



## AUTOSCREEN I/AUTOSCREEN II AUTOIMMUNE SCREENING TEST SYSTEM

For *In Vitro* Diagnostic Use

AD AS148 (Rat)	48 Tests	AD AS196 (Rat)	96 Tests
AD AS348 (Mouse)	48 Tests	AD AS396 (Mouse)	96 Tests
AD AS248 (Rat)	48 Tests	AD AS296 (Rat)	96 Tests
AD AS448 (Mouse)	48 Tests	AD AS496 (Mouse)	96 Tests

### Introduction:

The utilization of ALPHADIA's Fluorescent Autoimmune Antibody Screening Test will simultaneously detect circulating antinuclear (ANA), mitochondrial (MA), parietal cell (PCA), smooth muscle (SMA) and reticulin autoantibodies 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<sup>3</sup> 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.<sup>5</sup> 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.<sup>4,6,7</sup>

ANA, MA & 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.<sup>1,2</sup> Various drugs have been reported to include or activate SLE. Patients on these drugs often demonstrate varying levels of ANA in their serum.<sup>8</sup>

**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.<sup>13</sup> 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.<sup>19, 20</sup>

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.<sup>16, 21, 24</sup>

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.<sup>17, 18</sup>

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 facilitates this type of immediate differentiation in one well.<sup>25</sup>

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.<sup>15</sup>

**Smooth muscle antibodies (SMA)** can be demonstrated in patients with acute and chronic hepatitis; the highest titers occurring in chronic active hepatitis (CAH). All of the various forms of chronic liver disease show SMA titers not higher than 1:160, except for CAH where titers up to 1:1280 are found.

The differential diagnosis of CAH in patients with chronic liver disease is facilitated by titration of SMA using the indirect immunofluorescence method with rat or mouse stomach muscularis mucosa as the substrate.

There exist various forms of acute and chronic liver injury that are directly or indirectly related to hepatitis B(HB) infection. Both viral and autoantibody markers may be used to classify the different sub-groups of CAH and it has been demonstrated that most HB-antigen negative patients are SMA positive. Antinuclear antibodies (ANA), SMA and MA 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 demonstrated by immunofluorescent techniques.<sup>31-33</sup>

SMA tests have been found helpful in confirming the diagnosis of approximately 70% of these cases of CAH.<sup>27</sup> A positive SMA test rules out Systemic Lupus Erythematosus, since the AD AS148 Printed in U.S.A Rev I 9/19/17

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.<sup>28</sup> 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.<sup>32</sup>

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 also 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 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.<sup>35, 37</sup>

**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 and 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 anemia a probable diagnosis. In Type B gastritis, PCA is lacking and there is no association with pernicious anemia or other autoimmune endocrine disorders.<sup>44</sup>

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.<sup>41</sup>

PCA should be included in a differential work-up of patients with megaloblastic anemia since

93% of patients with pernicious anemia will be detected.<sup>43</sup> 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 PCA 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.<sup>42, 43</sup>

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 mucosae fluorescence, but a SMA may react with both muscularis mucosae and parietal cells.<sup>45</sup>

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 & Stability of Components:

- FITC IgG Conjugate No. AD CGER2 (3.0 ml) or AD CGER5 (5.0 ml) with Evans Blue Counterstain is to be stored at 2-8°C upon receipt. The conjugate is stable at this temperature until expiration date on the vial label.
- The antigen slides of rat or mouse kidney/stomach (Cat #: AD AS148, AD AS196, AD 348, AD AS398) or liver/ kidney/ stomach (Cat #: AD AS248, AD AS 296, AD AS448, AD AS496) sections must be stored at 2-8°C upon receipt. Check label for specific expiration date.
- ANA (Hom) positive control No. AD PCHOM (1.0 ml), MA positive control No. AD PCMI (1.0 ml) and SMA positive control No. AD PCSM (1.0 ml) should be stored at 2-8°C upon receipt. Check label for specific expiration date.
- Universal negative control No. AD NC (1.0 ml) should be stored at 2-8°C upon receipt. Check label for specific expiration date.
- Buffer Pack No. AD PBS1 - Phosphate Buffered Saline is stable at room temperature storage. Check label for specific expiration date. The reconstituted Buffer does not contain preservatives and should be stored at 2-8°C. Care should be taken to avoid contamination.
- Mounting Medium No. AD TMM3 is stable when stored at 2-8°C. Check label for specific expiration date.  
Note: All kit components are available separately. Please see the current ALPHADIA Catalog for more details.

### Additional Materials Required but not Provided:

- Test tubes and rack or microtiter system
- Disposable pipettes
- Staining Dish and Slide Forceps
- Moisture Chamber
- Volumetric Flask (500 ml)
- Distilled H<sub>2</sub>O
- Fluorescence Microscope
- Paper Towels - lint free

### Reagent Preparation:

1. FITC Conjugate FITC anti-IgG H&L with Evans blue No. AD CGER2 (3.0 ml) or
2. AD CGER5 (5.0ml). Ready for Use.
3. ANA (Homo) Positive Control No. AD PCHOM (1.0 ml). Ready for Use.
4. MA Positive Control No. AD PCMI (1.0 ml). Ready for Use.
5. SMA Positive Control No. AD PCSM (1.0 ml). Ready for Use.
6. Universal Negative Control No. AD NC (1.0 ml). Ready for Use.
7. Buffer Pack No. AD PBS1. 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 0.1% 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 30 µl) and controls over the antigen wells.
  3. Place slide with patient's serum and controls in a moist chamber for 30 minutes at room temperature (approximately 24°C).
  4. Remove slide from moisture. Using a wash bottle, gently rinse remaining sera from slide being careful not to aim the rinse stream directly on to the well.
  5. Wash in PBS for five minutes. Repeat using fresh PBS.
  6. Place a blotter on the lab table with absorbent side up. Remove slide from PBS and invert so that tissue side faces absorbent side of blotter. Line up wells to blotter holes. Place slide on top of blotter. **Do not allow tissue to dry.** Wipe back of slide with dry lint free paper towel. Apply sufficient pressure to slide while wiping to absorb buffer.
  7. Deliver 1 drop (20-30 µl) of conjugate per antigen well. Repeat steps 3-6.
  8. Place 4-5 drops of mounting medium on slide.
  9. Apply a 22 x 70 mm coverslip. Examine the slide under a fluorescent microscope.
- Note: To maintain fluorescence, store mounted slide in a moisture chamber placed in a dark refrigerator.

