Instructions For Use

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ANCA Dot

REF 4028

Dot Immunoassay for the determination of IgG antibodies against Myeloperoxidase (MPO), Proteinase 3 (PR3) and Glomerular Basement Membrane (GBM) in human serum

GA Generic Assays GmbH
Ludwig-Erhard-Ring 3
15827 Blankenfelde-Mahlow OT Dahlewitz
Germany

Phone.: +49 33708 9286 0
Fax: +49 33708 4417 25

info@genericassays.com
www.genericassays.com

IVD CE Σ 24 x 3 2°C–8°C

1 Intended Purpose

The ANCA Dot is a qualitative dot immunoassay for the determination of IgG antibodies against myeloperoxidase (MPO), proteinase 3 (PR3) and glomerular basement membrane (GBM) in human serum.

The ANCA Dot is intended as an aid in the diagnosis of systemic vasculitis and autoimmune renal disorders in conjunction with other clinical and laboratory findings.

The immunoassay is designed for manual professional *in vitro* diagnostic use.

2 Diagnostic Relevance

Anti-neutrophil cytoplasmic antibodies (ANCA) are a group of autoantibodies directed against cytoplasmatic components of neutrophilic granulocytes and are associated with inflammatory vascular diseases.

According to their immunofluorescence pattern on ethanol-fixed human granulocytes ANCA have been classified into cytoplasmic (cANCA) and perinuclear (pANCA) antibodies. Proteinase 3 (PR3), a serine proteinase with a molecular weight of 29 kDa, has been identified as the main autoantigen of cANCA. Antibodies to PR3 are highly specific for Wegener Granulomatosis, a granulomatous disease of nasopharynx, kidney and lung. Antibodies to MPO have been reported in a variety of vasculitides (Microscopic poyangitis, Churg-Strauss Syndrome, Polyarteritis nodosa) and glomerulonephritides (rapidly progressive glomerulonephritis).

Goodpasture syndrome is a medical emergency with a high fatality rate if not treated. The syndrome is characterized by glomerulonehritis, pulmonary haemorrhage and antibody formation against glomerular basement membrane (GBM). The antibodies are targeted to a GBM component, the collagen IV and specifically to the NC1 domain of the □3 chain (Goodpasture antigen). Anti-GBM antibodies are the primary pathogenic autoantibodies, binding along the glomerular basement membrane and inducing glomerulonephritis in all patients with Goodpasture syndrome. Their determination allows to differentiate the syndrome from other causes of glomerular nephritis and pulmonary haemorrhage.

3 Test Principle

Dot immunoassays are frequently used for the determination of specific antibodies directed against multiple antigens. The test strips are coated with various antigens in consistent intervals. If antibodies are present in the patient´s sample, they bind to the respective antigens. A secondary antibody conjugated with the enzyme alkaline phosphatase detects the generated immune complexes. A colorless substrate is converted into a colored, insoluble product. The signal intensity of the precipitated reaction product is proportional to the antibody activity in the sample.

4 Test Components

Component	Description
Test strips **A** **Ag | 24** 24 pieces	24 test strips (ready-to-use), each strip coated with highly purified - MPO (native human) - PR3 (native human) - GBM (recombinant human) as well as positive and negative control
Sample diluent **C** **DIL** 1 x 40 mL, yellow cap	Colored solution (ready-to-use; contains ProClin 300)
Wash buffer **B** **BUF|WASH|10x** 1 x 40 mL, blue cap	Concentrated solution (10x; contains ProClin 300)
Conjugate IgG **D** **CONJ** 1 x 40 mL, red cap	Colored solution of polyclonal anti-human IgG antibody conjugated to alkaline phosphatase (ready-to-use; contains ProClin 300)
Substrate **E** **SUB** 1 x 40 mL, black cap	Substrate solution of bromo-chloro-indolyl-phosphate (PCIP) and nitroblue tetrazolium (NBT; ready-to-use; contains sodium azide)
Incubation tray 3 pieces	Incubation tray for 8 test strips
Interpretation template 1 piece	Interpretation template for gluing of processed strips
QC Certificate 1 piece	-
Instructions for Use 1 piece	-

5 Materials required but not provided

- Common laboratory equipment
- Precision pipettes (5 – 1000 µL) and disposable tips
- Graduated cylinders (100 – 1000 mL)
- Rocking or horizontal plate shaker
- Plastic pincers
- Adsorbent paper or paper towel
- Distilled or de-ionized water

6 Storage and Stability

Upon receipt, all test components must be stored at 2 °C to 8 °C, preferably in the original kit box. If stored properly in their original containers, all components are stable until their expiry date. All components are stable for at least 2 months after opening when stored properly at 2 °C to 8 °C.

7 General Information

This product is for *in vitro* diagnostic use only. The instructions for use must be carefully read before use. They are valid only for the present product with the given composition and must be strictly followed to ensure reliable test results. Deviations can lead to erroneous test results. Components must not be exchanged by test reagents of different lots or of other manufacturers.

Contamination of reagents must be avoided by use of aseptic techniques when removing aliquots from the vials. After use, reagent vials must be tightly closed with their corresponding caps.

