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FLUORO nDNA TEST

Cat. No. 4270E: 80 wells kit
Cat. No. 4280E: 160 wells kit

MBL MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.

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English

Intended Use

The FLUORO nDNA TEST is intended for semi-quantitative detection of anti DNA antibodies in human serum. This product is only for in vitro diagnostic use. Do not use in human beings.

Summary and Explanation

Antinuclear antibodies can be found in the sera of patients with autoimmune diseases such as systemic lupus erythematosus (SLE). Especially, anti-native DNA antibodies (or anti-double stranded DNA antibodies) are highly specific for SLE. Therefore, detection of these antibodies is important in the diagnosis of SLE patients.

The mitochondrion (kinetoplast) of the *Crithidia luciliae* contains a large amount of ds-DNA but does not contain ss-DNA or histone. Therefore, the FLUORO nDNA TEST, which uses *Crithidia luciliae* as the substrate, is able to isolate and detect anti-nDNA antibodies with ease and accuracy.

Principle

The FLUORO nDNA TEST detects anti nDNA antibodies by the indirect immuno-fluorescence method. *Crithidia luciliae* is used as the substrate, and fluorescein isothiocyanate (FITC) is used as fluorescent dye.

Materials provided

Cat. No.	4270E 80wells	4280E 160 wells
Crithidia luciliae Substrate Slide	4 wells x 20 slides	8 wells x 20 slides
FITC conjugated goat anti-human immunoglobulins containing 2% BSA and 0.09% sodium azide, and Evans Blue	4.5 ml x 1 vial	8.5 ml x 1 vial
PBS Buffer	9.1g (for 1000ml) x 7 bags	9.1g (for 1000ml) x 7 bags
Positive Control Serum Human serum (anti nDNA positive) containing 2% BSA, 0.09% sodium azide	0.5 ml x 1 vial	0.5 ml x 1 vial
Negative Control Serum Human serum (anti nDNA negative) containing 2% BSA, 0.09% sodium azide	0.5 ml x 1 vial	0.5 ml x 1 vial
Mounting Medium Glycerol with Carbonate buffer containing 0.3% Trichloro Acetic Acid	3.0ml x 1 vial	3.0ml x 1 vial
Cover Slip	20 pcs	20 pcs
Blotting Paper	40 pcs	40 pcs

Materials required but not provided

500ml Beaker, Wash bottle, Magnetic stirrer, Moisture chamber, Staining basket, distilled or deionized water, Fluorescent microscope equipped with blue excitation filter unit

Precautions

- (1) Positive control serum and negative control serum are derived from human serum, in which HBs antigen, HIV (HIV-1 and HIV-2) antibodies and HCV antibodies have not been detected. However, it is strongly recommended that all clinical specimens and materials should be handled as if they are capable of transmitting infectious diseases.
- (2) FITC-conjugated antibody, negative control serum and positive control serum contain sodium azide (0.09%) as a preservative and must be handled with caution - do not ingest or allow contact with skin or mucous membranes. Sodium azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush with plenty of water when disposing materials containing sodium azide into a drain.
- (3) Some kit components contain animal origin materials, which are from non-infectious animals. These components, however, should be treated as potential biohazards in use and for disposal.
- (4) Mounting medium contains 0.3% trichloroacetic acid which is harmful to aquatic organisms and may cause long-term adverse effects in the aquatic environment. This material and its container must be disposed of as hazardous waste.

Storage and Stability

All kit components must be stored at 2-8°C. All reagents are stable for 12 months after manufacturing when stored at 2-8°C.

Procedure

1) Preparation of reagent

Bring substrate slides to room temperature prior to unsealing, in order to avoid moisture.

*Seal unused glass slides together with desiccant tightly in order to keep them dry during storage.

Prepare PBS by dissolving 1 bag of PBS powder in 1000ml of distilled water.

*Do not dilute the other kit components which are ready-for-use.

2) Preparation of samples

Use fresh patient sera.

a) Qualitative analysis: Dilute patient sera 1:5 with PBS.

b) Quantitative analysis: In the case of patient sera which were positive in the qualitative analysis, make serial dilutions of screening samples. (i.e. 1:10, 1:20, 1:40, 1:80)

*Do not repeat freezing and thawing of patient serum samples. This might result in decreased antibody titer or cause non-specific reactions.

*Lipemic sera should be avoided, because it causes non-specific reactions.

3) Addition of samples

Place one drop (20-30µl) each of diluted sera as well as positive and negative control sera over the antigen wells and place in a moisture chamber.

*Perform the analysis using the provided positive control sera and negative control sera as controls.

*Ensure that the added sample is not mixed with the sample in the next well. Also, in a quantitative analysis, add samples with lower concentration prior to samples with higher concentration.

4) Primary reaction

Incubate the slides in the moisture chamber for 20 minutes at room temperature (20-25°C).

