ANTI-nDNA ANTIBODY IFA TEST SYSTEM

REF 10-6050 **50 Tests**

Store +2 to +8°C

Pour d'autres langues
Für andere Sprachen
Para otras lenguas
Per le altre lingue
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INTENDED USE

These reagents are intended for use in the detection and quantitation of IgG antibody in human sera to native, double stranded, DNA (nDNA) by the indirect fluorescent antibody (IFA) procedure. ***For in vitro Diagnostic Use.*** High Complexity Test.

SUMMARY AND PRINCIPLES

Antinuclear antibody (ANA) is present in the blood of patients with certain connective tissue disorders. Systemic lupus erythematosus (SLE) patients produce many different types of ANA's. Antibody with a specificity for double stranded DNA (dsDNA) correlates highly with SLE (1,2). Antibodies directed to native dsDNA cannot be detected by standard immunofluorescent ANA methods which rely on different nuclear fluorescent patterns to determine antibody type. Antibodies that react with both double and single stranded DNA produce the same rim and/or homogenous patterns (3). Among the various ANA immunofluorescent patterns, the rim pattern confirms a clinical diagnosis of SLE, and as many as 33% of these patients have some renal disease. Tests which can unequivocally detect the presence of only native dsDNA antibodies should be performed to confirm the diagnosis of lupus nephritis (4,5,6).

Antibodies directed against native DNA (nDNA) are not species or organ specific. A useful test for detection of these antibodies is the immunofluorescent technique utilizing the giant mitochondrion (kinetoplast) of the non-pathogenic hemoflagellate, *Crithidia luciliae*, as a substrate for pure dsDNA (2). This procedure has proven to be very effective in the diagnosis of SLE. When used to quantitate (titrate) anti-dsDNA, it is also very helpful in the clinical management of SLE patients.

Good correlation has been found between the *C. luciliae* IFA test and the radioimmunoassay of Farr. The *C. luciliae* IFA test has been shown to have equivalent sensitivity to the Farr test, as well as the millipore filter KBDNA assay (7,12). The IFA method offers the advantages of simplicity, economy, and speed. It is also specific because the dsDNA of the *C. luciliae* kinetoplast appears to be free of single stranded DNA (ssDNA) and histone (12,2). In contrast, the Farr assay requires expensive materials and equipment and may have immunochemical problems due to the presence of ssDNA which may produce false positives (12,8).

The indirect fluorescent antibody (IFA) test is used for the detection of human IgG antibody to the native, dsDNA (nDNA) antigens located in the kinetoplasts of *C. luciliae.* Cultured organisms are washed and placed in the wells of specially prepared microscope slides. Dilutions of patient sera are placed on the antigen coated wells where antibody, if present, binds to the antigen. The reaction is visualized through the use of a conjugate. The conjugate is fluorescein isothiocyanate (FITC) labeled, anti-human IgG (gamma chain specific) absorbed with rodent serum. Excitation of FITC by ultraviolet (UV) light causes this dye to emit longer, visible, wavelengths of light in the yellow-green portion of the color spectrum. The conjugate will bind with human IgG antibodies attached to the kinetoplast DNA causing the kinetoplasts to fluoresce when viewed through a microscope equipped with a UV light source. A titer of antibody activity is determined by taking the reciprocal of the last dilution of patient sera showing fluorescence.

PRECAUTIONS

1. Follow the procedure instructions exactly as they appear in this insert to ensure valid results.
2. Always wear suitable protective clothing, gloves and eye/face protection when working with this product.
3. Thimerosal (Merthiolate), used as a preservative in some of the reagents, may be toxic if ingested, inhaled or absorbed through skin and is a reproductive hazard.
4. Some components contain less than 0.1% sodium azide which is toxic if ingested and forms potentially explosive copper and lead azide compounds in waste plumbing lines. Should the reagents come in contact with copper or lead waste plumbing, flush the waste line with large quantities of water to prevent the formation of potentially explosive compounds.
5. WARNING - POTENTIAL BIOHAZARDOUS MATERIAL. Each donor unit used in the preparation of this material was tested by an FDA approved method for the presence of antibody to HIV, as well as HBsAg, and found to be negative (were not repeatedly reactive). Because no test method can offer complete assurance that human immunodeficiency virus (HIV), hepatitis B virus, or other infectious agents are absent, these human control reagents should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories", 1999 (13).
6. The phosphate buffered saline and mounting medium found in this kit are irritating to the eyes, respiratory system and skin.
7. Some components in this kit contain 0.1% Proclin 300. At full strength Proclin 300 is corrosive and will cause burns and possibly sensitisation by skin contact.
8. The conjugate in this kit contains 0.015% Evan's Blue. Evan's Blue is a possible carcinogen and may cause reproductive harm.

The safety data sheet is available upon request.

