

ANCA Test System

RESULTS

Positive

A positive result is reported when the cytoplasm of the human granulocyte substrate displays 1 + or greater fluorescence. Differentiation between C-ANCA, P-ANCA and possible cross reactivity with GS-ANA (Granular Specific ANA) should be made. C-ANCA is defined as an uneven granular staining of the cytoplasm. P-ANCA is defined as a perinuclear / nuclear staining, and is best differentiated on an ANCA IFA slide specifically prepared for P-ANCA detection (cat no: SA007). P-ANCA at the screening dilution of 1:20 may appear dissociated on a standard ANCA slide (cat no: SA006/SA012/SA012). This reaction is caused by the inability of the ethanol fixation for C-ANCA to fix the P-ANCA antigen.

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 HEp2 substrate to avoid confusion with PSEUDO-ANCA. PSEUDO-ANCA will stain the cytoplasm of HEp2 cells whereas true ANCA will be negative on HEp2 unless the patient possesses both ANA and ANCA antibodies.

Positive results can be confirmed on an appropriate ELISA.

TEST LIMITATIONS

- No diagnosis should be based on a single serologic test since various host factors must be taken into consideration.

LITERATURE REFERENCES

- Wilk A and van der Woude F: Anti-neutrophil cytoplasmic antibodies (ANCA). APMIS 97 :7, 1989.
- Wilk A: Delineation of a standard procedure for indirect IF detection of ANCA. APMIS 97 :12-3, 1989.
- 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.
- 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 13:425-9, 1985.
- Falk R. & Jennette J.: ANCAs with specificity for myeloperoxidase in patients with systemic vasculitis and idiopathic necrotizing and crescentic glomerulonephritis. N Engl J Med 38(25):1651-7, 1988.
- Goldschmeding R., et al: Different immunological specificities and diseases associations of c-ANCA and p-ANCA. Neth J Med 36(3):121-5, 1990.



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INSTRUCTIONS FOR USE

Anti-Neutrophil Cytoplasm Antibody Test System

- **Ethanol Fixed (C-ANCA): SA060** (60 Tests); **SA0120** (120 Tests); **SA0150** (150 Tests)
- **Formalin Fixed (P-ANCA): SA070** - 60 Tests

Also for:

Human granulocyte slides (ethanol fixed)	SA006 (6 well)
Human granulocyte slides (formalin fixed)	SA007 (6 well)
C-ANCA Positive control	C023N / C023N-0.5
P-ANCA Positive control	C024N / C024N-0.5
ANCA Negative control	C025N / C025N-0.5
FITC Anti-IgG Conjugate for ANCA	J501A

INTENDED USE

The Bio-Diagnostics Anti-Neutrophil Cytoplasm Antibody (ANCA) Test kits are immunofluorescent antibody (IFA) tests to detect the presence of ANCA antibodies in human serum.

SUMMARY AND EXPLANATION

Autoantibodies specific for Wegener's granulomatosis (WG) were first reported in the late 1970's. Antibodies against the cytoplasmic components of the neutrophil granulocyte have also been detected in diseases other than WG, such as glomerulonephritis and/or systemic vasculitis, microscopic polyarteritis and idiopathic rapid progressive glomerulonephritis (1,2).

Standard IFA methods allow for the observation of several different patterns. Two patterns that have been well defined are C-ANCA (Cytoplasm) and P-ANCA (Perinuclear). The C-ANCA pattern shows an uneven granular staining of the cytoplasm. The P-ANCA has a perinuclear / nuclear staining pattern. During the 2nd International ANCA Workshop it was agreed that these two different patterns should be used to sub-classify the antibodies (7).

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.

PRINCIPLE OF THE TEST

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 occurs during the incubation period whilst the serum covers the antigen surface. A rinsing period is followed by a secondary reaction. The reagent used in the secondary reaction is a fluorescein labelled anti-human globulin conjugate. The antigen surface is then thoroughly rinsed free of unbound conjugate and viewed under an appropriate fluorescent microscope to visually identify various morphological patterns of nuclear fluorescence.