### Quality Control:

1. Positive and negative serum controls must be included in each day's testing to confirm reproducibility, sensitivity and specificity of the test procedure.
2. The negative serum control should result in little (+) or no fluorescence. If this control shows bright fluorescence, either the control, antigen, conjugate or technique may be at fault.
3. The positive serum control should result in bright 3+ to 4+ fluorescence. If this control shows little or no fluorescence, either the control, antigen, conjugate or technique may be at fault.
4. In addition to positive and negative serum controls, a PBS control should be run to establish that the conjugate is free from nonspecific staining of the antigen substrate. If the antigen shows bright fluorescence in the PBS control repeat using fresh conjugate. If the antigen still fluoresces, either the conjugate or antigen may be at fault.

### Results:

**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 Sjögren's Syndrome and Scleroderma.

### Titer Interpretation

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

Less than 1:20	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.<sup>23</sup>

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 non-granular fluorescence limited to the central (lumen) portion of the proximal tubules should not be reported as positive MA.<sup>14</sup>

### Titer Interpretation

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

Less than 1:20	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 1:6000 with about 50% of PBC patients having titers between 1:2,000 to 1:6,000. 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.

Less than 1:20	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.<sup>45</sup>

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.<sup>42</sup>

In addition to its diagnostic potential PCA testing is helpful in screening genetically determined high risk groups (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.<sup>46</sup>

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." (Immunofluorescence Detection of Autoimmune Disease. Immunology Series No. 7, U.S.D.H.E.W. CDC. 1976. p66).

### Limitations of Procedure:

#### ANA:

1. No diagnosis should be based upon a single ANA test result, since various host factors must be taken into consideration.
2. 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.<sup>10</sup>
3. Various autoimmune processes induce positive ANA tests.
4. Further evidence for a diagnosis of SLE is provided by low complement levels, particularly C1, C3 and C4.<sup>9</sup>
5. ANA tests may not agree with LE Prep test or with latex tests.
6. Presence of antibodies to double stranded native DNA is diagnosis for SLE.
7. Management of therapy should be based not only on positive serologic test for SLE, but should include the presence of active clinical disease.
8. Elderly patients with SLE have a better prognosis and their clinical symptoms differ substantially from those seen in younger patients.<sup>11</sup>
9. Although the predominant class of antinuclear antibodies (ANA) is immunoglobulin G, the presence of immunoglobulin E may be of pathogenic importance in SLE.<sup>12</sup>

#### MA:

1. No diagnosis should be based upon a single serologic test result since various host factors must be taken into consideration.
2. 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.
3. Liver and kidney microsomal antibody stains proximal tubules preferentially, whereas MA reacts with distal tubules more strongly than with proximal tubules.<sup>22</sup>
4. 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.
5. Anti-smooth muscle antibody can be detected in 30-50% and antinuclear antibody in 25-46% of patients with PBC.

#### SMA:

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

- SMA should be used as an aid in the diagnosis of liver disease.
  - Clinical manifestations such as liver biopsies and liver function tests should be considered in the final diagnosis of chronic active hepatitis.
  - 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.
  - Titers of some acute cases of viral hepatitis (AVH) can be as high as CAH cases but they decrease and disappear in a relatively short period while CAH titers remain high for prolonged periods.<sup>29, 30</sup>
  - 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.
  - 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.<sup>36</sup>
  - Drug induced CAH is rather rare but the drugs oxyphenisatin and methyl dopa have been associated with some cases of CAH.
  - Antibodies to native double-stranded DNA, initially considered specific for Systemic Lupus Erythematosus (SLE), are found in a variety of liver diseases, including CAH and cirrhosis.
- PCA:**
- No diagnosis should be based upon a single serologic test result since various host factors must be taken into consideration.
  - Additional confirming tests for pernicious anemia are: antibodies to intrinsic factor, vitamin B12 absorption or serum vitamin B12 activity.
  - PCA should be used as a diagnostic aid in establishing pernicious anemia as the cause of megaloblastic anemia.
  - PCA can be found in 16% of apparently normal individuals over the 60 year age group.
  - Conditions other than pernicious anemia may give positive PCA results.
  - 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.<sup>46</sup>
- Patients with Dermatitis herpetiformis can have PCA without any evidence of malabsorption of B12.

## US

<b>Component</b>	AD CGER2, AD CGER5, AD CGER10 FITC (IFA) Conjugate with Evans Blue Counterstain	<b>Precautionary Statement</b> <b>Prevention:</b> <ul style="list-style-type: none"> <li>Avoid breathing mists, vapors and/or sprays.</li> <li>Use only outdoors or in a well-ventilated area.</li> <li>Wash thoroughly after handling.</li> <li>Wear protective gloves/protective clothing/eye protection/face protection</li> </ul>
<b>Pictogram</b>		<b>Response:</b> <ul style="list-style-type: none"> <li>IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing. Call a POISON CENTER or doctor/physician if you feel unwell.</li> <li>IF ON SKIN: Wash with plenty of soap and water. Take off contaminated clothing and wash before reuse.</li> <li>Specific treatment, see supplemental first aid information.</li> <li>If skin irritation occurs, get medical advice/attention</li> <li>IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.</li> <li>If eye irritation persists: Get medical advice/attention.</li> </ul>
<b>Signal Word</b>	<b>WARNING</b>	<b>Storage/Disposal:</b> <ul style="list-style-type: none"> <li>Store in a well-ventilated place. Keep container tightly closed.</li> <li>Store locked up.</li> <li>Dispose of content and/or container in accordance with local, regional, national, and/or international regulations.</li> </ul>
<b>Hazard Statement</b>	Causes skin irritation. Causes serious eye irritation. May cause respiratory irritation	

<b>Component</b>	AD PBS1, PBS Powder Packets AD TMM3 Mounting Medium	<b>Precautionary Statement</b> <b>Prevention:</b> <ul style="list-style-type: none"> <li>Wash thoroughly after handling.</li> <li>Wear eye/face protection</li> </ul>
<b>Pictogram</b>		<b>Response:</b> <ul style="list-style-type: none"> <li>IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.</li> <li>If eye irritation persists: Get medical advice/attention.</li> </ul>
<b>Signal Word</b>	<b>WARNING</b>	<b>Storage/Disposal:</b> <ul style="list-style-type: none"> <li>Dispose of content and/or container in accordance with local, regional, national, and/or international regulations.</li> </ul>
<b>Hazard Statement</b>	Causes serious eye irritation.	

