CPAL
Central **P**ennsylvania **A**lliance
Laboratory

Technical Bulletin
No. 128

May 14, 2014

Anti-dsDNA
- Method Modified –

Contact: Steph Frey, 717.852.4768
Operations Manager, Clinical Pathology, CPAL

Dr. Jennifer Thebo, 717.851.3210
Director, Clinical Pathology, CPAL

Affected Tests:

Mnemonics:	**NDNA SCRN**	**NDNA QNT**
Test Name:	ANTI dsDNA ANTIBODY SCREEN (Native DNA)	ANTI dsDNA QUANT (Native DNA)
Test Number:	3000195	3000197
Specimen:	0.5 mL Serum, Refrig (2-8^{o}C) 7 days	

Effective Date: Anticipate testing will begin on May 27, 2014 with samples received beginning May 23, 2014.

Performed: Monday through Friday

Reference Range: Negative

Method Change:
The manufacturer has discontinued the previous testing kit for Anti-dsDNA due to international testing recommendations. The replacement kit uses goat anti-human IgG (***gamma chain specific***) for the conjugate. The previous kit used goat anti-human IgG (***heavy and light chain***) for the conjugate. Otherwise, the methods are the same. There should be no impact on patient results, according to the manufacturer.

Background:
Patients with systemic lupus erythematosus (SLE) 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, counterimmunoelectrophoresis, and immunodiffusion. The

Immuno Concepts IgG anti-nDNA test system 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.

Principle of Test:
The Immuno Concepts IgG anti-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. If anti-nDNA antibodies are present, a stable antigen-antibody complex is formed. After washing to remove non-specific antibodies, the substrate is incubated with an anti-human antibody reagent conjugated to fluorescein. 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. If the sample is negative for nDNA, the kinetoplast will show no fluorescence.

Results Interpretation:
Reference range: Negative

Limitations:
1. Diagnosis cannot be made on the basis of anti nDNA antibody detection alone. The physician must interpret these results in conjunction with the patient's history and symptoms, the physical findings, and other diagnostic procedures.
2. Treatment should not be initiated on the sole basis of a positive test for anti nDNA antibodies. Clinical indications, other laboratory findings, and the physician's clinical impression must be considered before any treatment is initiated.
3. Certain drugs, including procainamide and hydralazine, may induce a lupus erythematosus-like disease. Patients with drug-induced LE may demonstrate positive ANAs commonly directed against nuclear histones, although antibody to nDNA has also been reported.
4. Although a high-titered nDNA may be highly suggestive of SLE, it should not be considered diagnostic but rather viewed as a part of the overall clinical history of a patient. Low titers of nDNA antibodies are often present in the sera of patients with rheumatoid arthritis, Sjögren's syndrome, progressive systemic sclerosis, dermatomyositis, discoid lupus erythematosus, and mixed connective tissue disease.
5. Because of the many options available in fluorescent microscopes, it is recommended that light sources, filters, and optics be standardized when comparing patient titers between laboratories.
6. Patients undergoing steroid therapy may have negative results for nDNA antibody.

Validation Data:

Within Run Reproducibility Validation:
Three samples were run within the same run a total of three times. All three samples produced the same results when processed three times within the same run. (Table 1)

Table 1 Within Run Reproducibility

Sample	Rep 1	Rep 2	Rep 3
1	Negative	Negative	Negative
2	Positive 1:40	Positive 1:40	Positive 1:40
3	Positive 1:320	Positive 1:320	Positive 1:320

Between Run Reproducibility:
Three specimens were run on three different days. All three specimens produced the same results when run on three different days. (Table 2)

Table 2 Between Run Reproducibility

Sample	Day 1	Day 2	Day 3
1	Negative	Negative	Negative
2	Positive 1:40	Positive 1:40	Positive 1:40
3	Positive 1:320	Positive 1:160	Positive 1:320

Method Comparison
A total of 40 specimens were split and run using each testing kit. Samples were compared qualitatively for interpretation as a screen, i.e., Positive or Negative. There were 100% positive agreement (10 of 10) and 100% negative agreement (30 of 30) for this sample set.

Titers were performed by both methods on all 10 samples with positive screens. The titers spanned the reportable range of the method. One specimen had a titer that was two dilutions different between the two kits, and it was repeated by the old method. After resolution, all titers agreed within one dilution between the two methods (Figure 1).

Table 3 Anti-dsDNA Method Comparison
New kit (γ specific) vs Old kit (heavy & light chain)

Sample	heavy & light chain conjugate	γ specific conjugate
1	Positive 1:40	Positive 1:20
2	Positive 1:20	Positive 1:40
3	Positive 1:20	Positive 1:20
4	Positive 1:40	Positive 1:80
5	Positive 1:80	Positive 1:40
6	Positive 1:160	Positive 1:160
7	Positive 1:320	Positive 1:320
8	Positive 1:640	Positive 1:640
9	Positive 1:1280; repeat 1:2560	Positive ≥1:5120
10	Positive ≥1:5120	Positive ≥1:5120

References:

1. Immuno Concepts IgG ANTI-nDNA Fluorescent Test system package insert, Revision 2.0; 2011.
2. Cayce C. Horner; Southeast Regional Manager/Instrument Specialist Global Focus Marketing & Distribution, Ltd.