

MBL

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FLUORO HEPANA TEST

Cat. No. 4210E: 80 wells kit

Cat. No. 4220E: 160 wells kit

Cat. No. 4220-12E: 240 wells kit

Cat. No. 4221E: HEPANA Test Slide: 320 wells

Cat. No. 4213: Positive Control Serum

Cat. No. 4214: Negative Control Serum

Cat. No. 4222E: FITC conjugated goat anti-human immunoglobulins

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English

Intended Use

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

Summary and Explanation

Antinuclear antibodies (ANAs) are found in patients with autoimmune diseases such as systemic lupus erythematosus (SLE). Certain specific types of ANAs have been found to have important clinical correlations that make them useful for the diagnosis and treatment of patients with variety of rheumatic diseases.

In the FLUORO HEPANA TEST, HEp-2 cells which are derived from human pharyngeal cancer, are used as the nuclear substrate. Since the nuclei of HEp-2 cells are large, the staining pattern can be observed in detail. Moreover, since cells in various stages of the cell cycle coexist, detection of anti centromere antibodies and anti PCNA (proliferating cell nuclear antigens) antibodies is also possible with the FLUORO HEPANA TEST.

Principle

The FLUORO HEPANA TEST detects antinuclear antibodies by the indirect immunofluorescence. HEp-2 cells are used as nuclear substrates, and fluorescein isothiocyanate (FITC) is used as fluorescent dye.

Materials provided

Cat. No.	4210E 80wells	4220E 160 wells	4220-12E 240 wells	4221E 320 wells	4213	4214	4222E
HEp-2 Cell Substrate Slide	4 wells x 20 slides	8 wells x 20 slides	12 wells x 20 slides	8 wells x 40 slides			
FITC conjugated goat anti-human immunoglobulins containing 2% BSA, 0.09 % sodium azide, and Evans Blue	4.5 ml x 1 vial	8.5 ml x 1 vial	12.5 ml x 1 vial				8.5ml x1 vial
PBS Buffer	9.1g (for 1000ml) x 5 bags	9.1g (for 1000ml) x 5 bags	9.1g (for 1000ml) x 5 bags				
Positive Control Serum Human serum (ANA positive) containing 2% BSA, 0.09% sodium azide	0.5 ml x 1 vial	0.5 ml x 1 vial	0.5 ml x 1 vial		0.5 ml x 1 vial		
Negative Control Serum Human serum (ANA negative) containing 2% BSA, 0.09% sodium azide	0.5 ml x 1 vial	0.5 ml x 1 vial	0.5 ml x 1 vial			0.5 ml x 1 vial	
Mounting Medium Glycerol with Carbonate buffer containing 0.3%	3.0ml x 1 vial	3.0ml x 1 vial	3.0ml x 1 vial	3.0ml x 2 vial			

Trichloro Acetic Acid							
Cover Slip	20 pcs	20 pcs	20 pcs				
Blotting Paper	40 pcs	40 pcs	40 pcs				

Materials required but not provided

500 ml 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 antibody has 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 15 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 1000 ml 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 1:20 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. 1:40, 1:80, 1:160, 1:320)

*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 (30-40 μ l) each of diluted sera as well as positive control sera 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 to dry the substrate slides.

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 200 \times .

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

Interpretation of Results

1) Interpretation of negative or positive results

(-): No specific fluorescence is detected in cell nucleus.

(\pm): Although slight staining is detected in cell nucleus, staining pattern cannot be identified.

(+): Specific Fluorescence is clearly detected throughout the entire nucleus, or in a certain area of the nucleus.

When interpreted as antinuclear antibody positive, the pattern should be evaluated in accordance with step 2 below.

2) Evaluation of staining pattern

Staining patterns are mostly classified as follows. In some cases, multiple staining patterns coexist.

Peripheral pattern

Entire nucleus is stained smoothly, primarily around the outer region of the nucleus, with weaker staining toward the center of the nucleus. In a mitotic cell, chromosome region is strongly stained.

Homogeneous pattern

Entire nucleus is stained homogeneously and smoothly. Chromosome region in a mitotic cell shows the same or brighter fluorescence than that in interphase period.

Speckled pattern

Grainy fluorescence is detected inside nucleus, and staining is not smooth. The nucleoli are not usually stained. The chromosome region in a mitotic cell is not stained.

Nucleolar pattern

Nucleolus is stained as several large dots or clumps of granules inside nucleus. Usual number of dots is less than 6 per nucleus.

Discrete-speckled pattern

Homogeneously distributed and speckled fluorescence in nucleus is observed. The number of speckles is usually 40-60 grains per nucleus. Mitotic figures discrete speckles over the chromosome region.

Also, the following staining pattern is observed for each of the autoantibodies.

Anti-PCNA antibody

When positive cells and negative cells coexist, the presence of anti PCNA antibody is suspected. Staining pattern varies depending on cell cycle.

Anti-mitochondria antibody and anti-smooth muscle antibody

When staining is observed in cytoplasm, the presence of anti-mitochondria antibody or anti-smooth muscle antibody is suggested. Cytoplasm is stained in a granule-like-pattern with anti-mitochondria antibody and a fibrous pattern with anti-smooth muscle antibody. In this case, this kind of antibodies should be confirmed using substrates such as stomach or kidney of rat. (MBL AID-1 TEST, Cat.No.4250E)

Titer interpretation

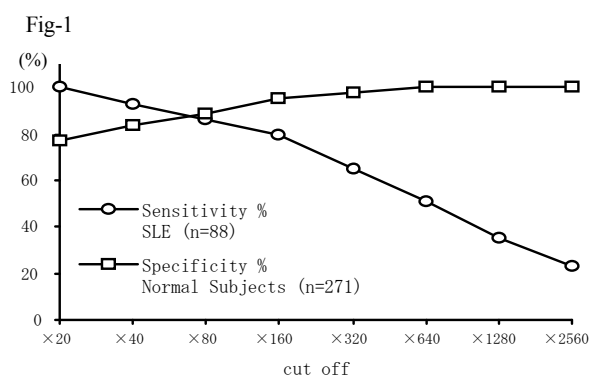


Fig-1 shows relation between sensitivity in 88 samples with SLE patients and specificity in 271 samples with normal subjects in accordance with the cut-off value. When samples were judged at 1:20 dilution, the positive percentage in SLE patients was 100%, and specificity in normal subjects was 77.4%. When samples were judged at 1:40 dilution, the positive percentage in SLE patients was 93.2%, and specificity in normal subjects was 84.1%. It is recommended that each laboratory determine its own cut-off value.

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

196 patients samples including 45 samples from SLE patients were tested by FLUORO HEPANA Test.

	N	anti-nuclear antibodies						anti-cytoplasmic antibody
		Peripheral	Homogeneous	Speckled	Nucleolar	Discrete-speckled	Negative	
SLE	45	7 (%)	64 (%)	27 (%)	7 (%)	0 (%)	7 (%)	31 (%)
PSS-diffuse	20	0	45	35	20	10	5	5
PSS-CREST	7	0	0	14	0	71	14	0
DM-PM	35	0	20	31	6	0	49	34
MCTD	7	0	29	100	0	0	0	0
Overlap	7	0	43	57	0	0	14	29
RA	75	0	17	3	5	0	68	20

271 normal samples were tested by FLUORO HEPANA Test.

	N	Positive	Negative
Normals	271	15.9%	84.1%

<Repeatability>

Sera of 4 different patients were tested 10 times.

No.	High Titer	Middle Titer	Low Titer	+/-
1	5,120	640	80	negative
2	5,120	640	80	negative
3	5,120	640	80	negative
4	5,120	640	80	negative
5	5,120	640	80	negative
6	5,120	640	80	negative
7	5,120	640	40	negative
8	5,120	640	80	negative
9	5,120	640	80	negative
10	5,120	640	80	negative

*Jpn J Med Pharm Sci; 19(5):1239-1242,1988

<Correlation>

40 samples of patients with collagen disease were measured using the FLUORO HEPANA TEST as well as FLUORO ANA TEST which uses rat liver section as substrate.

		FLUORO ANA TEST	
FLUORO HEPANA TEST		+	-
	+	34	3
	-	0	3

References

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2. Moroi Y., Peebles C., Frizler M.J., et al.: Proc. Natl. Acad. Sci. U.S.A., 77: 1627, 1980.
3. Tan E.M., Rodnan G.P., Garcia I., et al.: Arthritis Rheum., 23: 617, 1980.
4. Moroi Y., Hartman A., Nakane P.K., et al.: J. cell Biol., 90: 254, 1981.

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Cat. No. 4210E

[www.e-labeling.eu/ MBL006087](http://www.e-labeling.eu/MBL006087)

Cat. No. 4220E

[www.e-labeling.eu/ MBL008067](http://www.e-labeling.eu/MBL008067)

Cat. No. 4220-12E

[www.e-labeling.eu/ MBL008074](http://www.e-labeling.eu/MBL008074)

Cat. No. 4221E

[www.e-labeling.eu/ MBL009385](http://www.e-labeling.eu/MBL009385)

Cat. No. 4222E

[www.e-labeling.eu/ MBL009392](http://www.e-labeling.eu/MBL009392)



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