



CPAL

Central Pennsylvania Alliance
Laboratory

Technical Bulletin

No. 113

March 11, 2013

Immuno Concepts HEp-2000 ANA-Ro Assay

Contact:

Stephanie Frey, 717-851-1416
Operations Manager, Clinical Pathology, CPAL

Dr. Jeffrey Wisotzkey, 717-851-1416
Director, Molecular Pathology, CPAL

Effective Date:

March 19, 2013

Method Change:

CPAL is changing methods for Antinuclear Antibody (ANA) testing from the semi-automated Rhigene ImageTiter (MBL) Test System method to an automated methodology (Immuno Concepts Fluorescent ANA Test System using Immuno Concepts AFT2000 and HEp-2000 ANA-Ro kit. This change in methodology may result in slight differences between the previous and new methods.

NOTE:

1. ANA titers will only be reported from 1:40 to $\geq 1:1280$. The $\geq 1:2560$ dilution is discontinued.
2. The ANA substrate slides, which uses HEp-2000 cells (with mitotic figures) grown and stabilized directly on the test wells and transfected with the SSA/Ro autoantigen, allows for an added reportable pattern of SSA-Ro which previously may have been reported as Speckled.
3. Serum is the specimen type. CPAL will no longer accept other "body fluids" for ANA testing.

The Immuno Concepts Fluorescent ANA Test System uses the indirect fluorescent antibody technique. Patient samples are incubated with antigen substrate to allow specific binding of autoantibodies to cell nuclei. If ANA's are present, a stable antigen-antibody complex is formed. When results are positive, there is the formation of a stable three-part complex consisting of fluorescent antibody bound to human antinuclear antibody, which is bound to nuclear antigen. This complex can be visualized with the aid of a fluorescent microscope.

ANA method change-Technical Bulletin 113

Issued on: March 11, 2013

For questions about this and other information, call Central Pennsylvania Alliance Laboratory at 1-888-480-1422.

The Image Navigator is an automated microscope that captures images of autoantibody IFA slides. The Image Navigator presents these images to the technologist on separate review screens for easy and quick confirmation and reporting. The system includes a high quality fluorescent microscope, so samples can be observed by the conventional method as well as in the automated format. The Autoimmune Fast Track, AFT2000, is a reliable pipetting robot that automates IFA slides. Its precision, accuracy and tracking capabilities minimize errors, optimize traceability and provide consistent results. The AFT2000 processes ANA screens and titers.

Specimen:

Serum stored for 1 week at 2 - 10°C.

Supportive Data:

Forty negative and sixty-seven positive specimens were tested using both Immuno Concepts HEp-2000 ANA-Ro Assay and Rhigene ImageTiter ANA Test System. The results of the study are listed in the following table.

	HEp-2000 ANA-Ro positive (within 2x dilution)	HEp-2000 ANA-Ro Negative (including 1:40)
ImageTiter Positive (within 2x dilution)	67	0
ImageTiter Negative (including 1:40)	0	40

Forty negative specimens were run which produced the same results with both manufacturers' kits. A total of 67 positive specimens were run. Of those specimens, 54 matched both titer and pattern. The remaining twelve specimens either did not match the pattern, titer, or both. One of the specimens was negative by Immuno Concepts and 1:40 Nucleolar by MBL. A titer of 1:40 is the screening dilution and is not interpreted as a positive result until the titer is >1:80. Ten specimens differed in titer by no more than 2 dilutions. The difference in titer did not change the interpretation of the result i.e. all ten specimens were positive based on either titer result. Of those ten specimens, four differed in pattern. The pattern was interpreted as SSA/Ro with the IC kit and as Speckled with the MLB kit for two of the specimens. The IC HEp-2 cells have been transfected to detect the SSA/Ro autoantigen. The MBL cells are not transfected in which case the pattern will be expressed as speckled. The other two specimens were interpreted as speckled using IC's kit and homogenous using the MBL kit. Both patterns are indicative of SLE or other connective tissue diseases, therefore not changing the diagnosis for the patient. Intra- and inter-run reproducibility was 100% (including titer and pattern).