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AUTOIMMUNE ANTIBODY IFA SCREENING TEST SYSTEM

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INTENDED USE

The detection, differentiation and quantitation of circulating autoimmune antibodies in human serum utilizing indirect immunofluorescence (IFA***). For In Vitro Diagnostic Use.***

SUMMARY AND PRINCIPLES

Autoimmune disorders can be Non-organ Specific, Tissue or Organ Specific or Overlapping assiciated with both organ specific and non organ specific antibodies. The five most common autoimmune antibodies include:

1. Antinuclear antibodies (ANA)
2. Antimitochondrial antibody (MA)
3. Antismooth muscle antibodies (SMA)
4. Antiparietal cell antibodies (PCA)
5. Antireticulin antibody (RA)

ANTINUCLEAR ANTIBODIES

Tests for antinuclear antibody (ANA) 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. (1,2,3) The highest titers of ANA are found in active SLE and the presence of these antibodies is the second most common manifestation of SLE. (4) Immunofluorescence is the test of choice for screening the presence of ANA since it detects 95-100% of the active SLE cases. The presence of ANA has been well documented in different disease states as well as in healthy relatives of SLE patients. (5,6) The incidence of positive ANA varies with each disease (see Table 1).

TABLE 1
INCIDENCE OF ANA IN VARIOUS DISORDERS (5,6)

DISORDER	%INCIDENCE
Systemic lupus erythematosus	95-100
Lupoid hepatitis	95-100
Progressive systemic sclerosis (scleroderma)	75-80
Rheumatoid arthritis	25-60
Juvenile arthritis	15-30
Felty's syndrome	95-100
Sjogren's syndrome	40-75
Chronic discoid lupus	15-50
Dermatomyositis, polymyositis	10-30
Polyarteritis nodose	15-25
Rheumatic fever	5
Drug-associated SLE-like syndrome *(hydralazine: procainamide: isoniazid)*	50
Miscellaneous diseases	10-50
Generally normal values	5
Normal old age	40
Healthy relative of SLE patient	25

The "LE Prep" test is less sensitive than the immunofluorescence test as it will detect only 50-75& of active SLE cases. (5,7,8) The "LE Prep" is positive only when antibodies are present and will not react with all of the nuclear antibodies. The presence of "LE Cells" is not diagnostic of SLE and may be detected in a variety of other autoimmune diseases. (9)

The selected substrate used in immunofluorescence influences the identification and the titer of ANA. The use of rat liver nuclei, a commonly used substrate, reveals significantly higher ANA titers when compared to using human leucocytes which are subject to false positives due to the presence of leucocyte specific antigens. (4,10)

ANA antibodies are not organ or species specific.(6) The primary test reaction involves circulating antinuclear antibodies present in the patients serum which attach to their homologous nuclear 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 reagent used in the secondary reaction is a fluorescein labeled anti-human globulin conjugate. The antigen surface is then thoroughly rinsed free of unbound conjugate and viewed under an appropriate fluorescent microscope for various morphological patterns of nuclear fluorescence which can be visually identified. (10)

The clinical significance of the various nuclear immunofluorescent patterns is useful in evaluating patients for the presence of the connective tissue diseases. The homogenous pattern is the most common pattern and is associated with SLE. The peripheral pattern confirms a clinical diagnosis of SLE. True speckled nuclear fluorescence is seen in Scleroderma, Raynaud's disease, Rheumatoid Arthritis, and Sjogren's syndrome. Nucleolar fluorescence is seen mainly in Scleroderma and Sjogren's syndrome. (see Table 2)

TABLE 2

NUCLEAR PATTERN	NUCLEAR ANTIGEN
Homogenous (diffuse)	DNP
Speckled	Sm, RNP
Nucleolar	RNA
Peripheral (shaggy)	DNA

KNOWN PHARMACEUTICAL INTERACTIONS: *ANA*

Various drugs have been reported to induce or activate SLE. Patients being treated with these drugs often demonstrate varying levels of ANA in their serum. (11) (see Table 3)

