INSTRUCTIONS FOR USE

EN Anti-Neutrophil Cytoplasmic Antibodies (Formalin-fixed)

REF A060PL IVD Rx Only Σ 50 CE

INTENDED USE

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.
Anti-neutrophil cytoplasmic antibody (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 MPO-specific enzyme immunoassay (EIA) test has further enhanced the overall picture. The use of EIA and IFA together allows for the best possible patient assessment. The MPO and PR3 EIA allows for a very quick qualitative as well as quantitative report.

PRINCIPLE OF THE ASSAY

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.

REAGENT

Materials Provided:

1. FITC IgG conjugate (3 ml) is to be stored at 2 - 8°C upon receipt. The conjugate is stable at this temperature until the expiration date on the label.
2. The Formalin-Fixed antigen slides of ANCA (Human Granulocyte) substrate must be stored at 2 - 8°C upon receipt. Check the label for the specific expiration date.
3. P-ANCA positive control (0.5 ml), demonstrating a P-ANCA pattern, should be stored at 2-8° C upon receipt. Check the label for the specific expiration date.
4. ANCA negative control (0.5 ml) should be stored at 2-8° C upon receipt. Check the label for the specific expiration date.
5. Buffer Pack - Phosphate Buffered Saline is stable at room temperature. Check label for specific expiration date. Check label for the specific expiration date. Rehydrate buffer with 1 liter of DI H2O. 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 should be stored at 2-8° C. Check label for specific expiration date.

Each kit contains the following components in sufficient quantities to perform the number of tests indicated on the packaging label.

SLD	1	ANCA Substrate Slides: Ten, 5-well Slides with absorbent blotter and desiccant pouch.
CONJ	2	Conjugate: Goat anti-human IgG labeled with FITC. Contains phosphate buffer with BSA and counterstain. Two, amber bottles, containing 3 mL. Ready to use.
CTRL +	3	p-ANCA Positive Control: Contains p-ANCA-positive human serum, along with preservatives. One orange-capped microvial. 0.5mL, ready to use.
CTRL -	4	Negative Control: Contains human serum negative for ANCA, along with preservatives. One green-capped microvial. 0.5mL, ready to use.
COVGLS	5	Cover Glass. Package of twelve, 24 x 60 mm, Thickness #1.

BUF PBS	6	Phosphate-buffered saline (PBS): pH 7.2 ± 0.2. Empty the contents of each buffer packet into one liter of distilled or deionized water. Mix until all salts are thoroughly dissolved. Two packets, sufficient to prepare 2 liters.
MNTMED	7	Mounting Media (Buffered Glycerol): Two, white-capped 3 mL clear bottle, containing 3mL. Ready to use

NOTE: Conjugate and Controls contain a combination of Proclin (0.05% v/v) and Sodium Azide (<0.1% w/v) as preservatives

MATERIALS REQUIRED BUT NOT PROVIDED

1. Test tube rack or microtiter system.
2. Micro-pipettor of 2 - 20 µL range.
3. Graduated glass pipettes.
4. 1 L. graduated cylinder.
5. Staining dish.
6. Moist chamber.
7. Clean containers for diluted buffers.
8. Distilled or deionized water.
9. Fluorescent Microscope.
10. Lint free paper towel.
11. Timer (60 min. range).

STORAGE CONDITIONS

2°C – 8°C	Unopened Kit.
	The slides, controls, and conjugate should be stored at 2-8° C.
	Rehydrated PBS (Stable for 30 days).
*Once opened, slides must be used that day. Other ready-to-use reagents, except PBS, may be used until their stated expiration date.	

PRECAUTIONS

Caution:

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 (<0.1%) 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 ensure 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.
8. Use separate pipette tips for each sample and reagent to avoid cross contamination.
9. Reagents should be inspected for evidence of bacterial or fungal contamination.
10. Do not reuse the substrate slide.

SERUM 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 < 0.1% sodium azide is strongly recommended.

ASSAY PROCEDURE

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

Titration: Set up doubling dilutions of serum starting at 1:20 (i.e. 1:20, 1:40, 1:80, 1:160, 1:320, etc.). The slides are ready to use after they reach room temperature.

1. Allow the slide to reach room temperature before opening the envelope. Tear envelope at notch. Carefully remove the slide and avoid touching the antigen areas. The slide is now ready to use.
2. Place 25 µl of serum over the antigen wells.
3. Place slide with patient's serum and controls in a moist chamber for 35 +/- 5 minutes at room temperature (approximately 20-25° C).
4. Remove the slide from the moisture chamber. Using a wash bottle, gently rinse the remaining sera from the slide, being careful not to aim the stream directly on the well.
5. Wash in PBS (Cat. 1601) for two separate five-minute changes.
6. Place a blotter on the lab table with the absorbent side up. Remove slide from PBS and invert so that the substrate side faces the absorbent side of the blotter. Line up wells to blotter holes. Place the slide on top of the blotter. Do not allow the substrate to dry.
7. Wipe the back of the slide with a dry lint free paper towel. Apply sufficient pressure to slide while wiping to absorb buffer. Do not allow the substrate to dry.
8. Deliver 25 µl of conjugate per antigen well. Repeat steps 3-7.
9. Place 4-5 drops of mounting medium on the slide.
10. Apply a coverslip. Examine the slide under a fluorescent microscope.

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

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

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 substrate, 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 substrate shows bright fluorescence in the PBS control repeat using fresh conjugate. If the antigen substrate still fluoresces, either the conjugate or the antigen may be at fault.

INTERPRETATION OF 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 Granulomatosis with Polyangiitis (GPA).

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 atypical ANCA. Atypical ANCA will stain the cytoplasm of HEp-2 cells, whereas true ANCA will be negative on HEp2 unless the patient possesses both ANA and ANCA antibodies.

GLOSSARY OF SYMBOLS

The following symbols **may** have been used in the labelling of this product.

Symbol	Description	Symbol	Description
	Manufacturer		Keep away from sunlight
IVD	*In vitro* diagnostic medical device		Single use assay wells
REF	Catalogue number	COVGLS	Cover Glass

Σ n	Sufficient for *n* tests	SLD	ANCA Substrate (human granulocytes) Slide
LOT	Batch code	BUF PBS	PBS Buffer
	Use by	MNTMED	Mounting Media
	Storage Temperature limitations	CONJ	Conjugate
RX Only	For Prescription Use Only	CTRL +	Positive Control
	Consult electronic instructions for use	CTRL -	Negative Control
	Store in the upright position	Made in the USA	Made in the USA

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