

ALPHADIA IFA Anti P – ANCA (Human Granulocyte) IgG Assay

IMMUNOFLUORESCENCE ASSAY
FOR THE DETECTION
OF ANTI - NEUTROPHIL CYTOPLASMIC
ANTIBODIES (p - ANCA) IN HUMAN SERUM

CAT # AD PAN48 48 TESTS
CAT # AD PAN60 60 TESTS

FOR IN VITRO DIAGNOSTIC USE
CONS : 2 - 8°C

ALPHADIA sa/nv
DIAGNOSTIC PRODUCTS
AVENUE VESALE 26 - B1300 WAVRE
BELGIUM
TEL : 32 (0) 10 24 26 49
FAX : 32 (0) 10 24 55 99
contact@alphadia.be

INTRODUCTION

Standard IFA methods allow for the observation of several different patterns. Two patterns that have been well defined are C ANCA and P ANCA. The P ANCA pattern shows an uneven granular, staining of the cytoplasm. During the 2nd International ANCA Workshop it was agreed that these two different patterns should be used to subclassify the antibodies.

ANCA antibody detection by IFA methods has been a useful aid in the assessment of patient diagnosis, and to a certain extent their prognosis and response to therapy. Although there has been much work done with the IFA ANCA, the availability of an EIA MPO Test has further enhanced the overall picture. The use of EIA and IFA together allows for the best possible patient assessment. The MPO & ANCA (PR3) EIA allows for a very quick qualitative as well as quantitative report.

PRINCIPLES

The primary reaction in this assay involves human antibody (patient sera) and a specific antigen (human granulocytes). If ANCA antibody is present in the patient sera it will bind to form an antigen/antibody complex. This complex is then labeled with an FITC labeled antihuman conjugate that allows one to visualize the reaction through the microscope.

MATERIAL PROVIDED

1. FITC conjugate is to be stored at 2-8°C upon receipt. The conjugate is stable at this temperature until expiration date on label.
2. The antigen slides of ANCA (Human granulocyte) substrate must be stored at -20°C or lower upon receipt. Check label for specific expiration date. AD PAN48 - 12 slides x 4 wells AD PAN60 - 10 slides x 6 wells
3. P ANCA positive control N° PCPA (1 ml), demonstrating a P ANCA pattern, should be stored at 2-8°C upon receipt. Check label for specific expiration date.
4. ANCA negative control N° NCA (1 ml), should be stored at 2-8°C upon receipt. Check label for specific expiration date.
5. Buffer pack N° PBS1 - Phosphate Buffered Saline is stable at room temperature storage for 5 years. Check label for specific expiration date. Rehydrate buffer with 1 liter of sterile distilled water. The reconstituted buffer does not contain preservatives and should be stored at 2-8°C. Care should be taken to avoid contamination.
6. Mounting medium N° AMM3 should be stored at 2-8°C. Check label for specific expiration date.

GENERAL STORAGE OF THE KIT

The slides, controls and conjugate should be stored at 2-8°C.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

Test tube rack or microtiter system
Disposable pipettes
Staining dish and slide forceps
Fluorescence microscope
Moisture chamber
Volumetric flask (500 ml)
Distilled H₂O

ADDITIONAL COMPONENTS AVAILABLE

C ANCA positive control
Moisture chamber

SPECIMEN COLLECTION

Serological specimens should be collected under aseptic conditions. Hemolysis is avoided through prompt separation of the serum from the clot. Serum should be stored at 2-8°C if it is to be analyzed within a few days. Serum may be held for 3 to 6 months by storage at -20°C or lower. Lipemic and strongly hemolytic serum should be avoided. When specimens are shipped at ambient temperatures, addition of a preservative such as 0.01% thimerosal or 0.095% sodium azide is strongly recommended.

TEST INSTRUCTIONS

Screening : dilute test serums 1:20 (1 part patient sample to 19 part diluent) in PBS.

Titration : set up doubling dilutions of serum starting at 1:20 (ie 1:20, 1:40, 1:80...).

The slides are ready to use after they reach room temperature.

1. Allow slide to reach room temperature before opening envelope. Tear envelope at notch.
Carefully remove the slide and avoid touching the antigen areas. The slide is now ready to use.
2. Place a drop of diluent serum (20-30µl) over the antigen wells.
3. Place slide with patient's serum and controls in a moist chamber for 20 minutes at room temperature (approximately 19-24°C).
4. Remove slide from moisture chamber and tap the slide on its side to allow the serum to run off onto a piece of paper towel. Using a wash bottle, gently rinse remaining sera from slide being careful not to aim the stream directly on the well.
5. Wash in PBS for two separate five minute changes.
6. Place a blotter on the lab table with absorbent side up. Remove slide from PBS and invert so that substrate side faces absorbent side of blotter. Line up wells to blotter holes. Place slide on top of blotter. Do not allow substrate to dry.

