INTRODUCTION:
Standard IFA methods allow for the observation of different antigen areas. The slide is now ready to use.

Standard IFA methods allow for the observation of antigens. Check label for specific expiration date. Rehydrate buffer with 1 liter of sterile DI H2O. Moisten chamber for 20 minutes at room temperature (approximately 19-24°C). Place a blotter on the lab table with absorbent side up. Remove slide from PBS and invert so the patient possesses both ANA and ANCA antibodies.

PRINCIPLES:
The primary reaction in this assay involves human antibody (patient sera) and a specific antigen (human granulocytes). Care should be taken to avoid contamination. The slides, controls, and conjugate should be stored at 2-8º C. 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. A positive result is reported when the cytoplasm of the human granulocyte substrate displays a 1+ or greater fluorescence. P-ANCA and C-ANCA may occur together C-ANCA and P-ANCA may occur together. C-ANCA antibodies are associated with classic Wegener’s granulomatosis.

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 similar uneven granular staining of the cytoplasm, with A positive result is reported when the cytoplasm of the human granulocyte substrate displays a 1+ or greater fluorescence. P-ANCA and C-ANCA may occur together.

Wash in PBS (Cat. 1601) for two separate five minute changes. Apply sufficient pressure to slide while wiping to Carefully remove the slide and avoid touching the antigen areas. The slide is now ready to use. Be sure to not extend incubation or rinse times. The substrate will be affected and poor morphology will result.

RESULTS:
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 similar uneven granular staining of the cytoplasm, with. 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.

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. PSEUDO-ANCA will result in little (+) or no fluorescence. PSEUDO-ANCA will result in little (+) or no fluorescence. PSEUDO-ANCA will result in little (+) or no fluorescence. PSEUDO-ANCA will 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 (++) or (+) fluorescence. PSEUDO-ANCA will result in bright (++) or (+) fluorescence. PSEUDO-ANCA will result in bright (++) or (+) fluorescence. PSEUDO-ANCA will result in bright (++) or (+) fluorescence. PSEUDO-ANCA will result in bright (++) or (+) 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 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.

PRECAUTIONS:
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.

1. The sodium azide (<0.1%) included in the controls and conjugate is toxic if ingested.
2. Do not use components beyond their expiration date.
3. 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: