Autoscreen I/II Test System

RESULTS

ANA positive results are observed as one of the four basic staining patterns seen individually or in various combinations. The characteristic patterns are best seen when viewed using high dry objectives.

Homogenous	diffuse even, finely diffuse fluorescence of the entire nucleus is seen
Peripheral (rim, shaggy)	the nuclear membrane is more intensely fluorescent than the central areas
Speckled	the nuclei show numerous small "specks" of fluorescence throughout the nucleus
Nucleolar	the nucleoli are uniformly stained and appear as 1 to 5 large spherical areas of fluorescence scattered throughout the nucleus

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

Titre:	1/20 or less	Normal: virtually rules out active SLE if not on immunosuppressive therapy or in remission.
	1/20-1/80	Positive test often found in RA and other connective tissue diseases, a fresh sample should be tested in 2 weeks. If titre increases active SLE is suggested.
	1/160 or greater	Strongly suggests SLE although other autoimmune diseases and drugs may induce high titres.

Mitochondrial antibody (MA) positive results are observed as granular fluorescence in the cytoplasm of the renal tubules. The fluorescence is limited to the cytoplasm of the proximal and the distal tubular epithelium. Fluorescence of the cellular antigens such as nuclei, smooth muscle, or non-granular fluorescence limited to the central (lumen) portion of the proximal tubules should not be reported as positive MA (11).

The titre is the highest dilution of the patient's serum showing weak (1+) fluorescence of the renal tubular epithelium.

Titre:	1/10	Normal, negative
	1/20-1/80	Positive, suggestive of liver disease. Repeat with a fresh specimen in 2 weeks.
	1/160 or greater	Presumptive primary biliary cirrhosis.

Parietal Cell antibody (PCA) positive results are observed as bright granular cytoplasmic fluorescence of parietal cells of the rat gastric mucosa. Fluorescence of other cellular antigens such as nucleoli, smooth muscle or connective tissue should not be reported as positive PCA.

Smooth muscle antibody (SMA) positive results are observed as bright diffuse cytoplasmic staining of the smooth muscle layers of the muscular mucosae in the rat stomach. Fluorescence may also be evident in the capillary walls of the gastric layer and surrounding arteries or veins. Fluorescence of the other cellular antigens such as nucleoli, parietal cells or connective tissue should not be reported as positive SMA.

Titre:	1/10	Normal, negative
	1/20-1/80	Positive, suggestive of liver disease
	1/160 or greater	Suggestive of active chronic hepatitis

Liver kidney microsomal (LKM) positive results are observed as bright cytoplasmic fluorescence of the liver hepatocytes and the kidney proximal tubules with no staining of the kidney distal tubules or stomach tissue.

TEST LIMITATIONS

1. No diagnosis should be based upon a single serologic test result since various host factors must be taken into consideration. Among these host factors are age and sex. There is an increasing significance in positive ANA results in both males and females as age increases.
2. It is possible that substances such as drugs, viruses and bacteria may induce production of autoantibodies without there being any associated pathology or clinical condition.

LITERATURE REFERENCES

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INSTRUCTIONS FOR USE

Autoscreen I/II Autoimmune Test Systems

	48 Tests	96 Tests	50 Tests	100 Tests	250 Tests
Autoscreen I:					
- Rat Kidney / Stomach	**K4801**	**K9601**	**K5001**	**K0001**	
- Mouse Kidney / Stomach	**K4802**	**K9602**	**K5002**	**K0002**	
Autoscreen II:					
- Rat Liver / Kidney / Stomach	**K4803**	**K9603**	**K5003**	**K0003**	**K25003**
- Mouse Liver / Kidney / Stomach	**K4804**	**K9604**	**K5004**	**K0004**	**K25004**
- Rat Liver / Rat Kidney / Mouse Stomach	**K4821**	**K9621**	**K5021**	**K0021**	

Also for:	4 well	8 well	5 well	10 well
Rat Kidney/Stomach slides	**D4123**	**D8123**	**D5123**	**D0123**
Mouse Kidney/Stomach slides	**D4023**	**D8023**	**D5023**	**D0023**
Rat Liver/Kidney/Mouse Stomach/Rat Stomach slides			**Q5007**	**Q0007**
Rat Liver/Kidney/Stomach slides	**T4002**	**T8002/T8002-IN**	**T5002**	**T0002**
Mouse Liver/Kidney/Stomach slides	**T4004**	**T8004/T8004-IN**	**T5004**	**T0004**
Mouse Stomach / Rat Liver/Kidney slides	**T4007**	**T8007**	**T5007**	**T0007**
Mouse Liver	**S4001**	**S8001**	**S5001**	**S0001**

N.B. a number at the end of a code indicates number of slides, e.g. T500410 = pack of 10 x T5004 slides)

INTENDED USE

The Bio-Diagnostics Autoscreen I and II Test Systems are indirect immunofluorescent antibody (IFA) tests that will simultaneously detect antinuclear (ANA), mitochondrial (MA), parietal cell (PCA), smooth muscle (SMA), liver/kidney microsomal (LKM) and reticulin autoantibodies in human serum. All necessary tissue substrates are contained in each slide well of this system to perform the above antibody screening.

SUMMARY AND EXPLANATION

Antinuclear Antibody (ANA) tests are commonly performed on sera from patients with various connective tissue diseases, particularly in systemic lupus erythematosus (SLE), for diagnostic evidence, prognostic significance, and management of therapy. The highest titres of ANA are found in active SLE and the presence of these antibodies is the second most common manifestations of SLE (1). The presence of ANA has been well documented in different disease states as well as in healthy relatives of SLE patients. Rat or mouse liver is utilised for ANA detection (2).

Mitochondrial Antibody (MA) as a circulating autoantibody in chronic liver disease is of great clinical importance in the differential diagnosis of chronic active hepatitis (CAH) from chronic persistent hepatitis (CPH) and is particularly useful in the diagnosis of primary biliary cirrhosis (PBC). MA is present in sera of patients with a variety of liver disorders but studies have demonstrated that MA titres greater than 1/40 are found only in patients with PBC (3,4). Rat or mouse kidney is utilised for MA detection.

Smooth muscle antibodies (SMA) can be demonstrated in patients with acute and chronic hepatitis; the highest titres occurring in chronic active hepatitis (CAH). Most forms of chronic liver disease show SMA titres not higher than 1/160, except for CAH where titres up to 1/1280 are found. SMA is also found in approximately 50% of patients with primary biliary cirrhosis (PBC) and in up to 28% of patients with cryptogenic cirrhosis (5). 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.

Gastric autoimmune disease has been classified into Type A and Type B gastritis. Patients with antibodies to **parietal cells (PCA)** or intrinsic factor (or both) have atrophy of the fungal mucosa (Type A). A positive PCA is the presence of a megaloblastic anaemia makes pernicious anaemia a probable diagnosis. In type B gastritis, PCA is lacking and there is no association with pernicious anaemia or other autoimmune endocrine disorders (6). Conditions other than pernicious anaemia may give positive PCA results and in the normal population, PCA varies from 2% in the under 20 age group to 16% in the over 60 age group (7). The gastric mucosa of rat or mouse stomach is used for PCA detection.

There are 3 types of **Liver/kidney microsomal (LKM) antibodies** but LKM-1 is the most important and is associated with autoimmune hepatitis type II (8). These antibodies show homogenous staining of the liver hepatocytes and the proximal renal tubules although the distal tubules are negative. There is no reaction with stomach tissue. Due to the similarity of patterns, it is important to distinguish LKM-1 antibodies from mitochondrial antibodies which do react with the parietal cells of the stomach.

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PRINCIPLE OF THE TEST

Diluted human sera are incubated on the tissue sections. The primary test reaction involves circulating antibodies present in the patient's serum, which attach to their homologous antigens. This occurs during the incubation period while the serum covers the antigen surface.

ANA bind to nuclear antigens and are not organ or species specific.

MA bind to the inner lipoprotein membrane and cristae of mitochondria. These antibodies are not organ or tissue specific and may be found in many different tissues which are abundant in mitochondria such as the proximal and distal tubules of the rat kidney. MA are primarily IgG class but may also include IgA and IgM classes (9).

SMA associated with CAH bind to actin in smooth muscle cells. They are not organ or species specific and are found in tissues with smooth muscle areas. They are primarily the IgG class of immunoglobulin but may also occur as IgM.

PCA are organ specific and bind to intercytoplasmic components of the parietal call. **MA** will also react with parietal cells, resembling PCA fluorescence. To differentiate, a true PCA will not show renal tubular fluorescence on rat kidney while a MA will react with both kidney tubules and parietal cells (10).

LKM-1 are organ specific and bind to the liver hepatocytes and the proximal renal tubules although the distal tubules are negative. To differentiate, LKM-1 will react not with the parietal cells of the stomach while a MA will react with both kidney tubules and parietal cells.

A rinsing period is followed by a secondary reaction. The reagent used in the secondary reaction is a fluorescein labelled anti-human globulin conjugate. The antigen surface is then thoroughly rinsed free of unbound conjugate and viewed under an appropriate fluorescent microscope to visually identify various morphological patterns of nuclear fluorescence. With a positive reaction, the nuclear pattern appears apple-green when viewed under a fluorescent microscope, whilst a negative reaction appears black or greenish-black.

WARNINGS AND PRECAUTIONS

1. All human components in the controls have been screened and found to be negative or non-reactive for STS, HBsAg and antibodies to HIV-1, HIV-2 and for HCV, HCV Ab, RPR by an FDA registered laboratory. However, these tests cannot guarantee the absence of infectious agents. All human components should be handled with appropriate care.
2. The controls included in the kit contain either 0.1% sodium azide or 0.01% thiomersal as a preservative. 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 expiry 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	I: Rat or mouse kidney/stomach slides II: Rat liver / kidney / stomach slides or Mouse liver / kidney / stomach slides or Rat liver / Rat kidney / Mouse stomach 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 +	ANA homogenous antibody Positive Control no: C001N/C001N-0.5 OR Liver Kidney Microsomal Positive Control no: C049CE
CONTROL +	Mitochondrial antibody Positive Control no: C004N/C004N-0.5
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.

Autoscreen I/II Test System

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.

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. Remove slide from PBS and carefully wipe the underneath and around the wells 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. 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.