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FLUORO AID-1 TEST

Cat. No. 4250E: 40 wells kit

MBL MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.

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English

Intended Use

The FLUORO AID-1 TEST is intended for detection of anti-mitochondrial antibodies(AMA), anti smooth muscle antibodies (ASMA) and anti parietal cell antibodies (APCA) in human serum. This product is only for in vitro diagnostic use. Do not use in human beings.

Summary and Explanation

It is very useful for the diagnosis of hepatic diseases, in particular, primary biliary cirrhosis (PBC) and active chronic hepatitis, to detect AMA and ASMA.

AMA, which had been first reported by Dr. Mackay, was subsequently shown to have a strong specificity to PBC. Though low titer of AMA may be detected in other liver disorders which include chronic active hepatitis, cryptogenic cirrhosis and drug-induced hepatitis, high titers (x40 or more) present only in PBC.

Tests for AMA have been recommended as a substitute for surgical exploration of PBC.

ASMA was first reported by Dr. Johnson, and shown to have a strong specificity to lupoid hepatitis and active chronic hepatitis. As ASMA are not detected in SLE, it is very useful for differentiation SLE from lupoid hepatitis. It is reported that ASMA titer shows positive in an active stage of the disease and negative in a remissive stage of the disease.

APCA are associated with autoimmune gastritis type A (chronic atrophic gastritis) and pernicious anemia in about 90% of patients and helpful in diagnosis. It is reported that APCA shows positive in other autoimmune endocrine diseases such as thyroiditis and insulin dependent diabetes mellitus.

Principle

The FLUORO AID-1 TEST detects anti-mitochondrial antibodies, anti smooth muscle antibodies and anti parietal cell antibodies by the indirect immuno-fluorescence method. Rat stomach/kidney is used as substrates, and fluorescein isothiocyanate (FITC) is used as fluorescent dye.

Materials provided

	Cat.No.4250E 40wells
Rat stomach/kidney Substrate Slide	4 wells x 10 slides
FITC conjugated goat anti-human immunoglobulins containing 2% BSA and 0.09% sodium azide	4.5 ml x 1 vial
PBS Buffer	9.1g (for 1000ml) x 5 bags
AMA Positive Control Serum Human serum (AMA positive) containing 2% BSA, 0.09% sodium azide	0.5 ml x 1 vial
ASMA Positive Control Serum Human serum (ASMA positive) containing 2%	0.5 ml x 1 vial

BSA, 0.09% sodium azide	
APCA Positive Control Serum Human serum (APCA positive) containing 2% BSA, 0.09% sodium azide	0.5 ml x 1 vial
Mounting Medium Glycerol with Carbonate buffer containing 0.3% Trichloro Acetic Acid	3.0ml x 1 vial
Cover Slip	10 pcs
Blotting Paper	20 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) Each positive control serum is derived from human serum, in which HBs antigen, HIV antibody and HCV (HIV-1 and HIV-2) antibodies 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 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 1:20 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 (20-30 μ l) each of diluted sera as well as positive control over the antigen wells and place in a moisture chamber.

*Perform the analysis using the provided positive 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

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.

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 100×.

*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

(1) ASMA

(-): No specific fluorescence is seen in muscularis mucosae and muscularis.

(+): As in the positive control serum, specific fluorescence is seen in muscularis mucosae, muscularis, stomach sub-mucosa and renal vessel walls.

(2) AMA

(-): No specific fluorescence is seen in renal tubules in epithelial structures.

(+): As in the positive control serum, specific fluorescence is seen in cytoplasm of proximal and distal renal tubules and the cytoplasm of stomach parietal cells.

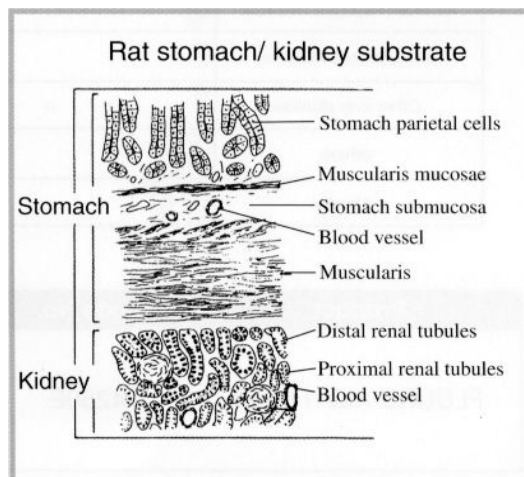
(3) APCA

(-): No specific fluorescence is seen in mucosae of stomach.

(+): As in the positive control serum, specific fluorescence is seen in cytoplasm of stomach parietal cells.

Since AMA also stain parietal cells, kidney tubules should also be checked before reporting APCA positive.

When interpreted as positive at a dilution of 1:20 or greater, the specimen is determined to be positive for AMA, ASMA or APCA.



Quality Control

Positive control serum which is 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 the clinical evaluation and other diagnostic information.

Expected Values and Performance Characteristics

Patients samples and normal samples were tested by FLUORO AID-1 Test.

		AMA		ASMA			
		positive	negative	positive	negative		
Autoimmune liver disease	Primary biliary cirrhosis		15	0	0	14	
	Autoimmune hepatitis		0	7	7	0	
Viral liver disease	Acute hepatitis	Type A		0	6	4	2
		Type B		0	2	2	0
		Non-A, Non-B		0	3	1	2
	Chronic hepatitis	Active	Type B, Non-B	0	6	0	6
		Inactive	Type B, Non-B	0	9	4	1
		Cirrhosis	Type B	0	2	0	2
			Non-B	0	9	4	5
Hepatoma		0	2	0	2		
Alcoholic hepatitis, cirrhosis		0	6	0	6		
Drug-induced hepatitis		0	3	0	3		
Other liver disease		0	5	2	3		
Others		0	15	0	15		
Normal		0	17	0	17		

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