Cross-contamination of samples or reagents can lead to inconsistent test results and must be avoided by use of consistent pipetting techniques.

Exposure of reagents to strong light must be avoided throughout the entire test procedure and storage.

Insufficient washing will result in poor precision and elevated measurement signals. After each washing step any residual fluid has to be removed completely.

8 Preparation

8.1 Preparation of Reagents

All components including the test strips must be brought to room temperature (RT: 18 °C to 25 °C) before use for at least 30 min. All liquid components must be mixed gently to ensure homogeneity.

8.1.1 Test Strips

The test strips are provided within an envelope. Unused test strips should always be stored refrigerated and protected from moisture in a plastic bag.

8.1.2 Sample Diluent

The sample diluent is ready-to-use.

8.1.3 Wash Buffer

The wash buffer is concentrated and must be diluted 1:10 with distilled water before use (e. g. 100 mL + 900 mL). A sufficient amount of washing solution must be prepared. The diluted washing solution can be stored at 2 °C to 8 °C up to 30 days.

8.1.4 Conjugate

The conjugate is ready-to-use.

8.1.5 Substrate

The substrate is ready-to-use. Exposure of the substrate solution to strong light should be avoided.

8.2 Preparation of Samples

8.2.1 Sample Material

The use of freshly collected serum from blood taken by venipuncture is recommended. The use of icteric, lipemic, hemolytic or bacterially contaminated samples should be avoided. Insoluble substances must be removed from the sample by centrifugation. Samples must not be thermally inactivated.

8.2.2 Sample Storage

Samples may be kept at 2 °C to 8 °C up to three days. Long-term storage requires -20 °C. Repeated freezing and thawing should be avoided. For multiple use, samples should be aliquoted and kept at -20 °C.

9 Test Performance

9.1 Procedure

Touch test strips with a plastic pincer only. The entire procedure has to be performed in incubation trays on a rocking or horizontal plate shaker. After each addition of solution to the wells, agitate the incubation tray manually to ensure strips are completely immersed and to remove any air bubbles, which may be trapped under the strip. The indicated incubation times and temperatures must be adhered to and significant time shifts during pipetting samples and reagents must be avoided.

Step	Description
1. Addition of washing solution	Place the strips with the reactive side up into the respective wells of the incubation tray. Add 2.0 mL washing solution into each well. Make sure all strips are completely covered with liquid.
2. Incubation	Incubate the strips for 10 min. at RT while shaking
3. Addition of sample diluent	Decant or aspirate the solution carefully. Remaining liquid has to be removed with an absorbent paper. Add 1.5 mL sample diluent into each well. Make sure all strips are completely covered with liquid.
4. Addition of samples	Add 10 µL of undiluted samples per well
5. Incubation	Incubate the strips for 30 min. at RT while shaking
6. Wash cycle	Decant or aspirate the solution carefully and wash 3 times for 3 min. with 1.5 mL washing solution while shaking. Decant the solution carefully. Remaining liquid has to be removed with an absorbent paper.
7. Addition of conjugate	Add 1.5 mL ready-to-use conjugate to each well
8. Incubation	Incubate the strips for 30 min. at RT while shaking
9. Wash cycle	Decant or aspirate the solution carefully and wash 3 times for 3 min. with 1.5 mL washing solution while shaking. Decant the solution carefully. Remaining liquid has to be removed with an absorbent paper.
10. Addition of substrate	Add 1.5 mL ready-to-use substrate to each well
11. Incubation	Incubate the strips for 10 – 12 min. at RT while shaking
12. Wash	Decant or aspirate the solution carefully and wash once for 3 min. with 1.5 mL washing solution at RT while shaking to stop the reaction.
13. Drying	Collect the strips from the wells and dry on absorbent paper for approximately 30 min.
14. Analysis	Glue the dried strips onto the template after approximately 30 min. and evaluate.

9.2 Automation

Automated processing of the immunoassays must be performed analogous to manual use and validated by the user.

10 Test Evaluation

10.1 Metrological Traceability

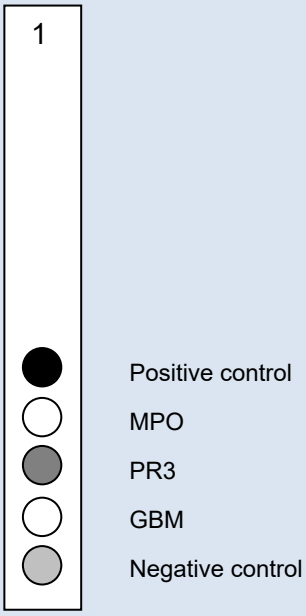
The immunoassay is calibrated using internal reference samples.

10.2 Evaluation

The qualitative evaluation of is performed by comparison of the antigen specific signal intensities of the patient´s sample with the negative control dot on the test strip. The negative control dot serves as a cut off control for qualitative evaluation.