TABLE 3
SLE INDUCING AGENTS

GROUP I Induced by Pharmacological Action	GROUP II Induced by Allergic Action
Hydralazine	Aminosalysilic Acid
Procainamide	Chlorthalidone
Anticonvulsants:	D-Penicillamine
Mephenytoin	Griseofulivin
Phenytoin	Isoquinazepon
Primidone	Levodope
Trimethadione	Methyldopa
Ethosuximide	Methysergidie
Carbamazepine	Methylthiouracil
Pheneturide	Oral Contraceptives
Isoniazid	Pencilillin
Chlorpromazine	Phenyibutazone
	Practolol
	Propylthiouracil
	Quinidine
	Reserpine
	Streptomycin
	Sulfonamides
	Tetracycline
	Tolezamide
	Oxyphenistan

MITOCHONDRIAL

The utilization of the indirect immunofluorescent test for the detection of circulating autoantibodies 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). (1) Tests for the detection of mitochondrial antibodies (MA) are recommended as an alternative to surgical exploration as the presence of high titer MA can provide confirmatory evidence for the diagnosis of PBC. (2,3) Both CAH and PBC have many overlapping immunologic features and may represent a continuum of a single disease entity. (4) MA titers in PBC do not appear to have any correlation with clinical activity since they do not vary with the severity or progression of the disease and cannot serve as a monitor of response to therapy or provide prognostic information. (5)

MA are present in sera of patients with a variety of liver disorders but are only present in high titer in the majority of patients with PBC. Recent studies have demonstrated that MA titers greater than 40 are found only in patients with PBC. (6) (See table 4)

TABLE 4
INCIDENCE OF MITOCHONDRIAL ANTIBODIES IN VARIOUS DISORDERS

DISORDER	INCIDENCE
Primary Biliary Cirrhosis	>90%
Chronic Active Hepatitis (HbsAg-Negative)	>50%
Chronic Active Hepatitis (HbsAg-Positive)	>60%
Cryptogenic Cirrhosis	>30%
Alcoholic Cirrhosis	30%
Chronic Persistant Hepatitis	<20%
Hemochromotosis	>50%
Cholangitis	23%
Hepatic Metastases	6%
Endocrine Disorders of Collangenoses	3-26%
Extra Hepatic Obstruction	<2%

SUMMARY: MITOCHONDRIAL (Continued)

The detection of MA by the indirect immunofluorescent technique is most useful in the differential diagnosis of extrahepatic obstruction in which only less than 2% of these patients possess this antibody and only at low titer. The utilization of frozen sections of rat kidney, as contained in this kit, has been the recommended substrate for IFA.

The MA reaction involves circulating antibodies that bind to the inner lipoprotein membrane and cristae of mitochondria. (7) These antibodies are not organ or tissue specific and may be found in many different tissues which are abundant in mitochondria. (8) Mitochondrial rich cells line the proximal and distal tubules of the rat kidney which is used as the test substrate in indirect immunofluorescent procedures. Ma are primarily of the IgG class but may also include IgA and IgM.(9)

Since MA will react with kidney tubules, thyroid epithelial cells and stomach parietal cells, a custom slide consisting of either rat kidney and monkey thyroid or rat kidney and rat stomach facilitates this type of immediate differentiation in one well.

The primary test reaction involves circulating mitochondrial antibodies present in the patient's serum which attach to their homologous mitochondrial antigens. This occurs during the incubation period while the serum covers the antigen surface. A secondary reaction then follows a rising period which removes the unbound human antibody. 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. Bright grandular cytoplasmic fluorescence of the renal tubules indicates a positive result. Fluorescence of the cellular antigens such as nuclei, smooth muscle, connective tissue or a nongrandular fluorescence limited to the central portion of the proximal tubules should not be reported as positive MA.