## EU

<b>Component</b>	AD PBS1 PBS Powder Packets AD TMM3 Mounting Medium	<b>Precautionary Statement</b> <b>Prevention:</b> P264 Wash thoroughly after handling. P280 Wear protective gloves and clothing.
<b>Pictogram</b>		<b>Response:</b> P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if easy to do. Continue rinsing. P337+P313 If irritation persists, get medical advice/attention.
<b>Signal Word</b>	<b>WARNING</b>	
<b>Hazard Statement</b>	H319 Causes serious eye irritation	

## Precautions:

- All human components have been tested by radioimmunoassay for (HB<sub>s</sub>A<sub>0</sub>) and HTLVIII/LAV by an FDA approved method and found to be negative. (Not repeatedly reactive). However, this does not assure the absence of HB<sub>s</sub>A<sub>0</sub> or HTLVIII/LAV. All human components should be handled with appropriate care.
- The sodium azide (0.1%) included in the controls and conjugate is toxic if ingested.
- Do not use components beyond their expiration date.
- Follow the procedural instructions exactly as they appear in this insert to insure valid results.

- For In Vitro Diagnostic Use.
- Handle slides by the edges since direct pressure on the antigen wells may damage the antigen.
- 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.
- Use separate pipette tips for each sample and reagent to avoid cross contamination.
- Reagents should be inspected for evidence of bacterial or fungal contamination.
- Do not reuse substrate slide.

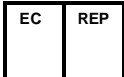
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## AUTOSCREEN I/AUTOSCREEN II AUTOIMMUNE SCREENING TEST SYSTEM

Réservé au diagnostic *in vitro*.

AD AS148 (Rat)	48 Tests	AD AS196 (Rat)	96 Tests
AD AS348 (Souris)	48 Tests	AD AS396 (Souris)	96 Tests
AD AS248 (Rat)	48 Tests	AD AS296 (Rat)	96 Tests
AD AS448 (Souris)	48 Tests	AD AS496 (Souris)	96 Tests

### Intitulé du test:

Le test d'immunofluorescence indirecte est destiné au screening des autoanticorps anti-nucléaires, anti-mitochondries, anti-cellules pariétales, anti-muscles lisses et anti-réticuline circulant dans le sérum de patients.

### Principe:

La réaction principale du test implique des anticorps circulant dans le sérum du patient qui se lient à leurs antigènes homologues. Ceci se produit pendant la période d'incubation lorsque le sérum recouvre la surface de l'antigène. Une deuxième réaction suit alors une étape de rinçage qui élimine tous les anticorps humains non fixés. Le réactif utilisé lors de la seconde incubation est un conjugué anti-globuline humaine marquée à la fluorescéine. La lame est ensuite soigneusement rincée pour éliminer l'excès de conjugué non fixé, et est observée à l'aide d'un microscope à fluorescence.

### Matériel fourni:

Conservation et stabilité des composants

- Lames de coupe de rat ou souris (rein/estomac). Conserver entre 2 et 8°C
- Lames de coupe de rat ou souris (foie/rein/estomac). Conserver entre 2 et 8°C
- Contrôle positif anti-mitochondries, conserver entre 2 et 8°C
- Contrôle positif anti-nucléaire - homogène, conserver entre 2 et 8°C.
- Contrôle positif anti-muscles lisses, conserver entre 2 et 8°C.
- Contrôle négatif universel, conserver entre 2 et 8°C.
- Conjugué ITCF anti-IgG H&L avec bleu d'Evans (AD CGER2-AD CGER5), conserver entre 2 et 8°C.
- Sachet de tampon No AD PBS1 - Tampon phosphate salin. le tampon reconstitué qui ne contient pas d'agents conservateurs doit être conservé à 2-8°C.
- Le milieu de montage ITCF No AD TMM3 est stable lorsqu'il est conservé entre 2 et 8°C

### Matériel supplémentaire requis mais non fourni:

Tubes à essai et portoir ou plaques de microtitration  
 Pipettes jetables.  
 Bac de coloration et pinces pour lames  
 Chambre humide  
 Ballon volumétrique (500 ml)  
 H2O distillée  
 Microscope à fluorescence  
 Serviettes en papier (non pelucheuses)

### Préparation des réactifs:

Sachet de tampon No AD PBS1. Réhydrater le tampon avec 1 litre d'eau distillée stérile

### Prélèvement des échantillons:

Les échantillons de sang doivent être prélevés dans des conditions aseptisées. Une hémolyse est évitée par une séparation rapide du sérum du caillot. Le sérum doit être conservé à 2-8°C en cas d'analyse prévue dans un délai de quelques jours. On peut garder le sérum pendant 3 à 6 mois en le conservant à une température maximale de -20°C. Éviter les sérums lipémiques et fortement hémolysés. Lorsque les échantillons sont expédiés à température ambiante, l'ajout d'un agent conservateur tel que 0,01 % de thimérol ou 0,095 % d'azide de sodium est fortement conseillé.

### Instructions du test:

**Test de screening:** diluer les sérums à 1:20 dans du PBS

**Titration:** préparer des dilutions sérielles du sérum à partir de 1:20 (à savoir, 1:20, 1:40, 1:80, 1:160, etc.)

- Une fois les lames revenues à température ambiante, ouvrir le sachet de lames en le déchirant à l'encoche. Retirer la lame avec soin et éviter de toucher les puits où se situent les antigènes. La lame est maintenant prête à l'emploi.
- Déposer une goutte de sérum dilué (20 à 30 µl) et des contrôles dans les puits contenant les antigènes.
- Placer la lame dans une chambre humide à température ambiante (environ 20-24°C) pendant 30 minutes.
- Enlever la lame de la chambre humide. À l'aide d'une pissette, rincer délicatement les puits en prenant soin de détourner le jet de rinçage des puits.
- Laver dans un bac de lavage avec du PBS pendant cinq minutes. Répéter l'opération en utilisant du PBS frais.
- Placer un buvard sur la table de laboratoire avec le côté absorbant tourné vers le haut. Retirer la lame du PBS et la retourner de manière à placer le côté frottis en face du côté absorbant du papier buvard. Aligner les puits pour absorber le contenu des trous à l'aide du buvard. Placer la lame sur le buvard. Ne pas laisser sécher les tissus. Essuyer le dos de la lame avec une serviette en papier non pelucheuse. Ne pas exercer trop de pression sur la lame tout en l'essuyant pour absorber le tampon.
- Déposer 1 goutte (20 à 30 µl) de conjugué dans chaque puits. Répéter les étapes 3 à 6.
- Déposer 4 à 5 gouttes de liquide de montage sur la lame.
- Déposer une lamelle couvre-objet de 22 x 70 mm. Examiner la lame sous un microscope à fluorescence.

**Remarque:** Pour maintenir la fluorescence, conserver la lame montée dans une chambre humide placée à l'obscurité dans un réfrigérateur.