*Incubation time should be between 20-30 min.

*Incubation temperature above or below normal room temperature (20-25°C), shorter or longer time periods of incubation may give erroneous results.

*Reaction should be performed in the moisture chamber with enough water poured not to dry the substrate slides.

5) Washing

(1) Place the PBS and the staining basket into a 500 ml beaker.

(2) Remove the slides from the moisture chamber one at a time and carefully rinse off the serum using a washing bottle filled with PBS.

*Do not squirt PBS directly on the wells.

*Do not take out all substrate slides at once, since this may lead to substrate drying out.

(3) Immediately stand the slides in the staining basket, prepared in step 1).

(4) After all the slides have been placed in the basket, wash them for 5 minutes using a magnetic stirrer.

*The amount of PBS used for washing is 500 ml per 10 glass slides.

6) Addition of FITC conjugated antibody

(1) After washing, remove the slides from the basket one at a time, and dry all parts other than the wells, using the enclosed blotting paper.

(2) Place the slides back into the moisture chamber, and add one drop of the secondary antibody (FITC-conjugated goat anti-human immunoglobulins) to each well on the slide.

*Never dry substrate slide, because this severely obstructs correct detection.

*Do not touch the well or remove PBS from well with blotting paper directly.

7) Secondary reaction

Incubate the slides in the moisture chamber for 20 minutes at room temperature (20-25°C).

*Incubation time should be between 20-30 min.

*Incubation temperature above or below normal room temperature (20-25°C), shorter or longer time periods of incubation may give erroneous results.

*Reaction should be performed in the moisture chamber with enough water poured not allowing the substrate slides to dry.

8) Washing

Wash the slides as in step 5.

9) Mount coverslip

After washing, remove the slides from the staining basket one at a time. Gently remove excess moisture with a piece of blotting paper and apply 2-3 drops of the mounting medium included in the kit. Carefully place coverslip in position.

*Be careful not to dry substrate slides.

10) Microscopic examination

Examine the slides using fluorescent microscope at a magnification of 400×.

*Examination should be performed promptly after mounting. If immediate examination is not possible, keep the slides in the cool, dark place, and perform examination within 24 hours.

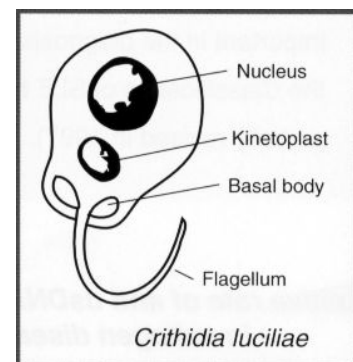
Interpretation of Results

Interpretation of negative or positive results

(-): As in the negative control serum, no specific fluorescence is seen in the kinetoplast.

There are 3 negative patterns:

- No fluorescence is seen in the *Crithidia luciliae* at all; the area of the kinetoplast looks like a black hole.
- Marked fluorescence in the basal body is seen, but no specific fluorescence in the kinetoplast.
- Specific fluorescence is seen in the nucleus, but no fluorescence in the kinetoplast.



(±): Slight specific fluorescence is seen in the kinetoplast.

(+): Weak but definite specific fluorescence is seen in the kinetoplast.

(++): Marked fluorescence is seen in the kinetoplast.

*The staining pattern of nuclei of *Crithidia luciliae* with the FLUORO nDNA TEST does not correspond to the results obtained by common ANA (anti nuclear antibodies) tests.

When interpreted as positive at a dilution of 1:5 or greater, the specimen is determined to be positive for anti-nDNA antibody.

Quality Control

Positive control serum and Negative control serum which are included in the kit should be tested in each run to insure that all reagents and procedures have performed properly.

Limitations

This product is only for diagnosis. Do not use in human beings. Test results should be used in conjunction with information available from clinical evaluation and other diagnostic information.

Expected Values and Performance Characteristics

75 patients samples and 40 normal samples were tested by FLUORO nDNA Test.

		SLE		RA	SjS	PSS	MCTD	Others	Normal
		active	inactive						
n		16	31	8	4	5	3	8	40
Positive	n	14	2	0	0	1	0	0	0
	%	87.5	6.5	0	0	20	0	0	0

RA: Rheumatoid arthritis, SjS: Sjogren's syndrome, PSS: Progressive systemic sclerosis, MCTD: Mixed connective tissue disease (Miura T. et al. Eisei Kensa 35(8): 1117-1122, 1986, Japanese)

<Correlation>

166 samples were measured using the FLUORO nDNA TEST as well as RIA kit.

		Fluoro nDNA	
		+	-
RIA DNA	+	47 (28%)	21 (12%)
	-	2 (1%)	96 (58%)

A result of 25 units/mL or higher was interpreted as positive for anti-dsDNA antibodies with the RIA method.

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