SMA

Smooth muscle antibodies (SMA) can be demonstrated in patients with acute and chronic hepatitis, the highest titers occurring in chronic active hepatitis (CAH).(1) 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.(2) The differential diagnosis of CAH in patients which chronic liver disease is facilitated by titration of SMA using the indirect immunofluorescence method with rat stomach muscularis mucosae, the most commonly recommended substrate employed in the detection of SMA. (3)

There exist various forms of acute and chronic liver injury that are directly or indirectly related to hepatitis B (HB) infection. (4,5,6) Both viral and auto antibody 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. (7,8) Antinuclear antibodies (ANA), SMA and AMA autoantibodies occur in CAH and form the basis of distinguishing different groups of autoimmune hepatitis. CAH patients which are ANA and SMA positive have high titers of these autoantibodies which are readily demonstrable by immunofluorescent techniques. (9)

SMA tests have been found helpful in confirming the diagnosis of approximately 70% of the cases 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 incidences of SMA have also been reported in serum of patients with infectious 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 erythematosis and in the normal population.

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 immunoglobulins but may also occur as IgM. Frozen sections of rat 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 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 fluorescence microscope. Bright cytoplasmic fluorescence of the smooth muscle layers of the muscularis mucosae indicates a positive result. (10)

Recent research has shown that the antigen active in the SMA reaction is actin. (11) Actin is found in such histological structures as: the capillary linings, platelets, brush borders of renal tubular epithelium and in the renal glomerular cells. SMA are non organ specific and will react with smooth muscle surrounding arteries, veins and other histological structures containing actin. (12) The reactivity of SMA for 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 . (13) However, studies using cultured fibroblasts reaffirm the actin specificity of SMA from CAH patients. (14) Attempts at classifying SMA by different immunofluorescent patterns have not yet provided a clear clinical correlation between distinct diseases and a particular fluorescent pattern. (15) 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.

PCA

Gastric autoimmune diseases have been classified into Type A and Type B gastritis based on the morphological changes of the fundus and antral portion of the stomach. (1) Patients with antibodies to parietal cells (PCA), intrinsic factor, or both have atrophy of the fundal mucosa (Type A) and a very high rate of pernicious anemia often associated with other autoimmune endocrine disorders. (2) A positive PCA in the presence of a megaloblastic anemia makes pernicious anemia a probable diagnosis. (3) In Type B gastritis, PCA is lacking thus, there is no association with pernicious anemia or other autoimmune endocrine disorders.(4)

Indirect immunofluorescence employing the gastric mucosa of the rat stomach as the substrate is the test of choice for detecting PCA. It has been demonstrated to be more sensitive than the CF method.

The incidence of PCA in patients with pernicious anemia is 93%. Conditions other than pernicious anemia which may give positive PCA results include: atrophic gastritis, diabetes mellitus, Hashimoto's disease, gastric ulcer, thyrotoxicosis, myasthenia gravis, iron deficiency anemia, idiopathic Addison's disease, primary myxedema, Sjogrens syndrome and rheumatiod arthritis. In normal population, PCA varies from 2% in under 20 age group to 16% in the over 60 age group.

PCA should be included in a differential work-up of patients megaloblastic anemia since 93% of patients with pernicious anemia will be detected.

The PCA reaction involves circulating antibodies to intercytoplasmic components of the parietal cell. PCA is organ specific, but not species specific. However, antimitchondrial antibody (MA) is not organ specific and will react with parietal cell and resemble PCA fluorescence. Therefore, in order to differentiate a true PCA from a MA the specimen showing PCA fluorescence should be tested on rat kidney section. A true PCA will not show renal tubular fluorescence while a MA will react with both kidney tubules and parietal cells.

Recent studies have demonstrated a potential pitfall in the detection of PCA. Smooth muscle antibodies (SMA) from patients with chronic acute hepatitis (CAH) bind to gastric parietal cells in an immunofluorescent pattern indistinguishable from PCA. Therefore, in order to differentiate a true PCA from a SMA, the specimen showing PCA fluorescence should be checked for a positive staining in muscularis mucosa. A true PCA will not show the stomach muscularis muscosal fluorescence, but a SMA may react with both muscularis mucosare and parietal cells. (5)

PCA is primarily IgG but may occasionally be found in IgM immunoglobulin fractions.

