TEST LIMITATIONS

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. Titres 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 titres 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 titres in CAH.
7. In CAH patients that are HB negative, the titres 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.

LITERATURE REFERENCES

1. Andersen, P., Pedersen, T.K. and Ladefoged, K.: Studies of smooth muscle antibodies in acute hepatitis. Acta Pathol Microbiol Scand (C) 84:365-71, 1976.
2. Pisi, E., Marchesini, C., Zauli, D. and Bianeki, F.B.: Smooth muscle antibody in chronic hepatitis. Chron Hep Intl Symp, P Gentini, H Popper and J Teodori, Eds., Monte-Catini pp 107-13, Karger, Basel, 1976.
3. Naccarato, R., Chiaramonte, M., Borelli, A. and Farini, R.: Circulating antibodies in chronic liver diseases. Chron Hep Intl Symp, P Gentini, H Popper and J Teodori, Eds. Monte-Catini, pp. 114-6. Karger, Basel, 1976.
4. Ishak, K.C.: Light microscopic morphology of viral hepatitis. Am J Clin Pathol 65:787-827, 1976.
5. Peters, R.L.: Viral hepatitis: A pathologic spectrum. Am J Med Sci 270: 17-31, 1975.
6. Huang, S.N., Neurath, A.R.: Immunohistologic demonstration of hepatitis B viral antigens in liver with reference to its significance in liver injury. Lab Invest 40: 1-17, 1979.
7. Doniach, D.: Immunofluorescent autoantibody studies in the diagnosis of chronic liver disorders. Rendic Gastroenterol ^:192-203, 1974.
8. Edgington, T.S. and Ritt, D.J.: Intrahepatic expression of serum hepatitis virus-associated antigens: Immunologic aspects of hepatitis B virus infection. Am J Med Sci 270-213, 1975.
9. Ferguson, A. and MacSween, R.N.M.: Autoimmune diseases of the liver. In: Immunological Aspects of Liver and Gastrointestinal Tract. University Park Press, Baltimore, p. 346-86 1976.
10. Lindman, K., Biberfeld, G. et al: Anti-actin specificity of human smooth muscle antibodies in chronic active hepatitis. Clin Exp Immunol 24:266-72, 1976.
11. Biberfeld, G. and Sterner, G.: Smooth muscle antibodies in mycoplasma pneumoniae infection. Clin Exp Immunol 24:287-91, 1976.
12. Andersen, P., Small, J.V. and Soliceszek, A.: Studies on the specificity of smooth muscle antibody. Clin Exp Immunol 26:57-60, 1976.
13. Bottazzo, G.F., Christensen A.F. et al: Classification of smooth muscle autoantibodies detected by immunofluorescence. J Clin Pathol 29:403-10, 1976.

EC REP Advena Ltd. Tower Business Centre, 2nd Flr., Tower Street, Swatar, BKR 4013 Malta

INSTRUCTIONS FOR USE

Anti-Smooth Muscle Antibody Test System

	48 Tests	96 Tests	50 Tests	100 Tests
Rat Stomach system	**K4809**	**K9609**	**K5009**	**K0009**
Mouse Stomach system	**K4810**	**K9610**	**K5010**	**K0010**
Also for:	4 well	8 well	5 well	10 well
Mouse Stomach slides	**S4003**	**S8003**	**S5003**	**S0003**
Rat Stomach slides	**S4103**	**S8103**	**S5103**	**S0103**

INTENDED USE

The Bio-Diagnostics Anti-Smooth Muscle Antibody Test kit is an immunofluorescent antibody (IFA) test to detect the presence of antibodies to smooth muscle in human serum.

SUMARY AND EXPLANATION

Smooth muscle antibodies (SMA) can be demonstrated in patients with acute and chronic hepatitis; the highest titres occurring in chronic active hepatitis (CAH). All of the various forms of chronic liver disease show SMA titres not higher than 1:160, except for CAH where titres 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 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, alcoholic cirrhosis, lupus erythematosus and in the normal population. Rat or mouse stomach is utilised for SMA detection in this test system.