### Contrôle de qualité:

- Des contrôles de sérum positif et négatif doivent être inclus dans chaque série

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- pour confirmer la reproductibilité, sensibilité et spécificité du test.
- Le contrôle de sérum négatif doit faire apparaître peu (1+) ou pas de fluorescence. La mise en évidence d'une fluorescence vive par ce contrôle peut être due au contrôle, de l'antigène, du conjugué ou de la technique.
- Le contrôle de sérum positif doit montrer une fluorescence vive de 3+ à 4+. La mise en évidence d'une fluorescence faible ou inexistante par ce contrôle peut être due au contrôle, de l'antigène, du conjugué ou de la technique.
- En plus des contrôles de sérums positifs et négatifs, effectuer un contrôle PBS pour s'assurer que le conjugué ne provoque pas de coloration non spécifique du substrat antigénique. Si l'antigène montre une fluorescence vive avec le contrôle PBS, répéter l'opération à l'aide d'un nouveau conjugué. Une fluorescence de l'antigène peut être due à une dégradation du conjugué ou de l'antigène.

### Interprétation du titre:

**ANA:** Le titre est la dilution de sérum la plus élevée qui montre encore une faible fluorescence (1+).

Inférieur à 1:20	Exclut pratiquement le LED en phase active à condition que le patient ne soit pas sous thérapie immuno-suppressive ou en rémission.
De 1:20 à 1:80	Test positif fréquemment observé dans le cas de la PR et celui d'autres maladies des tissus conjonctifs. Tester un nouvel échantillon endéans les deux semaines. Une augmentation du titre suggère un LED évolutif. L'absence de changement de titre indique une autre maladie auto-immune possible non évolutive, un LED traité et stabilisé ou un autre processus d'auto immunisation.
1:160 ou supérieur	Suggère fortement un LED bien que d'autres maladies auto-immunes et médicaments puissent induire ces titres élevés.

**MA:** Auto anticorps anti-mitochondries (MA). La cirrhose biliaire primitive (CBP) est une maladie hépatique chronique se traduisant par une cholestase intra hépatique. On l'observe plus fréquemment chez la femme que chez l'homme avec une prédominance dans le groupe des 30-60 ans. Le diagnostic est établi sur base des données cliniques, des observations histologiques, de l'augmentation de la phosphatase alcaline, du taux d'IgM élevés et de la présence d'anticorps anti-mitochondries. Un résultat positif montre une fluorescence cytoplasmique des tubules rénaux (proximaux et distaux). Toute autre fluorescence ne peut être considérée comme étant un anti mitochondrie.

< 20:	Normal, négatif
20-80:	Positif. Suggère une atteinte hépatique. Répéter le test avec un nouvel échantillon après 2 semaines.
160 ou plus:	CBP probable.

Près de 50% des CBP ont des titres compris entre 1:2000 et 1: 6000. Ces titres sont en général stables et ne sont pas modifiés par le traitement ou l'évolution de la maladie.

**SMA:** L'hépatite chronique active (ACH) est une maladie chronique du foie qui touche généralement les femmes jeunes mais aussi les hommes et à tous les âges. Elle est caractérisée (biopsie du foie) par une détérioration de la fonction hépatique due à une nécrose des cellules hépatiques parenchymateuses dans les régions d'infiltration des lymphocytes et des cellules plasmiques.

Un résultat positif montre une coloration cytoplasmique diffuse des couches de muscles lisses de la muscularis mucosae de l'estomac du rat ou de la souris. Une fluorescence peut aussi être présente dans les parois capillaires de la couche gastrique et les artères ou veines environnantes. Une fluorescence des autres antigènes cellulaires tels que les noyaux, les cellules pariétales ou le tissu conjonctif ne doit pas être considérée comme étant un test anti muscles lisses positif.

Le titre est la dilution la plus élevée du sérum du patient montrant une fluorescence faible (1+) de la muscularis mucosae.

< 1:20	Normal, négatif
De 1:20 - 1:80	Positif. Signes d'une maladie du foie. Répéter avec un nouvel échantillon après 2 semaines.
1:160 ou supérieur	Signe d'une hépatite chronique active.

### Résultats:


Autoanticorps anti-nucléaires: On observe diverses images appartenant aux 4 familles classiques mais pouvant être associées.

- Homogène (diffus): On voit une fluorescence homogène, légèrement diffuse du noyau entier.
- Périphérique (bord, irrégulière): La membrane nucléaire montre une fluorescence plus intense que la partie centrale.
- Mouchetée: De nombreuses petites « taches » de fluorescence sur l'ensemble du noyau.
- Nucléolaire: Les nucléoles sont colorés de manière uniforme et se présentent sous forme de 1 à 5 larges zones sphériques de fluorescence dispersées sur l'ensemble du noyau.

### Précautions:

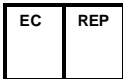
- HBsAg et HTLV-III/LAV ont été testés par dosage radioimmunologique pour tous les composants d'origine humaine par une méthode approuvée par la FDA, et se sont avérés négatifs. Ceci ne garantit toutefois pas l'absence de HBsAg ou de HTLV-III/LAV. Tous les composants d'origine humaine doivent être manipulés avec les précautions appropriées.
- Les contrôles et le conjugué contiennent du sodium azide (0,095%).
- Ne pas utiliser les composants après leur date de péremption.
- Suivre les instructions du test exactement comme elles figurent dans cette notice afin de garantir des résultats valables.
- Réservé au diagnostic *in vitro*.
- Manipuler les lames par les bords car une pression appliquée directement sur les

7. puits contenant les antigènes peut altérer la structure de l'antigène.  
Une fois la procédure lancée, ne pas laisser sécher les antigènes des puits. Ceci peut entraîner l'obtention de résultats faussement négatifs ou l'apparition d'artefacts superflus.
8. Utiliser des pointes de tips pour chaque échantillon et réactif pour éviter des contaminations.
9. Les réactifs doivent être inspectés afin d'éliminer une contamination bactérienne ou contamination fongique.
10. Ne pas réutiliser les lames après usage.

<b>Composants</b>	AD PBS1 PBS Poudre sachet AD TMM3 Liquide de montage	<b>Déclaration de précaution</b>
<b>Pictogramme</b>		<b>Prévention:</b> P264 Se laver les mains soigneusement après manipulation. P280 Porter des gants de protection/des vêtements de protection/un équipement de protection des yeux/ du visage.
<b>Mot de signal</b>	<b>ATTENTION</b>	<b>Réponse:</b> P305+P351+P338 EN CAS DE CONTACT AVEC LES YEUX: rincer avec précaution à l'eau pendant plusieurs minutes. Enlever les lentilles de contact si la victime en porte et si elles peuvent être facilement enlevées. Continuer à rincer. P337+P313 Si l'irritation oculaire persiste: consulter un médecin.
<b>Mention de danger</b>	H319 Provoque une sévère irritation des yeux.	



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Deutsch



## AUTOSCREEN I/AUTOSCREEN II AUTOIMMUNE SCREENING TEST SYSTEM

Die Tests sind für die *diagnostische Verwendung in vitro* bestimmt.

