Introduction:
Smooth muscle antibodies (SMA) can be demonstrated in patients with acute and chronic hepatitis; the highest titers occurring in chronic active hepatitis (CAH). All of the various forms of chronic liver disease show SMA titers not higher than 1:160, except for CAH where titers up to 1:1280 are found. The differential diagnosis of CAH in patients with chronic liver disease is facilitated by titration of SMA using the indirect immunofluorescence method with rat or mouse stomach muscularis mucosa as the substrate.

There exist various forms of acute and chronic liver injury that are directly or indirectly related to hepatitis B(HB) infection. Both viral and autoantibody markers may be used to classify the different sub-groups of CAH and it has been demonstrated that most HB-antigen negative patients are SMA positive.

SMA tests have been found helpful in confirming the diagnosis of approximately 70% of CAH. A positive SMA test rules out Systemic Lupus Erythematosus, since the SMA test is generally negative in SLE. It is also found in approximately 50% of patients with primary biliary cirrhosis (PBC) and in up to 28% of patients with cryptogenic cirrhosis. High incidence of SMA have also been reported in serum of patients with infective mononucleosis. Diseases including carcinoma of the breast, malignant melanoma and ovarian carcinoma have been reported to contain SMA.

SMA is rarely found (less than 2%) in patients with bile duct obstruction, acholic cirrhosis, lupus erythematosus and in the normal population. Rat or mouse stomach is utilized for SMA detection in this test system.

Principles:

The SMA reaction involves circulating antibodies to a normal component of the smooth muscle cell. These antibodies are not organ or species specific and may be found in tissues with smooth muscle areas. They are primarily of the IgG class of immuno-globulins but may also occur as IgM. Sections of rat or mouse stomach are used as the antigen substrate.

The primary reaction involves circulating antibodies in the patient's serum which attach to their homologous smooth muscle antigens. This occurs during the incubation period while the serum covers the antigen surface. A secondary reaction then follows a rinsing period which removes all unbound human antibody.

The secondary reaction is a fluorescein labelled anti-human globulin conjugate and is viewed under an appropriate fluorescent microscope. Bright cytoplasmic fluorescence of the smooth muscle layers of the muscularis mucosae indicates a positive result.

Research has shown that the antigen active in the SMA reaction is actin. Actin is found in such histological structures as: the capillary linings, platelets, brush borders of renal tubular epithelium and in the renal glomerular cells. These antibodies are non-organ specific and will react with smooth muscle surrounding arteries, veins and other histological structures containing actin. The reactivity of SMA from CAH patients is rather broad and includes many of these "non-muscle" tissues. SMA can be actin or non-actin specific and it is the former that is associated with CAH. However, studies using cultured fibroblasts reaffirm the actin specificity of SMA from CAH patients. Attempts at classifying SMA by different immunofluorescent patterns have not yet provided a clear clinical

correlation between distinct diseases and a particular fluorescent pattern. Fluorescence of the gastric mucosal cells (parietal or chief cells) or nuclear staining in ANA positive sera should not be reported as positive SMA reactions.

Materials Provided:

Storage & Stability of Components:

1. FITC Conjugate No. 1501L (3.0 ml)/1533L (5.0 ml) with Evans Blue Counterstain is to be stored at 2-8 C upon receipt. The conjugate is stable at this temperature until expiration date on the vial label. This reagent contains antibodies which will react with the human IgG, IgM and IgA Immunoglobulin classes.

2. The antigen slides of rat or mouse stomach sections must be stored at 2-8 C or lower upon receipt. Check label for specific expiration date.

3. SMA positive control No. 3202L (1.0 ml) should be stored at 2-8 C upon receipt. Check label for specific expiration date.

4. Universal negative control No. 1000L (1.0 ml) should be stored at 2-8 C or lower upon receipt. Check label for specific expiration date.

5. Buffer Pack No. 1601 - Phosphate Buffered Saline is stable at room temperature storage for 5 years. The reconstituted Buffer does not contain preservatives and should be stored at 2-8 C. Care should be taken to avoid contamination.

6. Mounting Medium No. 1610 is stable when stored at 2-8 C. Check label for specific expiration date.

Note: All kit components are available separately. Please see current SCIMEDX Corporation Catalog for more details.

Additional Materials Required but not Provided:

Test tubes and rack or microtiter system
Disposable pipettes
Staining Dish and Slide Forceps
Moisture Chamber
Volumetric Flask (500 ml)
Distilled H_20
Fluorescence Microscope
Paper Towels - lint free

Reagent Preparation:

1. Buffer Pack No. 1601 Rehydrate buffer with 1 liter of sterile distilled water.

Specimen Collection:
Serological specimens should be collected under aseptic conditions. Hemolysis is avoided through prompt separation of the serum from the clot. Serum should be stored at 2-8 C if it is to be analyzed within a few 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, addition of a preservative such as 0.01% (thimerosal) or 0.095% sodium azide is strongly recommended.

Test Instruction:
Screening: dilute test serums 1:20 (1 part patient sample to 19 part diluent) in PBS.
Titration: set up doubling dilutions of serum starting at 1:20 (i.e., 1:20, 1:40, 1:80, 1:160, 1:320, etc.

