

# ALPHADIA IFA Autoscreen I IgG ALPHADIA IFA Autoscreen II IgG

## Autoscreen I

RAT STOMACH - KIDNEY

AD AS148 48 tests

AD AS196 96 tests

MOUSE STOMACH - KIDNEY

AD AS348 48 tests

AD AS396 96 tests

## Autoscreen II

RAT STOMACH - KIDNEY - LIVER

AD AS248 48 tests

AD AS296 96 tests

MOUSE STOMACH - KIDNEY - LIVER

AD AS448 48 tests

AD AS496 96 tests

FOR IN VITRO DIAGNOSTIC USE

CONS : 2 - 8°C

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## INTRODUCTION

The utilization of ALPHADIA fluorescent autoimmune antibody screening test will simultaneously detect circulating antinuclear (ANA), mitochondrial (MA), parietal cell (PCA), smooth muscle (SMA) and reticulin antibodies in an indirect immunofluorescent test system. All necessary tissue substrates are contained in each slide well of this system to perform the above antibody screening.

## PRINCIPLES

**Antinuclear (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 titers of ANA are found in active SLE. The presence of these antibodies is the second most common manifestations of SLE. Immunofluorescence is the test of choice for screening for the presence of ANA since it detects 95-100% of active SLE cases. The presence of ANA has been well documented in different disease states as well as in healthy relatives of SLE patients. The incidence of positive ANA varies with each disease. Rat or mouse kidney/liver is utilized for ANA detection in this test system.

ANA, MA and SMA antibodies are not organ or species specific. 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. 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 fluorescent microscope for various morphological patterns of nuclear fluorescence which can be visually identified.

The clinical significance of the various nuclear immunofluorescent patterns is useful in evaluating patients for the presence of one of the connective tissue diseases. The homogeneous 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. Nuclear fluorescence is seen mainly in scleroderma and Sjogren's syndrome.

Various drugs have been reported to include or activate SLE. Patients on these drugs often demonstrate varying levels of ANA in their serum.

**Mitochondrial antibody (MA)**, 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). 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. Both CAH and PBC have many overlapping immunologic features and may represent a continuum of a single disease entity. 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.

MA is 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 1:40 are found only in patients with PBC.

The detection of MA by the indirect immunofluorescent technique is most useful in the differential diagnosis of extrahepatic obstruction in which less than 2% of these patients possess this antibody and only at low titer. Rat or mouse kidney is utilized for MA detection in this system.

The MA reaction involves circulating antibodies that 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. Mitochondrial rich cells line the proximal and distal tubules of the rat or mouse kidney which is used as the test substrate in indirect immunofluorescent procedures. MA are primarily IgG class but may also include IgA and IgM.

Since MA will react with kidney tubules, thyroid epithelial cells, and stomach parietal cells, ALPHADIA offers two (kidney/stomach) and three (kidney/stomach/thyroid) sections per well to help differentiate organ specific antibodies.

A rat kidney and monkey thyroid slide facilitates this type of immediate differentiation in one well.

Bright granular cytoplasmic fluorescence of renal tubules indicates a positive result. Fluorescence of other cellular antigens such as nuclei, smooth muscle, connective tissue or a non-granular fluorescence limited to the central portion of the proximal tubules should not be reported as positive MA.

**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 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. Antinuclear antibodies (ANA), SMA and MA auto antibodies 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 demonstrated by immunofluorescent techniques.

SMA tests have been found helpful in confirming the diagnosis of approximately 70% of these 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 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 utilized for SMA detection in this test system.

The SMA reaction involves circulating antibodies to a normal component of the smooth muscle cell. These antibodies are not organ or species and may be found in tissues with smooth muscle areas. They are primarily of the IgG class of immunoglobulins but may occur as IgM.

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 immunofluorescence 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.

**Parietal cell antibody (PCA):** Gastric autoimmune disease have been classified into type A and type B gastritis based on the morphological changes of the fundus antral portion of the stomach. Patients with antibodies to parietal cells (PCA) or 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. A positive PCA in the presence of a megaloblastic anemia makes pernicious a probable

diagnosis. In type B gastritis, PCA is lacking and there is no association with pernicious anemia or other autoimmune endocrine disorders.

The indirect immunofluorescent method is the test of choice for detecting PCA and it is more sensitive than the CF method. The gastric mucosa of rat or mouse stomach is utilized for PCA detection in this test system.

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

PCA should be included in a differential work-up of patients with 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, anti-mitochondrial antibody (MA) is not organ specific and will react with parietal cells and resemble PCS fluorescence. Therefore, in order to differentiate a true PCA from a MA the specimen showing PCA, fluorescence should be tested on rat or mouse 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 the muscularis mucosa. A true PCA will not show the stomach muscularis mucosal fluorescence, but a SMA may react with both muscularis mucosae and parietal cells.

In order to facilitate this type of differentiation ALPHADIA offers custom component slides

containing two and three sections per well. Slides are available containing three sections per well, i.e. monkey thyroid/rat stomach/rat kidney which allows for immediate differentiation of thyroid reactions as well as PCA from non-organ specific MA reactions in one well.

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

## MATERIALS PROVIDED

### Storage and stability of components

1. FITC Conjugate N°CGER2 - 3 ml or CGER5 - 5ml 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. The conjugate may be aliquoted into small amounts and frozen once for extended storage. This reagent contains antibodies which will react with the human IgG, IgM and IgA immunoglobulin classes. Ready for use.