AD AS148 (Ratte)	48 Tests	AD AS196 (Ratte)	96 Tests
AD AS348 (Maus)	48 Tests	AD AS396 (Maus)	96 Tests
AD AS248 (Ratte)	48 Tests	AD AS296 (Ratte)	96 Tests
AD AS448 (Maus)	48 Tests	AD AS496 (Maus)	96 Tests

### Verwendungszweck:

Der indirekte Immunofluoreszenztest wird als Screeningtest für zirkulierende antinucleare (ANA), mitochondriale (MA), Parietalzellen- (PCA), Glatte-Muskel- (SMA) und Retikulin-Autoantikörper im Patientenserum empfohlen.

### Prinzip:

Die primäre Testreaktion erfasst im Serum des Patienten zirkulierende Antikörper, die sich an ihre homologen Antigene anlagern. Dies findet in der Inkubationszeit statt, während das Serum die Antigenoberfläche bedeckt. Auf einen Auswaschvorgang, in dem alle ungebundenen humanen Antikörper entfernt werden, folgt eine sekundäre Reaktion. Das in der sekundären Reaktion verwendete Reagens ist ein fluoresceinmarkiertes Antihumanglobulin-konjugat. Die Antigenoberfläche wird danach vollständig von ungebundenem Konjugat freigespült und unter einem geeigneten Fluoreszenzmikroskop betrachtet.

### Bereitgestellte Materialien:

Lagerung und Stabilität von Komponenten

1. Ratte oder Maus (Niere-, Magen-Gewebeschnitte) (bei 2-8°C lagern)
2. Ratte oder Maus (Leber-, Niere-, Magen-Gewebeschnitte) (bei 2-8°C lagern)
3. MA Positivkontrolle (bei 2-8°C lagern)
4. ANA homogene Positivkontrolle (bei 2-8°C lagern)
5. SMA Glatte-Muskulatur-Positivkontrolle (bei 2-8°C lagern)
6. Universelle Negativkontrolle (bei 2-8°C lagern)
7. FITC IgG H&L Konjugat mit Evans-Blau (bei 2-8 °C lagern) (AD CGER2-
8. AD CGER5),
9. Pufferpackung Nr. AD PBS1 . Phosphatgepufferte Salzlösung (rekonstituierender Puffer enthält keine Konservierungsmittel und sollte bei 2-8°C gelagert werden).
10. FITC Einbettungsmittel Nr. AD TMM3 lässt sich bei 2-8°C stabil lagern.

### Weitere erforderliche, aber nicht bereitgestellte Materialien:

Reagenzgläser und Gestell- oder Mikrotitersystem  
Einmalgebrauchspipetten  
Färbeschale und Objektträgerpinzette  
Feuchtkammer  
Messkolben (500 ml)  
Destilliertes H<sub>2</sub>O  
Fluoreszenzmikroskop  
Nichtfasermde Papiertücher

### Reagensvorbereitung:

Pufferpackung Nr. AD PBS1. Puffer mit 1 Liter sterilem destilliertem Wasser rehydratisieren.

### Probennahme:

Serologische Proben sollten unter aseptischen Bedingungen genommen werden. Hämolyse wird durch umgehende Trennung des Serums vom Koagulat vermieden. Serum sollte bei 2-8 °C gelagert werden, wenn es innerhalb weniger Tage analysiert werden soll. Serum lässt sich bei -20 °C oder darunter 3 bis 6 Monate lang lagern. Lipämisches und stark hämolytisches Serum sollte vermieden werden. Wenn Proben bei Raumtemperatur bereitgestellt werden, wird die Zugabe eines Konservierungsmittels wie 0,01% (Thimerosal) oder 0,095% Natriumazid sehr empfohlen.

### Testanweisung:

**Screening:** verdünnen Sie Testsera 1:20 in PBS.

**Titrationen:** setzen Sie die Serumverdünnungen in jeweils zweier Verdünnungsstufen an, beginnend bei 1:20 (d. h. 1:20, 1:40, 1:80, 1:160 usw.)

1. Wenn die Objektträger Raumtemperatur erreicht haben, reißen Sie die Objektträgergehülfe an der Kerbe auf. Entnehmen Sie den Träger vorsichtig, ohne die Antigenbereiche zu berühren. Der Objektträger ist nun einsatzbereit.
2. Geben Sie jeweils einen Tropfen gelöstes Serum (20 bis 30  $\mu$ l) und Kontrolle auf die Antigenkavitäten.
3. Legen Sie den Objektträger mit dem Patientenserum und den Kontrollen 30 Minuten lang in eine Feuchtkammer bei Raumtemperatur (ungefähr 20-24 °C).
4. Nehmen Sie den Objektträger aus der Feuchtkammer. Spülen Sie verbleibende Sera mit einer Waschflasche vom Objektträger, wobei Sie sorgfältig darauf achten, den Spülstrahl nicht direkt auf die Kavität zu richten.
5. Fünf Minuten in PBS waschen. Wiederholen Sie den Vorgang mit frischem PBS.
6. Legen Sie ein Löschblatt auf den Labortisch, mit der absorbierenden Seite nach oben. Nehmen Sie den Objektträger aus dem PBS und drehen Sie ihn um, so dass die Gewebeseite der absorbierenden Seite des Löschblatts zugewandt ist. Richten Sie die Kavitäten auf die Löschblattlöcher aus. Legen Sie den Objektträger auf die Löschblattoberseite. Das Gewebe darf nicht austrocknen. Wischen Sie die Tr.gerrückseite mit einem nichtfasermde Papiertuch ab. Üben Sie beim Abwischen das Absorbieren des Puffers genügend Druck auf den Objektträger aus.
7. Geben Sie 1 Tropfen (20-30  $\mu$ l) Konjugat auf jede Antigenkavität. Wiederholen Sie die Schritte 3-6.
8. Geben Sie 4-5 Tropfen Einbettungsmittel auf den Objektträger.
9. Setzen Sie ein Deckglas von 22 x 70 mm auf. Untersuchen Sie den Objektträger unter einem Fluoreszenzmikroskop.

**Hinweis:** Um die Fluoreszenz aufrechtzuerhalten, lagern Sie den präparierten Objektträger in

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einer Feuchtkammer in einem dunklen Kühlschrank.