1. Once slides reach room temperature tear slide envelope at notch. Carefully remove the slide and avoid touching the antigen areas. The slide is now ready to use.

2. Place a drop of diluted serum (20 to 30 µl) and controls over the antigen wells.

3. Place slide with patient's serum and controls in a moist chamber for 30 minutes at room temperature (approximately 24 C).

4. Remove slide from moisture chamber and tap the slide on its side to allow the serum to run off onto a piece of paper towel. Using a wash bottle, gently rinse remaining sera from slide being careful not to aim the rinse stream directly on to the well.

5. Wash in PBS for five minutes. Repeat using fresh PBS.

6. Place a blotter on the lab table with absorbent side up. Remove slide from PBS and invert so that tissue side faces absorbent side of blotter. Line up wells to blotter holes. Place slide on top of blotter. **Do not allow tissue to dry.** Wipe back of slide with dry lint free paper towel. Apply sufficient pressure to slide while wiping to absorb buffer.

7. Deliver 1 drop (20-30 µl) of conjugate per antigen well. Repeat steps 3-6.

8. Place 4-5 drops of mounting medium on slide.

9. Apply a 22 x 70 mm coverslip. Examine the slide under a fluorescent microscope. Note: To maintain fluorescence, store mounted slide in a moisture chamber placed in a dark refrigerator.

Quality Control:

1. Positive and negative serum controls must be included in each day's testing to confirm reproducibility, sensitivity and specificity of the test procedure.

2. The negative serum control should result in little (+) or no fluorescence. If this control shows bright fluorescence, either the control, antigen, conjugate or technique may be at fault.
3. The positive serum control should result in bright 3+ to 4+ fluorescence. If this control shows little or no fluorescence, either the control, antigen, conjugate or technique may be at fault.

4. In addition to positive and negative serum controls, a PBS control should be run to establish that the conjugate is free from nonspecific staining of the antigen substrate. If the antigen shows bright fluorescence in the PBS control repeat using fresh conjugate. If the antigen still fluoresces, either the conjugate or antigen may be at fault.

Results:
ACH is a chronic disease of the liver mainly affecting young females but also affecting both sexes and all ages. It is characterized in liver biopsies of deterioration of liver function due to necrosis of hepatic parenchymal cells in areas of lymphocytic and plasma cell infiltration.

A positive result is observed as bright diffused cytoplasmic staining of the smooth muscle layers of the muscularis mucosae found in the rat or mouse stomach. Fluorescence may also be evident in the capillary walls of the gastric layer and surrounding arteries or veins. Fluorescence of other cellular antigens such as nuclei, parietal cells or connective tissue should not be reported as positive SMA.

The titer is the highest dilution of the patient's serum showing weak (1 +) fluorescence of the muscularis mucosae.

Less than 1:20 or less - Normal, negative

1:20 - 1:80 - Positive. Suggestive of liver disease. Repeat with fresh specimen in two weeks.

1:160 or greater -Suggestive of active chronic hepatitis.

Limitations of Procedure:

1. No diagnosis should be based upon a single SMA test result, since various host factors must be taken into consideration.

2. SMA should be used as an aid in the diagnosis of liver disease.

3. Clinical manifestations such as liver biopsies and liver function tests should be considered in the final diagnosis of chronic active hepatitis.

4. SMA can be found in: primary biliary cirrhosis (PBC), cryptogenic cirrhosis, infective mononucleosis, asthma, yellow fever, acute infective hepatitis, carcinoma of the breast, malignant melanoma and ovarian carcinoma.

5. Titers of some acute cases of viral hepatitis (AVH) can be as high as CAH cases but they decrease and disappear in a relatively short period while CAH titers remain high for prolonged periods.

6. SMA represents a family of antibodies directed against contractile proteins present in different tissues. The non-homogenous glomerular pattern has never been found in cirrhotic patients and this pattern is always associated with high SMA titers in CAH.

7. In CAH patients that are HB negative, the titers of the IgG-SMA and IgG-ANA seem to be related to the degree of inflammatory activity but no prognostic importance can be associated with these phenomena.

8. Drug induced CAH is rather rare but the drugs oxypenisatin and methyl dopa have been associated with some cases of CAH.

Precautions:

1. All human components have been tested by radio-immunoassay for (HB_SA_g) and HTLVIII/LAV by an FDA approved method and found to be negative. (Not repeatedly reactive). However, this does not assure the absence of HB_SA_g or HTLVIII/LAV. All human components should be handled with appropriate care.

2. The sodium azide (0.095%) included in the controls and conjugate is toxic if ingested.

3. Do not use components beyond their expiration date.

4. Follow the procedural instructions exactly as they appear in this insert to insure valid results.

5. For In Vitro Diagnostic use.

6. Handle slides by the edges since direct pressure on the antigen wells may damage the antigen.

7. Once the procedure has started do not allow the antigen in the wells to dry out. This may result in false negative test results, or unnecessary artifacts.

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