WARNING
Some components of this kit contain 0.1% ProClin 300®, a biocidal preservative that may cause sensitization by skin contact; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals.
H317: May cause an allergic skin reaction.
H335: May cause respiratory irritation.
P280: Wear protective gloves / protective clothing / eye protection / face protection.
P302 + P352: IF ON SKIN: Wash with plenty of soap and water.
P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention.
P501: Dispose of contents and container in accordance to local, regional, national and international regulations.

WARNING
Some components of this kit contain < 0.1% sodium azide.
H302: Harmful if swallowed.
P264: Wash thoroughly with plenty of soap and water after handling.
P270: Do not eat, drink or smoke when using this product.
P301+P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.
P330: If swallowed, rinse mouth.
P501: Dispose of contents/container to in accordance to local, regional, national and international regulations.

MATERIALS PROVIDED

Prod#	Description	Qty
10-6005	nDNA, *C. luciliae* Slides, 5 Well	10 ea
10-6202	nDNA Positive Control	0.5 mL
10-1201	Autoimmune Negative Control	0.5 mL
10-1501	FITC IgG Conj. Rodent Ads. w/ Evans' Blue	4.0 mL
90-1610	FITC Mounting Medium (pH 7.5)	3.0 mL
90-1607	Phosphate Buffered Saline (pH 7.5)	2x10 g
90-1700	Coverslips, 70x22 mm	12 ea
90-1705	Blotters, 5 Well	10 ea

REAGENT PREPARATION

1. Allow all reagents to come to room temperature before use.
2. *C. luciliae* nDNA slides (Prod #10-6005) should be brought to room temperature prior to breaking the package seal. Peel back the top portion of the package and remove the slide without touching the antigen wells. The slide is now ready to use.
3. Reconstitute each 10 gram vial of PBS (Prod #90-1607) with 1.0 L distilled water.
4. Conjugate (Prod# 10-1501) is supplied at working dilution. The recommended working dilution of the conjugate was established using reference reagents and recommended optical systems. Note: The conjugate may require retitration. Variations in absolute fluorescence between microscopes can be expected due to the variation in the optical sensitivity of the microscope components including light source, objective lenses, ocular lenses, total magnification, etc. If the controls consistently yield results higher or lower than expected, the conjugate may require retitration. This is accomplished by retesting the controls at appropriate two-fold dilutions of the conjugate using PBS as a conjugate diluent. If retitration of conjugate is required, please call the MarDx technical support department for assistance.
5. The mounting medium (Prod #90-1610) is used at the concentration provided.

ADDITIONAL MATERIALS REQUIRED BUT NOT SUPPLIED

1. Test tubes, test tube rack, pipettes, or a microtiter system for preparing titrations.
2. Volumetric flask (1 liter) for PBS.
3. Moist incubation chamber.
4. Slide washing chamber.
5. Fluorescence microscope equipped with FITC filters.
6. Microscope slide roller.
7. Distilled water.

STORAGE AND STABILITY

1. *C. luciliae* nDNA, 5 Well, Slides (Prod #10-6005): Store at +2 to +8°C or lower upon receipt. All antigens are stable until the expiration date printed on the product label, when stored at the recommended temperature.
2. *C. luciliae* nDNA Positive Control Serum (Prod #10-6202) and Autoimmune Negative Control Serum (Prod #10-1201): Control sera should be stored at +2 to +8°C. Refer to the product label for expiration date. Aliquots may be stored at -20°C or lower for up to 2 years.
3. FITC Labeled, Rodent Absorbed, Anti-human IgG (gamma chain specific) with Evans' Blue Counterstain (Prod #10-1501): Conjugate should be stored at +2 to +8°C. Refer to the product label for expiration date. Aliquots may be stored at -20°C or lower for up to 2 years.
4. Phosphate Buffered Saline, pH 7.5 (Prod #90-1607): PBS is stable at room temperature in its unreconstituted form. Refer to label for expiration date. PBS contains no preservative and should be stored at +2 to +8°C after it is reconstituted. Discard if turbidity develops.
5. Mounting Medium, pH 7.5 (Prod #90-1610): Store at +2 to +8°C. Refer to product label for expiration.

SPECIMEN COLLECTION

Serological specimens should be collected under aseptic conditions. Avoid hemolysis by prompt separation of the serum from the clot. Serum should be stored at +2 to +8°C if it is to be analyzed within 4-7 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, additions of a preservative such as 0.01% thimerosal (merthiolate) or 0.1% sodium azide is strongly recommended (14).

PREPARATION OF CONTROLS

Include the positive and negative controls in each run. Both controls are to be used at the concentration provided.

PREPARATION OF SPECIMENS

Make dilutions of patient sera in PBS. Dilute test sera 1:10 if testing is being performed for screening purposes. Sera positive at 1:10 should be titrated to endpoint. For titrations, set up two-fold dilutions of sera starting at 1:10, i.e., 1:10, 1:20, 1:40, 1:80, etc.