The primary reaction involves circulating PCA antibodies present in the patient's serum which attach to their homologous parietal cell 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 reagent used in the secondary reaction is a fluorescein labelled anti-human conjugate. The antigen surface is then thoroughly rinsed free of unbound conjugate and viewed under an appropriate fluorescence microscope. Bright granular cytoplasmic fluorescence limited to the parietal cells of the rat stomach gastric mucosa indicates a positive result. Fluorescence of other cellular antigens such as nuclei, smooth muscle, connective tissue or chief cells should not be reported as positive PCA.

PRECAUTIONS

1. Each donor unit used in the preparation of this material was tested by an FDA approved method for the presence of the antibody to HIV as well as for HBsAg and found to be negative (were not repeatedly reactive). WARNING - POTENTIAL BIOHAZARDOUS MATERIAL Because no test method can offer complete assurance that human immunodeficiency virus (HIV), hepatitis B virus, or other infectious agents are absent, these human control reagents should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health Manual (*Biosafety in Microbiological and Biomedical Laboratories*, 4th Edition, U.S. Dept. of Health and Human Services, Public Health Service 1999).
2. All reagents must be brought to 20 to 25°C before performing the test procedure.
3. Always wear suitable protective clothing, gloves and eye/face protection when working with this product.
4. The phosphate buffered saline and mounting medium found in this kit are irritating to the eyes, respiratory system and skin.
5. Some components in this kit contain 0.1% Proclin 300. At full strength Proclin 300 is corrosive and will cause burns and possibly sensitization by skin contact.
6. The conjugate in this kit contains 0.015% Evan's Blue. Evan's Blue is a possible carcinogen and may cause reproductive harm.
7. Some components in this kit contain 0.02% Thimerosal. Thimerosal is toxic by inhalation, in contact with skin, and if swallowed, and is a reproductive hazard.
8. Some components in this kit contain sodium azide at a concentration of less than 0.1 %. Sodium azide is toxic if ingested and forms
   potentially explosive copper and lead azide compounds in waste plumbing lines. Should the reagents come in contact with copper or lead waste plumbing, flush the waste line with large quantities of water to prevent the formation of potentially explosive compounds.
9. Do not use components beyond their expiration date.
10. Follow the procedural instructions exactly as they appear in this insert to insure valid results.
11. For vitro diagnostic use.
12. Handle slides by the edges since direct pressure on the antigen wells may damage the antigen.
13. Once the procedure has been started do not allow antigen in the wells to dry out. This may result in false negative test results, or unnecessary artifacts.

The safety data sheet is available upon request.

WARNING

Some components of this kit contain 0.1% ProClin 300®, a biocidal preservative that may cause sensitization by skin contact; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals.

H317: May cause an allergic skin reaction.
H335: May cause respiratory irritation.
P280: Wear protective gloves / protective clothing / eye protection / face protection.
P302 + P352: IF ON SKIN: Wash with plenty of soap and water.
P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention.
P501: Dispose of contents and container in accordance to local, regional, national and international regulations.

WARNING

Some components of this kit contain < 0.1% sodium azide.

H302: Harmful if swallowed.
P264: Wash thoroughly with plenty of soap and water after handling.
P270: Do not eat, drink or smoke when using this product.
P301+P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.
P330: If swallowed, rinse mouth.
P501: Dispose of contents/container to in accordance to local, regional, national and international regulations.