### Qualitätskontrolle:

1. Positive und negative Serumkontrollen müssen täglich beim Testen einbezogen werden, um die Reproduzierbarkeit, Empfindlichkeit und Spezifität der Testprozedur zu bestätigen.
2. Die negative Serumkontrolle sollte zu geringer (+) oder ausbleibender Fluoreszenz führen. Sollte sich bei dieser Kontrolle helle Fluoreszenz zeigen, ist die Kontrollprobe, das Antigen, das Konjugat oder die Vorgehensweise möglicherweise fehlerhaft.
3. Die positive Serumkontrolle sollte zu heller Fluoreszenz von 3+ bis 4+ führen. Sollte sich bei dieser Kontrolle geringe oder keine Fluoreszenz zeigen, ist die Kontrollprobe, das Antigen, das Konjugat oder die Vorgehensweise möglicherweise fehlerhaft.
4. Zusätzlich zu positiven und negativen Serumkontrollen sollte eine PBS-Kontrolle durchgeführt werden, um sicherzustellen, dass das Konjugat frei von unspezifischer Färbung des Antigensubstrats ist. Wenn das Antigen bei der PBS-Kontrolle helle Fluoreszenz zeigt, wiederholen Sie mit frischem Konjugat. Wenn das Antigen noch immer fluoresziert, ist das Konjugat oder das Antigen möglicherweise fehlerhaft.

### Titer-Interpretation:

**ANA:** Der Titer ist die höchste Verdünnung von Patientenserum, bei der sich schwache Fluoreszenz (1+) zeigt.

Weniger als 1:20

Negativ: schließt aktive SLE praktisch aus, vorausgesetzt, der Patient befindet sich nicht in immunsuppressiver Therapie oder in Remission.

1:20 - 1:80

Positiver Test, der häufig bei RA und anderen Bindegewebskrankheiten vorkommt. Nach zwei Wochen sollte eine frische Probe getestet werden. Zunehmender Titer lässt auf aktive SLE schließen. Unveränderter Titer zeigt mögliche andere Autoimmunkrankheit in statischem Zustand oder einen behandelten und kontrollierten SLE-Fall oder einen anderen Autoimmunprozess an.

1:160 oder darüber

Positiv: Starker Hinweis auf SLE, auch wenn andere Autoimmunkrankheiten und Medikamente diese hohen Titer hervorrufen können.

**MA:** Die Primäre-biliäre Zirrhose (PBC) ist eine chronische intrahepatische Cholestase, die im Lebensabschnitt von 30-60 Jahren vermehrt bei Frauen als bei Männern beobachtet wird. Die Diagnose von PBC wird gestützt durch klinische Beobachtungen, histologische Befunde der Leberbiopsie, durch vermehrte Alkalische Phosphataseaktivität, erhöhte IgM Titer und Vorhandensein von mitochondrialen Antikörpern. Als positiv wird eine granuläre Fluoreszenz des Zytoplasmas der Nierentubuli gewertet. Meist ist die Fluoreszenz auf das Zytoplasma der proximalen und distalen Tubuli Epithelien beschränkt. Eine Fluoreszenz anderer zellulärer Antigene wie Nukleole, glatte Muskulatur, oder eine nicht-granuläre Fluoreszenz, die auf das Lumen der proximalen Tubulie beschränkt bleibt, sollte nicht als MA positiv gewertet werden.

Es wird die Titerstufe angegeben bei der die höchste Verdünnung des Patientenserums eine schwache Fluoreszenz (1+) des Epitheliums der Nierentubuli zeigt.

Kleiner als 1:20

Normal, negativ

1:20 - 1:80

Positiv, Verdacht auf eine Lebererkrankung. In zwei Wochen mit einem frischen Serum Test wiederholen.

1:160 oder größer

Verdacht auf eine aktive Primär-biliäre Zirrhose.

Bei PBC können Titer von 1:10 bis 1:6.000 vorkommen. 50% der PBC Patienten haben Titer von 1:2000 bis 1:6000. MA Titer verändern sich nicht im Zeitverlauf oder unter Therapie. Daher können sie nicht zur Therapiekontrolle herangezogen werden.

**SMA:** ACH ist eine chronische Lebererkrankung, an der vor allem junge Frauen leiden, an der aber auch beide Geschlechter und alle Altersgruppen erkranken können. Sie zeichnet sich in Leberbiopsien durch eine verschlechterte Leberfunktion aufgrund einer Nekrose hepatischer Parenchymzellen in Bereichen der lymphozytären und Plasmazellinfiltration aus.

Ein positives Ergebnis zeigt sich als helle diffuse zytoplasmatische Verfärbung der glatten Muskelschichten der Lamina muscularis mucosae in Ratten- und Mäusemägen. Eine Fluoreszenz kann auch in den Kapillarwänden der Magenwand und den umgebenden Arterien und Venen auftreten. Eine Fluoreszenz anderer zellulärer Antigene wie von Zellkernen, Parietalzellen oder Bindegewebe sollte nicht als positives SMA-Ergebnis gemeldet werden.

Der Titer ist die höchste Verdünnung des Patientenserums, das eine schwache (1+) Fluoreszenz der Lamina muscularis mucosae aufweist.

Unter als 1:20 oder weniger

normal, negativ

1:20 - 1:80

positiv. Suggestiert eine Lebererkrankung. Mit einer frischen Probe zwei Wochen später wiederholen.

1: 160 oder mehr

Suggestiert eine aktive, chronische Hepatitis.

### Ergebnisse:


**ANA:** Ein Ergebnis ist positiv, wenn eines der vier grundlegenden Fluoreszenzmuster einzeln oder in verschiedenen Kombinationen auftreten. Die charakteristischen Muster lassen sich am besten mit stark vergrößerten Trockenobjektiven beobachten.

1. Homogen (diffus) . Es zeigt sich eine gleichmäßige, fein verteilte Fluoreszenz des gesamten Nucleus.
2. Peripher (randständig, zottig) . Die Nucleusmembran fluoresziert intensiver als der zentrale Bereich.
3. Gefleckt . Zahlreiche kleine "Flecken" von Fluoreszenz über den gesamten Nucleus.
4. Nucleolär . Die Nucleoli sind gleichmäßig gefärbt und erscheinen als 1 bis 5

große kugelförmige Fluoreszenzbereiche, die über den Nucleus verstreut sind.