ANCA Test System

WARNINGS AND PRECAUTIONS

1. The human components of the controls have been tested and found to be negative or non-reactive for HBsAg, HCV antibodies, PRP, HIV-1 and HIV-2 antibodies and HIV1 p24 antigen. However, these tests cannot guarantee the absence of infectious agents. All human components should be handled with appropriate care.
2. The conjugates and controls included in the kit contain 0.1% sodium azide or 0.05% Proclin as preservatives. Although these are at low concentrations, these reagents should be considered toxic. They should not be ingested or allowed to come into contact with either the skin or the mucous membranes. Sodium azide may also cause the formation of potentially explosive lead or copper azides in sinks.
3. Do not use components beyond their expiration date.
4. Follow the procedural instructions exactly as they appear in this insert to ensure valid results.
5. For *in vitro* diagnostic use only.
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 artefacts.

KIT CONTENTS

SA060/SA0150/SA0120 kit:

SLIDE ANCA (human granulocyte - ethanol fixed) substrate antigen slides (SA006)

CONTROL + C-ANCA Positive Control no: C023N / C023N-0.5

SA070 kit:

SLIDE ANCA (human granulocyte - formalin fixed) substrate antigen slides (SA007)

CONTROL + P-ANCA Positive Control no: C024N / C024N-0.5

In both kits:

CONJ IgG FITC Conjugate with Evans Blue Counterstain: J501A. This reagent contains antibodies that will react with the human IgG (H+L) Immunoglobulin class.

CONTROL - ANCA Negative Control no: C025N / C025N-0.5

IFA/DFA PBS Buffer Pack no: R002

MM Mounting Medium no: R009

Note: All kit components are available separately. Please see the Bio-Diagnostics Ltd catalogue for more details.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

Test tubes and rack or microtitre system	Disposable pipettes	Distilled Water
Staining Dish and Slide Forceps	Moisture Chamber	Volumetric Flask (500 ml)
Fluorescence Microscope	Paper Towels - lint free	

REAGENT PREPARATION

1. Buffer Pack no: R002. Rehydrate buffer with 1 litre of sterile distilled water.

KEY FOR OTHER SYMBOLS

Used in this instruction leaflet and on accompanying product packaging:

Manufacturer Contains sufficient for <n> tests Ready for Use
 Temperature limitation In vitro diagnostic medical device

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STORAGE AND STABILITY

The IFA Test System components (except PBS) must be stored at a temperature of +2°C to +8°C. Do not freeze the test kit. The stability of the kit is as indicated by the expiry date on the packaging under the above storage conditions. This applies to unopened and opened reagents.

Phosphate Buffered Saline is stable at room temperature storage. The reconstituted Buffer does not contain preservatives and should be stored at 2-8°C. Care should be taken to avoid contamination.

SPECIMEN COLLECTION

Serological specimens should be collected under aseptic conditions. Haemolysis is avoided through prompt separation of the serum from the clot. Serum should be stored at 2-8°C if it is to be analysed within a few days. Serum may be held for 3 to 6 months by storage at -20°C or lower. Lipaemic and strongly haemolytic serum should be avoided. When specimens are shipped at ambient temperatures, addition of a preservative such as 0.01% thiomersal or 0.1% sodium azide is strongly recommended.

TEST INSTRUCTIONS

Screening: dilute test serums 1/20 (1 part patient sample to 19 parts diluent) in PBS. N.B. Although this dilution factor is suggested, each laboratory should determine their individual screening dilution.

Titration: set up doubling dilutions of serum starting at 1/20, (i.e. 1/20, 1/40, 1/80, 1/160, 1/320, etc.).

1. Once slides reach room temperature tear slide 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 diluted serum (20 to 30µl) and controls over the antigen wells.
3. Place slide with patient's serum and controls in a moist chamber for 30 minutes at room temperature (approximately 18-24°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 rinse stream directly onto the well.
5. Wash in PBS for 5 minutes. Repeat using fresh PBS.
6. Carefully dry the back and edges of the slide using a paper towel. **Do not allow tissue to dry.**
7. Deliver 1 drop (20-30µl) of conjugate per antigen well. Repeat steps 3-6.
8. Place 4-5 drops of mounting medium on slide.
9. 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.

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 controls should result in bright 3+ to 4+ fluorescence. If these controls show 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 non-specific staining of the antigen substrate. If the antigen shows bright fluorescence in the PBS control repeat using fresh conjugate. If the antigen still fluoresces, either the conjugate or antigen may be at fault.