MATERIALS PROVIDED

Prod#	Description	Quantity
10-2308	ANA Rat Stomach/Kidney 8 well Slide	12 ea
10-1201	Autoimmune Negative Control	0.5mL
10-1202	ANA (4+) Homogenous Positive Control	0.5mL
10-2202	Mitochondiral Positive Control	0.5mL
10-3202	Smooth Muscle Positive Control	0.5mL
10-1501	FITC IgG Conj. Rodent Ads. w/ Evans' Blue	4.0mL
90-1607	Phosphate Buffered Saline (pH 7.5)	2x10 g
90-1610	FITC Mounting Medium (pH 7.5)	3.0mL
90-1700	Coverslips, 70 x 22mm	12 ea
90-1708	Blotters, 8 well	12 ea

Note: Only reconstitution required is for 1 x 10g PBS Buffer (90-1607) with 1 Liter of DI Water.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

1. Test tubes, test tube rack, pipettes.
2. Volumetric flask (1000mL)
3. Staining dish.
4. Epifluorescence microscope
5. Microscope Slide Roller

STORAGE AND STABILITY

1. Antigen slides Prod# 10-2308 should be stored at +2 to +8°C or lower. Slides are stable until their expiration date on the product label.
2. Positive controls Prod# 10-1202, 10-2202 and 10-3202 should be stored at +2 to +8°C. Refer to expiration date on label.
3. Universal negative control Prod# 10-1201 should be stored at +2 to +8°C. Refer to expiration date on label.
4. FITC labeled anti-human conjugate Prod# 10-1501 should be stored at +2 to +8°C. Refer to expiration date on label.
5. Mounting Media Prod# 90-1610 should be stored at +2 to +8°C. Refer to the expiration date on label.
6. Phosphate buffered saline buffer salts pH 7.5 Prod# 90-1607 are stable at room temperature. Reconstitute each vial of PBS buffer salts with 1.0L of distilled water. The PBS contains no preservative and should be stored at +2 to +8°C. Discard if turbidity develops.

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 to 8°C if it is to be analyzed within 4-7 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, additions of a preservative such as 0.01% thimerosal (merthiolate) or 0.1% sodium azide is strongly recommended.

TEST PROCEDURE

Dilute test serums 1:20 in PBS if testing is being performed for screening purposes. For titrations set up doubling dilutions of serum starting at 1:20, (i.e. 1:20, 1:40, 1:80, 1:160, 1:320,etc). The slide, controls and conjugate are ready to use.

1. Tear 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 (15 to 20µL) over the antigen wells.
3. Place slide with patient's serum and controls in a moist chamber for 30 minutes at room temperature. (approximately 20°C)
4. Remove slide from moisture chamber. Using a wash bottle, gently rinse remaining sera from slides being careful not to aim the stream directly on the well.
5. Wash in PBS for two separate five minute changes.
6. Remove the slides from PBS and place slide antigen side facing up on a dry paper towel. Carefully place the blotter over the slide so that the blotter is indexed to the surface of the microscope slide. Hold one edge of the blotter with one hand to keep the blotter in place and apply sufficient gentle pressure with the microscope slide roller to remove the moisture between the antigen wells. DO NOT ALLOW THE ANTIGEN WELLS TO DRY.
7. Using dispenser provided*, deliver 1 drop (25µL) of conjugate per antigen well. Repeat steps 3-6.
8. Place 4-5 drops of mounting medium on slide.
9. Apply a 22 x 70mm cover glass. Examine the slide under a fluorescent microscope. Note: To maintain fluorescence, store mounted slide in a humid chamber placed in a dark refrigerator.

* The conjugate dispenser is provided with a calibrated tip and allows quantitative delivery of reagents from the storage bottle. To use, wipe the tip with a paper towel, invert the bottle and squeeze gently to release one drop. If the tip contains an air bubble, tap the bottle gently to remove air bubble which will ensure precise drop delivery.

TITER INTERPRETATION: *ANA*

A positive result is 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 at magnification of 400x.

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

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

1:20 or less.	Normal; virtually rules out active SLE provided patient is not on immunosupressive 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 two weeks. If the titer increases active SLE is suggested. No change in titer indicates possible other autoimmune disease in a static condition or a controlled SLE patient. A decrease in titer indicates an SLE case in remission, treated controlled SLE or another autoimmune process.
1:160 or greater.	Strongly suggests SLE although other autoimmune diseases and drugs may induce these high titers.