**Vorsichtsmaßnahmen:**

1. Alle humanen Bestandteile wurden mit Radioimmuntest auf (HBsAg) und HTLVIII/LAV mit einer FDA-anerkannten Methode negativ getestet. (Nicht wiederholt reaktiv.) Dies gewährleistet jedoch nicht die Abwesenheit von HBsAg oder HTLVIII/LAV. Alle humanen Bestandteile sollten mit angemessener Sorgfalt gehandhabt werden.
2. Den Kontrollproben und dem Konjugat ist sodium azide (0,095%) beigefügt.
3. Verwenden Sie keine Bestandteile über das Verfallsdatum hinaus.
4. Befolgen Sie die methodischen Anweisungen genau wie in dieser Beilage beschrieben, um gültige Ergebnisse zu gewährleisten.
5. Die Tests sind für die diagnostische Verwendung in vitro bestimmt.
6. Fassen Sie die Objektträger an den Kanten an, da direkter Druck auf die Antigenkavitäten das Antigen beschädigen kann.
7. Nach Beginn der Prozedur darf das Antigen in den Kavitäten nicht austrocknen. Dies kann zu falsch negativen Testergebnissen oder unnötigen Artefakten führen.
8. Verwenden Sie getrennte Pipette Tips für jede Probe und Reagenz zu treffen, um Kreuzkontaminationen zu vermeiden.
9. Reagenzien geprüft werden sollen auf Anzeichen von Bakterien- oder Pilzbefall.
10. Nicht wiederverwenden Objektträger

<b>Komponente</b>	AD PBS1 PBS Pufferpackung  AD TMM3 Einbettungsmittel	<b>Sicherheitshinweis</b> <b>Prävention:</b> P264 Nach Gebrauch ö gründlich waschen. P280 Schutzhandschuhe/Schutzkleidung/Augenschutz/Gesichtsschutz tragen.
<b>Piktogramm</b>		<b>Antwort:</b> P305+P351+P338 BEI KONTAKT MIT DEN AUGEN: Einige Minuten lang behutsam mit Wasser spülen. Vorhandene Kontaktlinsen nach Möglichkeit entfernen. Weiter spülen.
<b>Signalwort</b>	<b>ACHTUNG</b>	
<b>Gefahrenhinweis</b>	H319 Verursacht schwere Augenreizung.	P337+P313 Bei anhaltender Augenreizung: Ärztlichen Rat einholen/ärztliche Hilfe hinzuziehen.

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L'italiano



## AUTOSCREEN I/AUTOSCREEN II AUTOIMMUNE SCREENING TEST SYSTEM

Per uso *diagnostico in vitro*.

AD AS148 (Ratto)	48 Tests	AD AS196 (Ratto)	96 Tests
AD AS348 (Topo)	48 Tests	AD AS396 (Topo)	96 Tests
AD AS248 (Ratto)	48 Tests	AD AS296 (Ratto)	96 Tests
AD AS448 (Topo)	48 Tests	AD AS496 (Topo)	96 Tests

### Uso previsto:

Il test di immunofluorescenza indiretta è raccomandato come test di screening per gli autoanticorpi antinucleari (ANA), antimitocondriali (MA), anticellula parietale (PCA) antimuscolatura liscia (SMA) e antireticolina in circolazione nel siero del paziente.

### Principi:

La reazione primaria del test implica la circolazione nel siero del paziente di anticorpi che si legano ai loro antigeni omologhi. Ciò si verifica durante il periodo di incubazione, quando il siero ricopre la superficie dell'antigene. Successivamente ad un periodo di risciacquo necessario per la rimozione degli anticorpi umani non legati, avviene una reazione secondaria. Il reagente utilizzato nella reazione secondaria è un coniugato antiglobulina umana marcato con fluoresceina. La superficie dell'antigene viene quindi completamente risciacquata in modo da eliminare il coniugato non legato e quindi osservata con un idoneo microscopio a fluorescenza.

### Materiali in dotazione:

Conservazione e stabilità dei componenti

1. Rene/stomaco di ratto o topo (conservare a una temperatura di 2-8°C).
2. Fegato/rene/stomaco di ratto o topo (conservare a una temperatura di 2-8°C).
3. Controllo positivo MA (conservare a una temperatura di 2-8°C).
4. Controllo positivo ANA di tipo omogeneo (conservare a una temperatura di 2-8°C).
5. Controllo positivo SMA (conservare a una temperatura di 2-8°C).
6. Controllo negativo universale (conservare a una temperatura di 2-8°C).
7. Coniugato FITC IgG H&L con Evans Blue (AD CGER2-AD CGER5), (conservare a una temperatura di 2-8°C).
8. Confezione tampone n. AD PBS1 - Tampone fosfato. Il tampone ricostituito non contiene conservanti e deve essere conservato a una temperatura di 2-8°C.
9. La soluzione di montaggio FITC n. AD TMM3 rimane stabile se conservata a una temperatura di 2-8°C.

### Materiali aggiuntivi richiesti ma non in dotazione:

Provette per test e cestello o sistema per microtitolazione  
Pipette monouso  
Vaschetta per colorazione e pinze per vetrini  
Camera umida  
Pallone volumetrico (500 ml)  
Acqua distillata  
Microscopio a fluorescenza  
Carta assorbente che non lasci residui

### Preparazione del reagente:

Confezione tampone n. AD PBS1. Reidratare il tampone con 1 litro di acqua distillata sterile.

### Raccolta dei campioni:

Raccogliere i campioni sierologici in condizioni asettiche. L'emolisi viene evitata separando tempestivamente il siero dal coagulo. Conservare il siero a una temperatura di 2-8°C se questo deve essere analizzato entro pochi giorni. È possibile conservare il siero per un periodo di 3-6 mesi a una temperatura pari o inferiore a -20°C. Evitare il siero lipemico e fortemente emolitico. Se i campioni vengono spediti a temperatura ambiente, si raccomanda l'aggiunta di un conservante quale (timerosal) 0,01% o sodio azide 0,095%.

### Istruzioni per il test:

**Screening:** diluire i sieri da testare 1:20 in tampone fosfato.

**Titolazioni:** impostare diluizioni di siero al raddoppio a partire da 1:20 (cioè 1:20, 1:40, 1:80, 1:160, 1:320, ecc.)

1. Quando i vetrini raggiungono la temperatura ambiente, strapparne l'involucro in corrispondenza dell'apposita tacca. Rimuovere con cautela il vetrino dall'involucro evitando di toccare le aree su cui è presente l'antigene. Il vetrino è pronto per l'uso.
2. Versare una goccia di siero diluito (da 20 a 30  $\mu$ l) e i controlli sui pozzetti dell'antigene.
3. Posizionare il vetrino con il siero del paziente e i controlli in una camera umida a temperatura ambiente (circa 20-24°C) per 30 minuti.
4. Rimuovere il vetrino dalla camera umida. Risciacquare delicatamente il siero rimanente sul vetrino con spruzzetta per lavaggio facendo attenzione a non dirigere il getto direttamente sul pozzetto.
5. Lavare in tampone fosfato per cinque minuti. Ripetere la procedura utilizzando tampone fosfato fresco.
6. Posizionare un tampone di carta assorbente sul tavolo del laboratorio con il lato assorbente rivolto verso l'alto. Rimuovere il vetrino dal tampone fosfato e capovolgere in modo che il lato su cui è applicato il campione di tessuto sia a contatto con il lato assorbente del tampone. Allineare i pozzetti con i fori del tampone. Posizionare il vetrino sulla parte superiore del tampone. Non lasciare asciugare il tessuto. Pulire la parte posteriore del vetrino con carta assorbente asciutta che non lasci residui. Assorbire il tampone fosfato con la carta esercitando una leggera pressione ed accertarsi che il vetrino sia asciutto.
7. Versare 1 goccia (20-30  $\mu$ l) di coniugato in ciascun pozzetto di antigene. Ripetere le fasi da 3 a 6.
8. Versare 4-5 gocce di soluzione di montaggio sul vetrino.
9. Applicare un vetrino coprioggetto da 22 x 70 mm. Esaminare il vetrino al microscopio a

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fluorescenza. Nota: per mantenere la fluorescenza, conservare il vetrino montato in una camera umida all'interno di un frigorifero al buio.