7. Wipe back of slide with dry lint free paper towel. Apply sufficient pressure to slide while wiping to absorb buffer. Do not allow substrate to dry.

8. Deliver 1 drop (20-30µl) of conjugate per antigen well. Repeat steps 3-7.

9. Place 4-5 drops of mounting medium on slide.

10. Apply a 22 x 70 mm coverslip. Examine the slide under a fluorescent microscope.

Note : To maintain fluorescence, store mounted slide in a moisture chamber placed in a dark refrigerator.

Note : Caution should be taken to not extend incubation or rinse times. The substrate will be effected and poor morphology will result.

RESULTS

Positive

A positive result is reported when the cytoplasm of the human granulocyte substrate displays a 1+ or greater fluorescence. P ANCA and C ANCA will give a similar uneven granular staining of the cytoplasm, with formalin fixation.

C ANCA and P ANCA may occur together.

C ANCA antibodies are associated with classic Wegener's granulomatosis.

P ANCA (MPO) antibodies are associated with renal limited diseases.

Negative

A serum is considered negative for ANCA if the cytoplasm fluorescence is less than 1+. Patients should be screened on ANA Hep 2 substrate to avoid confusion with PSEUDO ANCA. PSEUDO ANCA will stain the cytoplasm of Hep 2 cells whereas true ANCA will be negative on Hep 2 unless the patient possesses both ANA and ANCA antibodies.

QUALITY CONTROL

1. Positive and negative serum controls must be included in each day's testing to confirm reproducibility, sensitivity and specificity of the test procedure.
2. The negative serum control should result in little (+) or no fluorescence. If this control shows bright fluorescence, either the control antigen, conjugate or technique may be at fault.
3. The positive serum control should result in bright 3+ to 4+ fluorescence. If this control shows little or no fluorescence, either the control, antigen, conjugate or technique may be at fault.
4. In addition to positive and negative serum controls, a PBS control should be run to establish that the conjugate is free from nonspecific staining of the antigen substrate. If the antigen still fluoresces, either the conjugate or antigen may be at fault.

PRECAUTIONS

1. All human components have been tested for (HBsAg) and HTLVIII/LAV by an FDA approved method and found to be negative (not repeatedly reactive). However, this does not assure the absence of HBsAg or HTLVIII/LAV. All human components should be handled with appropriate care.
2. The sodium azide (0.095%) included in the controls and conjugate is toxic if ingested.
3. Do not use components beyond their expiration date.
4. Follow the procedural instructions exactly as they appear in this insert to insure valid results.
5. For in vitro diagnostic use.
6. Handle slides by the edges since direct pressure on the antigen wells may damage the antigen.
7. Once the procedure has started do not allow the antigen in the wells to dry out. This may result in false negative test results, or unnecessary artifacts.

BIBLIOGRAPHY

1. Wilk A and van der Woude F : Anti-neutrophil cytoplasmic antibodies (ANCA) : A historic review. APMIS 97 (Suppl 6):7, 1989.
2. Wilk A: Delineation of a standard procedure for indirect immunofluorescence detection of ANCA. APMIS 97 (Suppl 6):12-3, 1989.
3. Rasmussen N and Wilk A: Indirect immunofluorescence examination for IgG-ANCA in sera submitted for the 1st international workshop on ANCA, 1988. APMIS 97 (Suppl 6): 16-20, 1989.
4. van der Woude FJ, et al: Autoantibodies against neutrophils and monocytes: Tools for diagnosis and marker of disease activity in Wegener's granulomatosis. Lancet, Feb 13:429-9, 1985.
5. Falk R.J. and Jennette Jc. : Anti-neutrophil cytoplasmic autoantibodies with specificity for myeloperoxidase in patients with systemic vasculitis and idiopathic necrotizing and crescentic glomerulonephritis. N Engl J Med 38 (25):1651-7, 1988.
6. Goldschmeding R., et al:Different immunological specificities and diseases associations of c ANCA and p ANCA.

Neth J Med 36 (3):121-5, 1990.



ALPHADIA sa/nv
DIAGNOSTIC PRODUCTS
AVENUE VESALE 26 - B1300 WAVRE
BELGIUM
TEL : 32 (0) 10 24 26 49
FAX : 32 (0) 10 24 55 99
contact@alphadia.be