TITER INTERPRETATION: *MA*

Primary Biliary Cirrhosis (PBC) is a chronic intrahepatic cholestasis found more frequently in woman than in men with an incidence which is the highest in the 30 -60 year age group. The diagnosis of PBC is based upon clinical observations, histologic findings on the liver biopsy, increased alkaline phosphates activity, elevated IgM levels, and the presence of mitochondria antibodies.

A positive result is observed as granular fluorescence in the cytoplasm of the renal tubules, limited to the cytoplasm of the proximal and distal tubular epithelium. Fluorescence limited to the central (lumen) portion of the proximal tubules should not be reported as positive MA.

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

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

The titer range in PBC is from 1:10 to 1:6,000 with about 50% of PBC patients having titers between 1:200 to 1:6,000. MA titers do not appear to change with time of therapy and cannot serve as monitors of response to therapy.

TITER INTERPRETATION: *SMA*

ACH is a chronic disease of the liver mainly affecting young females but has also been reported as affecting both sexes and all ages. It is characterized in liver biopsies by 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 diffuse cytoplasmic staining of the smooth muscle layers of the muscularis mucosae found in the rat 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.

1:10 or less	Normal, negative
1:20 to 1:80	Positive. Suggestive of liver disease. Repeat with a fresh specimen in two weeks.
1:160 or greater	Suggestive of active chronic hepatitis.

The titer in ACH may reach 1:640. However, they generally range from 1:80 to 1:320 and persist for years. In viral hepatitis the titers are generally below 1:80 and are transient. The titers in PBC are also low, ranging from 1:10 to 1:40

TITER INTERPRETATION: *PCA*

Pernicious anemia is a megaloblastic anemia. A positive PCA test from a patient with a megaloblastic anemia helps establish a presumptive diagnosis of pernicious anemia or pernicious anemia associated with a second disease. Additional confirming tests for pernicious anemia are: antibodies to intrinsic factor, vitamin B12 absorption or serum vitamin B12 activity. A key factor in differentiating between pernicious anemia and simple atrophic gastritis is the lack of antibody to intrinsic factor in atrophic gastritis.

On the basis of PCA alone one may assume some form of atrophic gastritis which may or may not be related to pernicious anemia. PCA are generally associated with some degree of hypochlohydria.

In addition to its diagnostic potential PCA testing is helpful in screening genetically determined high risk groups (ie. relatives of thyroid patients and pernicious anemia patients) for asymptomatic chronic atrophic gastritis and for early recognition of atrophic gastritis and pernicious anemia.

A positive result is observed as bright granular cytoplasmic fluorescence of parietal cells of the rat gastric mucosa. Fluorescence of other cellular antigens such as nuclei, smooth muscle, or connective tissue should not be reported as positive PCA.

TITER INTERPRETATION: *PCA (*continued)

The titer is the highest dilution of the patient's serum showing weak 1+ fluorescence of the parietal cell.

"The clinical significance of the PCA titer has no relation to the severity or duration of the disease state. Thus, one cannot predict or assume on the basis of PCA titer alone the degree of impaired secretion of intrinsic factor or the extent of histopathologic changes." (Immunofluorescence detection of autoimmune disease. Immunology Series No.7, U.S.D.H.E.W.CDC.1976.p66).

PATTERN INTERPRETATION: *ANA*

The nuclear immunofluorescent patterns found in SLE can be of prognostic significance. Peripheral-Confirms clinical diagnosis of SLE. Renal involvement, confirmed by anti-DNA tests, is associated with an intermediate prognosis. Homogenous-High titer anti-DNA antibodies suggest SLE with probable renal involvement and is associated with an intermediate prognosis. Large and Small Speckles-Seen in very benign SLE and associated with a good prognosis. Nucleolar-High titers are associated with Sjogren's syndrome and Scleroderma.