PRINCIPLE OF THE TEST

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 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 which 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.

Bio-Diagnostics Ltd, Upton Industrial Estate, Rectory Road, Upton-upon-Severn, Worcestershire, WR8 0LX, United Kingdom. ☎+44 (0) 1684 592262 email: enquiries@bio-diagnostics.co.uk

WARNINGS AND PRECAUTIONS

1. All human components have been tested by radioimmunoassay for (HBsAg) and HTLVIII/LAV by an FDA approved method and found to be negative (not repeatedly reactive). However, this does not assure the absence of (HBsAg) or HTLVIII/LAV. All human components should be handled with appropriate care.
2. The reagents included in the kit contain 0.1% sodium azide or 0.01% thiomersal as preservatives. Although this is at a low concentration, these reagents should be considered toxic. They should not be ingested or allowed to come into contact with either the skin or the mucous membranes. Sodium azide may also cause the formation of potentially explosive lead or copper azides in sinks.
3. Do not use components beyond their expiration date.
4. Follow the procedural instructions exactly as they appear in this insert to ensure 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 artefacts.

KIT CONTENTS

SLIDE	Rat or mouse stomach substrate antigen slides
CONJ **IgG**	FITC Conjugate with Evans Blue Counterstain: J501/J501-5.This reagent contains antibodies that will react with the human IgG (H+L) Immunoglobulin class.
CONTROL +	Smooth muscle antibody Positive Control no: C005N/C005N-0.5
CONTROL -	Universal Negative Control no: C000N/C000N-0.5
IFA/DFA **PBS**	Buffer Pack no: R002
MM	Mounting Medium no: R005

Note: All kit components are available separately. Please see the Bio-Diagnostics Ltd catalogue for more details.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

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

REAGENT PREPARATION

1. Buffer Pack no: R002. Rehydrate buffer with 1 litre of sterile distilled water.

KEY FOR OTHER SYMBOLS

Used in this instruction leaflet and on accompanying product packaging:

Manufacturer — Contains sufficient for <n> tests — **RFU** Ready for use

Temperature limitation — **IVD** In vitro diagnostic medical device

STORAGE AND STABILITY

The IFA Test System components (except PBS) must be stored at a temperature of +2ºC to +8ºC. Do not freeze the test kit. The stability of the kit is as indicated by the expiry date on the packaging under the above storage conditions. This applies to unopened and opened reagents.

Phosphate Buffered Saline is stable at room temperature storage. The reconstituted Buffer does not contain preservatives and should be stored at 2-8°C. Care should be taken to avoid contamination.

SPECIMEN COLLECTION

Serological specimens should be collected under aseptic conditions. Haemolysis is avoided through prompt separation of the serum from the clot. Serum should be stored at 2-8°C if it is to be analysed within a few days. Serum may be held for 3 to 6 months by storage at -20°C or lower. Lipaemic and strongly haemolytic serum should be avoided. When specimens are shipped at ambient temperatures, addition of a preservative such as 0.01% thiomersal or 0.1% sodium azide is strongly recommended.

Anti-Smooth Muscle Antibody Test System

TEST INSTRUCTIONS

Screening: dilute test serums 1/20 (1 part patient sample to 19 parts diluent) in PBS. N.B. Although this dilution factor is suggested, each laboratory should determine their individual screening dilution.

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 30µl) 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 18-24ºC).
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 onto the well.
5. Wash in PBS for 5 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 the wells to blotter holes. Place slide on top of the blotter. Wipe the back of the slide with dry lint free paper towel. Apply sufficient pressure to slide while wiping to absorb buffer. **Do not allow tissue to dry.**
7. Deliver 1 drop (20-30µl) 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 controls should result in bright 3+ to 4+ fluorescence. If these controls show 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 non-specific 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 characterised 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.

Titre Interpretation:

The titre 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. May be suggestive of liver disease. Repeat with fresh specimen in two weeks.
1/160 or greater	- Suggestive of active chronic hepatitis