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Immuno Concepts Fluorescent nDNA Test System - METHOD CHANGE -

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Method Change:

CPAL is changing methods for anti-native DNA (nDNA) antibody testing from the manual IFA method using Zeus's IFA reagents to an automated methodology (Immuno Concepts nDNA Test System). This change in methodology may result is slight differences between the previous and new methods.

The nDNA titer range is 1:10 to ≥ 1:5120. Some of the many factors that may affect nDNA titer results include, but are not limited to: the type of light source used, the type of excitation filter used, the numerical aperture of the objective, suppression filters, and precision and accuracy of the dilution technique. The specimens tested with the Zeus kit (previous) were read on a microscope with a mercury bulb, while the specimens tested with the Immuno Concepts kit (new), are read on the Image Navigator which uses a LED light source. In addition, the titers for the Immuno Concepts kit are diluted on an automated platform whilst the specimens tested with Zeus kit were manually diluted. The nDNA results are not diagnostic by themselves. They must be interpreted in conjunction with the patient's history and symptoms, the physical findings, and other diagnostic procedures. Treatment should not be initiated on the sole basis of a positive test for anti nDNA antibodies.

Automated Methodology:

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 nDNA screens and titers.

The Immuno Concepts nDNA test uses the indirect fluorescent antibody technique, first described by Weller and Coons. Patient samples are incubated with antigen substrate to allow specific binding of autoantibodies to kinetoplast nDNA. When results are positive, there is the formation of the stable three-part complex consisting of fluorescent antibody bound to human anti-nDNA antibody, which is bound to nDNA antigen. This complex can be visualized with the aid of a fluorescent microscope. In positive samples, the kinetoplast or the kinetoplast and nucleus will show a bright apple green fluorescence within the *Crithidia luciliae* organisms.

The ImageNavigator is a high quality fluorescent microscope which uses an LED light source.

Supportive Data:

Fifty-seven negative and twenty-four positive specimens were tested using both Immuno Concepts nDNA Assay and Zeus' IFA reagents. The results of the study are listed in the following table. The overall agreement with both kits is 96.3%. Intra- and inter- run reproducibility was 100% (including titer).

	Immuno Concepts nDNA positive	Immuno Concepts nDNA Negative
Zeus nDNA Positive	21	3
Zeus nDNA Negative	0	57

In an additional study, three positive CAP proficiency specimens were run using Immuno Concepts kit. All three results were positive and matched the expected titer.

Specimen:

Serum stored for 1 week at 2 - 10°C.

Background:

Antinuclear antibody (ANA) is a general term used to describe autoantibodies against various cell nuclear proteins. Early studies of these autoantibodies, using immunoflourescent techniques, revealed a select few nuclear protein specificities. Because of the high correlation of positive ANA with systemic lupus erythematosus (SLE), a negative ANA essentially ruled out the disease.

Although antibodies specific to DNA continue to show a high disease correlation with SLE, in recent years a number of nuclear and cytoplasmic macromolecules have been detected and associated with other connective tissue diseases. Because a number of these antibodies appear

to be of diagnostic and/or prognostic use in progressive systemic sclerosis, mixed connective tissue disease, Sjögren's syndrome, polymyositis, and rheumatoid arthritis, ANA testing is now recognized as a general screening tool for connective tissue disease.

SLE patients may produce antibodies to a variety of nuclear antigens, but antibodies directed against Sm (Smith antigen) and nDNA show the highest correlation with disease. Antibodies directed against Sm demonstrate a speckled ANA staining pattern while antibodies directed against nDNA generally demonstrate a homogeneous ANA staining pattern. Although low levels of nDNA antibodies may be present in the serum of patients with rheumatoid arthritis, Sjögren's syndrome, progressive systemic sclerosis, dermatomyositis, discoid lupus erythematosus, and mixed connective tissue disease, high levels of nDNA antibodies are seen almost exclusively in SLE. Antibodies against nDNA are thought to be involved in the pathogenesis of the most severe variants of SLE when deposited as immune complexes. Antibodies to nDNA occur in high titer, and, because they correlate with disease activity, their detection is important in the management of SLE patients.

Several assays are available for the detection of nDNA antibodies. The most commonly used methods include indirect immunofluorescence, radioimmunoassay, counter-immuno electrophoresis, and immunodiffusion. The Immuno Concepts nDNA test system that CPAL has adopted is an indirect fluorescent antibody (IFA) method. Serum antibody, reactive to nDNA, is detected by staining of the kinetoplast within the organism *Crithidia luciliae*. *C. luciliae* is a parasite of the blowfly and is non-pathogenic to humans. The kinetoplast of these hemoflagellates is part of the large mitochondrion in which the helical nDNA is concentrated. In electron micrographs, the kinetoplast appears as a slightly concave, disc-shaped structure containing mitochondrial cristae and a fibrous DNA mass. The kinetoplast is found between the centrally located nucleus and the basal body of the flagellum. Because the kinetoplast nDNA contains no single-stranded DNA (ssDNA) contaminants, potential problems of ssDNA false-positive reactions, which may occur with calf thymus DNA radioimmunoassay, are virtually eliminated.