LIMITATIONS OF PROCEDURE: *ANA*

1. No diagnosis should be based upon a single ANA test result, since various host factors must be take into consideration.
2. Among these host factors are sex and age. There is an increase in positive ANA results in both males and females as age increases (10). Normal females between 20-60 have 7% incidence of ANA; normal males, a 3% incidence. Normal males and females over 80 years of age have a 50% incidence of ANA.
3. Various medications including antibiotics, tranquilizers, aspirin and birth control pills can induce a Lupus-like condition resulting in high ANA titers (11). (See table 3). Drug- induced Lupus generally goes into sustained clinical remission following removal of the triggering medication.
4. Various autoimmune processes induce positive ANA tests.
5. Further evidence for a diagnosis of SLE is provided by low complement levels, particularly C1Q, C3 and C4. (12).
6. ANA tests may not agree with LE Prep tests or with latex tests.
7. Presence of antibodies to double-stranded native DNA is diagnostic for SLE.
8. Management of therapy should be based not only on positive serologic tests for SLE, but should include the presence of active clinical disease. (13).
9. Elderly patients with SLE have a better prognosis and their clinical symptoms differ substantially from those seen in younger patients. (14).
10. Although the predominant class of antinuclear antibodies (ANA) is immunoglobulin G, the presence of immunoglobulin E may be a pathogenic importance in SLE (15).

LIMITATIONS OF PROCEDURE: *MA*

1. Clinical manifestations, histologic findings on liver biopsies, elelvation of IgM and increased alkaline phosphatase values should be considered in the final diagnosis of PBC.
2. Liver and kidney miscrosomal antibodies preferentially stain proximal tubules whereas mitochondrial antibodies react with distal tubules more strongly.(11)
3. A normal serum IgM is strong evidence against the diagnosis of PBC as increased concentration of this immunoglobulin is the dominant abnormality in this disease.
4. Anti-smooth muscle antibody can be detected in 30-50% and antinuclear antibody in 25-46% of patients with PBC. (15)

LIMITATIONS OF PROCEDURE: *SMA*

1. SMA should be used as an aid in the diagnosis of liver disease.
2. Clinical manifestations such as liver biopsies and liver function tests should be considered in the final diagnosis of chronic active hepatitis.
3. SMA can be found in: Primary biliary cirrhosis (PBC), cryptogenic cirrhosis, infectious mononucleosis, asthma, yellow fever, acute infectious hepatitis, carcinoma of the breast, malignant melanoma and ovarian carcinoma.
4. Titers of some cases of acute 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. (2)
5. SMA represents a family of antibodies directed against contractile proteins present in different tissues.(7) The non homogenous glomerular pattern has never been found in cirrhotic patients and this pattern is always associated with high SMA titers in CAH.
6. 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.
7. Drug induced CAH is rather rare; however, oxypenisatin and methyl dopa have been associated with some cases of CAH.
8. Antibodies to native double-stranded DNA, initially considered specific for Systemic Lupus Erythmatosus (SLE), are found in a variety of liver diseases, including CAH and cirrhosis. (14)

LIMITATIONS OF PROCEDURE: *PCA*

1. Additional confirming tests for pernicious anemia are: antibodies to intrinsic factor, vitamin B12 absorption or serum vitamin B12 activity.
2. PCA should be used as a diagnostic aid in establishing pernicious anemia as the cause of megaloblastic anemia.
3. PCA can be found in 16% of apparently normal individuals over the 60 year age group.
4. Conditions other than pernicious anemia may give positive PCA results.
5. The presence of intrinsic factor autoantibodies is considered to be diagnostic for pernicious anemia and for rare cases of endocrine disorders associated with gastric atrophy. (6)
6. Patients with dermatitis herpetiformis can have PCA without any evidence of malabsorption of B12.

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 of the nuclei. If this control shows bright fluorescence either the control or the antigen may be at fault.
3. The positive serum control should result in 3+ to 4+ fluorescence of the type specified on the label. If this control shows little or no fluorescence either the control, antigen, conjugate or technique may be a 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 the antigen may be at fault.

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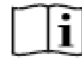

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Temperature limitation

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WARNING

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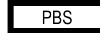

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