The test strips are coated with antigens. The color intensity is proportional to the specific antibody activity in the sample. A sample is considered to be positive for a specific antigen, if the coloration of the antigen dot shows a more intense coloration than the negative control dot on the test strip. A sample is considered to be negative for a specific antigen, if the coloration of the antigen dot shows a less intense or equal coloration compared to the negative control dot on the test strip.

Example of a processed ANCA Dot test strip



10.3 Criteria of Validity

Test runs are only valid if the following criteria of validity are fulfilled:

- Intensity negative control < intensity positive control
- The positive control must be evaluated positive.

The coloration of these controls ensures that the test has been performed correctly. If these criteria are not met, the test is not valid and must be repeated.

10.4 Troubleshooting

In case of an invalid test run, the expiry dates and storage conditions, incubation times and temperatures, and precise calibration of all instruments used should be verified. If no reason for an invalid test run could be identified, please contact the supplier or manufacturer of the product.

10.5 Reference Ranges

As a result of different seroprevalences in individual regions, each laboratory should verify the reference ranges by own analysis and adapt, if necessary.

10.6 Interpretation of Test Results

A positive test result indicates the presence of specific antibodies. A negative result indicates the absence of specific antibodies, but does not exclude the possibility of an autoimmune reaction. In case of a borderline test result, a reliable evaluation is not possible.

10.7 Limitations of the Method

The interpretation of test results must always be considered in combination with the clinical picture of the patient. The diagnosis should not be based on the results of a sole diagnostic method. All clinical and laboratory findings should be evaluated to state a diagnosis. For confirmation, further investigations should be carried out.

11 Performance Characteristics

11.1 Analytical Performance Characteristics

11.1.1 Precision

The precision of test results was assessed by the determination of the intra- and interassay variation by the analysis of multiple samples with different antibody activities. No differences in the qualitative evaluation have been observed.

11.2 Diagnostic Performance Characteristics

11.2.1 Diagnostic Sensitivity and Specificity

The sensitivity and specificity were assessed by the analysis of characterized samples (confirmed positive or negative for specific antibodies by reference laboratories and/or methodologies).

	Sensitivity	Specificity
MPO	93 %	98 %
PR3	89 %	> 99 %
GBM	> 99 %	99 %

12 Warnings and Precautions

The product is designed exclusively for *in vitro* diagnostic use by qualified, authorized and trained personnel. All test components and human samples should be handled with care as potentially hazardous. Good laboratory practices (GLP) and all relevant regulations should be adhered to.

In case the product is damaged or product information including labelling is wrong or incorrect, please contact the manufacturer or supplier.

This product contains preparations of human and / or animal origin. Any material derived from human body fluids or organs used for the preparation of components were tested and found negative for HBsAg (Hepatitis B-Virus-surface Antigen) and anti-HIV as well as anti-HCV antibodies. However, all components and all patient samples should be handled as potentially hazardous in accordance with national laws and appropriate guidelines on biological safety.

As the product contains potentially hazardous materials, the following precautions should be followed: Do not smoke, eat or drink while handling kit material or samples. Avoid direct contact to kit material or samples by wearing protective gloves laboratory coat and safety glasses. Never pipette material by mouth. Wipe up spills promptly and wash the affected surface thoroughly with a decontaminant. Wash hands thoroughly after use.

Some of the reagents contain ProClin (< 0.0015 %) as a preservative, and must not be swallowed or allowed to come into contact with skin or mucosa.

Some of the reagents contain sodium azide (< 0.1 %) as a preservative and must not be swallowed or allowed to come into contact with skin or mucosa. The possible formation of heavy metal azides in the drainage has to be prevented by sufficient rinsing with water.

The information in the safety data sheet on possible hazards, first aid measures, measures in the event of the unintentional release of large quantities, handling and storage, personal protective equipment, information on disposal as well as information on toxicology must be observed.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent

authority of the member state in which the user and/or the patient is established.

13 Disposal

For decontamination and disposal the recommendations of the CDC as well as the relevant local and national environmental guidelines and regulations should be adhered to. Samples, potentially contaminated materials and infectious waste must be decontaminated, e.g. by autoclaving for 20 min. at 121 °C.

14 References

- Gross W.L., Czernok E., Helmchen U.: Antineutrophil cytoplasmic antibodies, autoantigens and systemic vasculitis. APMIS 1995, 103, 81 – 97.
- Hellmark T. et al: Goodpasture disease. Characterization of a single conformational epitope as the target of pathogenic autoantibodies. J. Biol. Chem. 1999, 274, 25862 – 8.

15 Symbols

	Manufacturer
CE	CE marking of conformity
IVD	*In vitro* diagnostic medical device
REF	Catalogue number
UDI	Unique device identifier
LOT	Batch code
	Temperature limit
	Use-by date
	Consult instructions for use
Σ	Contains sufficient for <n> tests
	Do not re-use
	Caution
	Biological risk
	Keep away from sunlight
Ag 24	Test strips
DIL	Sample diluent
CONJ	Conjugate
BUF WASH 10x	Wash buffer
SUB	Substrate

16 Changes

Changes in current Instructions for Use	
Current Version	004/09.2022
Summary of Changes	Editorial changes in all sections