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

3. ANA (homogeneous), MA and SMA positive controls - 1 ml should be stored at 2-8°C upon receipt. Check label for specific expiration date. The control may be aliquoted into small amounts and frozen once for extended storage. Ready for use.

4. Universal negative control N°ADNC - 1 ml should be stored at 2-8°C upon receipt. Check label for specific expiration date. The control may be aliquoted into small amounts and frozen once for extended storage. Ready for use.

5. Buffer pack N° PBS1 - 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 N° TMM3 - 3 ml is stable when stored at 2-8°C. Check label for specific expiration date. Ready for use.

Note : All kit components are available separately.

#### Additional Materials required but not provided

Test tubes and rack or microtiter system  
Disposable pipettes  
Staining dish and slide forceps  
Moisture chamber  
Distilled water  
Fluorescence microscope  
Paper towels

#### REAGENT PREPARATION

##### Buffer pack :

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 in PBS

**Titration** : set up doubling dilutions of serum starting at 1:20, 1:40, 1:80, 1:160 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 control 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 (25-30µl) 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 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

**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.

1. Homogeneous (diffuse) - An even, finely diffused fluorescence of the entire nucleus is seen.
2. Peripheral (Rim, shaggy) - The nuclear membrane is more intensely fluorescent than the central area.
3. Speckled - 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.

#### **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.

*Homogeneous* - High titer anti-DNA antibodies suggest SLE with probable renal involvement and is associated with an intermediate prognosis.

*Speckled* - Large and small speckles seen in benign SLE and associated with good prognosis.

*Nucleolar* - High titers are associated with Sjogren's Syndrome and Scleroderma.

#### Titer Interpretation

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 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 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 treated and controlled SLE case or another autoimmune process.

*1:160 or greater* - Strongly suggests SLE although other autoimmune diseases and drugs may induce these high titers.

**MA** : Primary Biliary Cirrhosis (PBC) is a chronic intrahepatic cholestasis found more frequently in women than in men with an incidence which is highest in the 30-60 year age group. The diagnosis of PBC is based upon clinical observations, histologic findings on liver biopsy, increased alkaline phosphatase 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. The fluorescence is limited to the cytoplasm of the proximal and distal tubular epithelium. Fluorescence of other cellular antigens such as nuclei, smooth muscle, or nongranular fluorescence limited to the central (lumen) portion of the proximal tubules should not be reported as positive MA.

#### Titer Interpretation

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 PBC is from 1:10 to 1:6000 with about 50% of PBC patients having titers between 1:2000 to 1:6000. MA titers do not appear to change with time or therapy and cannot serve as monitors of response to therapy.

**SMA** : 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.

#### Titer Interpretation

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 - 1:80* - Positive. Suggestive of liver disease. Repeat with 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.

**PCA** : Pernicious anemia is a megaloblastic anemia. A positive 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 not be related to pernicious anemia. PCA are generally associated with some degree of hypochlorhydria.

In addition to its diagnostic potential PCA testing is helpful in screening genetically determined high risk group (i.e. 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 or mouse gastric mucosa. Fluorescence of other cellular antigens such as nuclei, smooth muscle or connective tissue should not be reported as positive PCA.

#### Titer Interpretation

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 and 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 ».

#### LIMITATIONS OF PROCEDURE

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

#### **ANA**

1. Among these host factors are age and sex. There is an increasing incidence in positive ANA results in both males and females as age increases. Normal females between 20-60% have a 7% incidence of ANA; normal males, a 3% incidence. Normal males and females over 80 years of age have a 50% incidence of ANA.

2. Various autoimmune processes induce positive ANA tests.

3. Further evidence for a diagnosis of SLE is provided by low complement levels, particularly C1, C3 and C4.

4. ANA tests may not agree with LE Prep tests or with latex tests.

5. Presence of antibodies to double stranded native DNA is diagnostic for SLE.

6. Management of therapy should be based not only on positive serologic tests for SLE, but should include the presence of active clinical disease.

7. Elderly patients with SLE have a better prognosis and their clinical symptoms differ substantially from those seen in younger patients.

8. Although the predominant class of antinuclear antibodies (ANA) is immunoglobulin G, the presence of immunoglobulin E may be of pathogenic importance in SLE.

#### **MA**

1. Clinical manifestations, histologic finding on liver biopsies, elevation of IgM and increased alkaline phosphatase values should all be considered in the final diagnosis of PBC.

2. Liver and kidney microsomal antibody stains proximal tubules preferentially, whereas MA reacts with distal tubules more strongly than with proximal tubules.
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.

### **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, cryptogenic cirrhosis, infective mononucleosis, asthma, yellow fever, acute infective hepatitis, carcinoma of the breast, malignant and ovarian carcinoma.
4. Titers of some acute cases of viral hepatitis 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.
5. SMA represents a family of antibodies directed against contractile proteins present in different tissues. The non-homogeneous 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 but the drugs oxyphenisatin and methyl dopa have been associated with some cases of CAH.
8. Antibodies to native double-stranded DNA, initially considered specific for Systemic Lupus Erythematosus, are found in a variety of liver diseases, including CAH and cirrhosis.

### **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. Patients with dermatitis herpetiformis can have PCA without any evidence of malabsorption of B12.

### **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 thimerosal 0.01% 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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