TEST PROCEDURE

1. Remove the number of slides needed from the sealed pouches and mark them with a marking pen as necessary.
2. Add controls and diluted serum (15-20 µL) to antigen wells.
3. Incubate slides in a moist chamber at room temperature for 30 minutes.
4. After incubation with sera the slides should be tapped onto a piece of paper toweling in such a way as to prevent the serum of one well coming into contact with any of the other wells. Direct a gentle stream of PBS over the slide using a wash bottle. Do not aim the stream of PBS directly onto the wells.
5. Place the slides in a wash chamber filled with PBS for 5 minutes. Replace wash chamber with fresh PBS and wash slides for another 5 minutes.
6. Remove the slides from the PBS and place, antigen side up, on a dry paper towel. Carefully place the 5 well blotter over the slide, positioned so as not to come into contact with the reaction wells. Hold one edge of the blotter with one hand to keep the blotter in place and apply sufficient gentle pressure with the microscope slide roller to remove the moisture surrounding antigen wells. DO NOT ALLOW THE ANTIGEN WELLS TO DRY.
7. Using dispenser provided, deliver 1 drop of conjugate per antigen well. The conjugate dispenser is provided with a calibrated tip and allows quantitative delivery of reagents from the storage bottle. To use, wipe the tip with a paper towel, invert the bottle and squeeze gently to release one drop. If the tip contains an air bubble, tap the bottle gently to remove air bubble which will ensure precise drop delivery.
8. Incubate slides as described above (#3).
9. Rinse, wash and blot slides as described above (#4, #5, #6). DO NOT ALLOW THE ANTIGEN WELLS TO DRY.
10. Place 2 to 3 drops of mounting medium on slide and cover with a coverslip avoiding air bubbles.
11. Read slides with a fluorescence microscope.

READING SLIDES

1. Do not attempt to read the slides before the microscope has been switched on for at least 5 minutes.
2. Read slides within one hour. Slides may be read within 24 hours if stored refrigerated in a moist chamber. Allow refrigerated slides to warm to room temperature before reading.
3. The slides should be examined at a total magnification of 400X or with immersion oil at 1000X.
4. Drying may disturb the most peripherally situated organisms in the well, therefore disregard the reactions seen with these organisms.
5. A positive reaction is seen as a fluorescent circular structure (kinetoplast) which is located against the cytoplasmic membrane in between the nuclear and flagellar ends of the organism. Frequently, a serum will produce both nuclear and kinetoplast fluorescence simultaneously. This reaction is also regarded as positive. Negative reactions include nuclear fluorescence alone or basal body (polar) fluorescence. Occasionally, non-specific cytoplasmic staining can produce diffuse staining which surrounds the negative image of the kinetoplast and the nucleus, but this is not read as a positive reaction.
6. Record reaction intensity at each dilution using the following criteria:
   4+ = Brilliant yellow-green staining.
   3+ = Bright yellow-green staining.
   2+ = Definite but dull yellow-green staining.
   1+ = Dim yellow-green staining, diffuse staining.
   Negative = Absence of yellow green specific fluorescence.
7. Read the controls before proceeding to the test sera.

QUALITY CONTROL

1. Positive and negative controls must be included in each run to confirm reproducibility, sensitivity and specificity of the test procedure.
2. The negative control serum should demonstrate no fluorescence of the kinetoplast.
3. The positive control serum should demonstrate 3+ to 4+ fluorescence of the kinetoplast.
4. The positive and negative controls must demonstrate appropriate reactions otherwise the run is considered invalid and must be repeated.
5. Reading of test serum end-points with each microscope assembly must be made with reference to the reactivities of the control sera with the antigen slides and conjugate provided.

INTERPRETATION OF RESULTS

The titer is the highest dilution of the patient's serum showing a positive (1+ to 4+) reaction of the kinetoplast. A positive reaction at 1:10 and above is significant.

LIMITATIONS OF PROCEDURE

1. A diagnosis should not be based on a single serologic test since various host factors must be taken into consideration.
2. Additional confirmatory tests for SLE include ANA procedures, complement level determinations, kidney biopsy and skin biopsy (7).
3. Drug induced SLE can give a positive reaction (9).
4. The class of circulating antinuclear antibody from patients with lupus nephritis is mostly IgG (in particular, subclasses IgG-1 and IgG-3), the dominant complement fixing class in humans. If only IgM anti-DNA is present, renal disease does not occur (10). This test procedure detects only IgG antibody.
5. The *C. luciliae* assay does not show a good correlation with the activity of renal disease in patients on immunosupressive therapy (11).

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Consult Instructions for Use

EC REP	Authorized Representative in the European Community
REF	Product Number
LOT	Lot Number
IVD	In Vitro Diagnostic Medical Device
	Use By
	Caution, consult accompanying documents
	Temperature limitation
	Manufacturer
 or 	WARNING
CONTROL -	Negative Control
CONTROL +	Positive Control
CONJ	Conjugate
PBS	Phosphate Buffered Saline
MTMED	Mounting Medium
CVRSLP	Coverslips
BLTRS 5W	Blotters, 5 Well
SLD 5W	Slide, 5 Well

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