; van Es, L.A.; Daha, M.R.; Van der Woude, F.J. Antineutrophil cytoplasmic autoantibodies: a review of the antigens involved, the assay, and the clinical and possible pathogenic consequences. *Blood*, Vol. 81(8):1996-2002, 1993
3. Jennette, C.J.; Falk, R.H.; Andrassy, K.; Bacon, P.A. et al. Nomenclature of systemic vasculitides. Proposal of an international consensus conference. *Arthritis Rheum.* Vol. 37, 187-192, 1994
4. Jenette, J.C.; Falk, R.J. Antineutrophil cytoplasmic autoantibodies and associated diseases. A review. *Am. J. Kidney Dis.*, Vol. 15, 517-529, 1990
5. Kallenberg, C.G. Laboratory findings in the vasculitides. *Baillieres Clin. Rheumatol.*, Vol. 11, 395-421, 1997
6. Hudson, B.G.; Wieslander, J.; Wisdom, B.J.; Noelken, M.E. Goodpasture syndrome: Molecular architecture and function of basement membrane antigen. *Lab. Invest.*, Vol. 61, 256-269, 1989
7. Goodpasture, E.W. The significance of certain pulmonary lesions to the etiology of influenza. *Am. J. Med. Sci.*, Vol. 158, 864-870, 1919
8. Daly, C.; Conlon, P.J.; Medwar, W.; Walshe, J.J. Characteristics and outcome of anti-glomerular basement membrane disease: a single center experience. *Ren. Fail.*; Vol. 18, 105- 112, 1996
9. Bolton, W.K. Goodpasture's syndrome. *Kidney Int.*; Vol. 50, 1753-1766, 1996
10. Hellmark, T.; Segelmark, M.; Bygren, P.; Wieslander, J. Glomerular basement membrane autoantibodies. in: Peter, J.B.; Shoenfeld, Y. (publisher), *Autoantibodies*, S. 291-298, 1996

INCUBATION SCHEME

- ① Add **blot strip** into the incubation tray
 - Add **1000 µl** sample buffer per strip into the incubation tray
 - Shake **5 minutes** while incubating

- ② Add **10 µl** patient sample and resuspend
 - Shake **60 minutes** while incubating
 - Discard content and wash 3 times for **5 minutes** with **1000 µl** wash buffer, discard wash

- ③ Add **1000 µl** enzyme conjugate solution per strip
 - Shake **30 minutes** while incubating
 - Discard content and wash 3 times for **5 minutes** with **1000 µl** wash buffer, discard wash

- ④ Add **500 µl** substrate per strip
 - Shake **10 minutes** while incubating
 - Discard content and wash 3 times for **5 minutes** with **1000 µl distilled water**, dry blot strips. Read after complete drying, only