### Controllo di qualità:

1. I controlli di siero positivo e negativo devono essere inclusi in tutti i test del giorno per confermare la riproducibilità, la sensibilità e la specificità della procedura. Il controllo di siero negativo deve visualizzare una fluorescenza minima (+) o nulla. Una eventuale fluorescenza evidente di questo controllo indica un problema a livello di controllo, di antigene, di coniugato o di procedura tecnica.
2. Il controllo di siero positivo deve visualizzare una fluorescenza evidente da 3+ a 4+. Una eventuale fluorescenza minima o nulla di questo controllo indica un problema a livello di controllo, di antigene, di coniugato o di procedura tecnica.
3. In aggiunta ai controlli di siero positivi e negativi, eseguire un controllo con tampone fosfato per stabilire se il coniugato è libero da colorazioni non specifiche del substrato dell'antigene. Se l'antigene mostra una fluorescenza evidente nel controllo con tampone fosfato, ripetere la procedura utilizzando coniugato fresco. La presenza continua di fluorescenza indica un problema a livello del coniugato o dell'antigene stesso.

### Interpretazione del titolo:

**ANA:** Il titolo è la diluizione più alta del siero del paziente che mostra una fluorescenza debole (1+).

Inferiore a 1:20

Normale: praticamente esclude il lupus eritematoso sistemico (LES) attivo se il paziente non è stato sottoposto a terapia immunosoppressiva o non è in fase di remissione.

1:20 - 1:80

Test positivo spesso riscontrato nell'artrite reumatoide e in altre malattie del tessuto connettivo. Ripetere il test su un campione di siero fresco dopo due settimane. Un aumento del titolo suggerisce LES attivo. L'assenza di cambiamenti nel titolo indica una possibile altra malattia autoimmune in una condizione statica, un caso di LES trattato o un altro processo autoimmune.

1:160 o superiore

Elevata possibilità di LES, anche se altre malattie autoimmuni e alcuni farmaci possono indurre titoli elevati.

**MA:** Il titolo è la più alta diluizione del siero del paziente, che mostra fluorescenza debole (1+) dell'epitelio del tubulo renale.

Inferiore a 1:20

Normale, negativo

1:20 - 1:80

Positivo, Suggestivo di malattia epatica. Ripetere con un nuovo campione entro due settimane.

1:160 o maggiore

Presuntivamente cirrosi biliari primaria.

La gamma dei titoli nei casi di CBP varia da 1:10 a 1:6.000 con circa il 50% dei pazienti con CPB, che mostrano titoli compresi tra 1:2.000 a 1:6.000. I titoli nei casi di MA non mostrano di variare con il tempo o con la terapia e non possono essere utilizzati per monitorare la risposta terapeutica.

**SMA:** L'AQCH è una malattia cronica del fegato che colpisce principalmente le giovani donne come pure entrambi i sessi a tutte le età. Nelle biopsie epatiche è caratterizzata dal deterioramento della funzione del fegato, dovuta alla necrosi delle cellule del parenchima epatico in aree di infiltrazione linfocitaria e di cellule del plasma.

Il risultato positivo è evidenziato da una colorazione citoplasmatica brillante e diffusa degli strati della muscolatura mucosae del muscolo liscio, che si trova nello stomaco del ratto o del topo. La fluorescenza può essere evidenziata anche sulle pareti dei capillari dello strato gastrico che circonda arterie e vene. La fluorescenza di altri antigeni cellulari come i nuclei, le cellule parietali o il tessuto connettivo non devono essere referati come SMA positivi.

Il titolo è la più alta diluizione del siero del paziente, che mostra una fluorescenza debole (+1) della muscolatura mucosae.

Inferiore a 1:20 o meno

Normale, negativo

1:20 - 1:80

Positivo. Suggestivo di malattia epatica. Ripetere con un campione fresco entro 2 settimane.

1:160 o maggiore

Suggestivo di epatite attiva o cronica.

### Risultati:


**ANA:** Risultato positivo se viene osservato uno dei quattro quadri di colorazione di base individualmente o in diverse combinazioni. I quadri caratteristici vengono meglio osservati con obiettivi a secco ad alto ingrandimento.

1. Omogeneo (diffuso) - fluorescenza dell'intero nucleo uniforme e diffusa.
2. Periferico (bordato, irregolare) - la membrana nucleare ha una fluorescenza più intensa dell'area centrale.
3. Granulare - numerose piccole "macchie" di fluorescenza in tutto il nucleo.
4. Nucleolare - I nucleoli sono colorati uniformemente e nei nuclei si osservano da 1 a 5 grandi aree sferiche di fluorescenza.

### Precauzioni:

1. Tutti i componenti umani sono stati testati mediante test radioimmunologico per (HBsAg) e HTLVIII/LAV con metodo approvato dalla FDA e sono risultati negativi (non reattivi ripetutamente). Tuttavia, questo non garantisce l'assenza di HBsAg o HTLVIII/LAV. Tutti i componenti umani devono essere manipolati con estrema cautela.
2. Sodio azide (0,095%) è compresa in controlli e coniugato.
3. Non utilizzare componenti scaduti.
4. Per garantire risultati validi, seguire le istruzioni procedurali esattamente come vengono descritte in questo inserto.
5. Per uso diagnostico in vitro.
6. Manipolare i vetrini prendendoli dai bordi in quanto la pressione diretta sui pozzetti dell'antigene può danneggiare l'antigene stesso.
7. Dopo aver iniziato la procedura, non fare asciugare l'antigene nel pozzetto. Ciò

- può comportare risultati falsi negativi o artefatti inutili.
8. Separate le punte della pipetta per ogni campione e dei reagenti per evitare la contaminazione incrociata.
  9. I reattivi devono essere controllati per la prova di contaminazione batterica o fungina.
  10. Non riutilizzare vetrini con substrate.

<b>Componente</b>	AD PBS1 PBS Confezione tampone AD TMM3 soluzione di montaggio	<b>Consiglio di prudenza</b> <b>Prevenzione:</b> P264 Lavare accuratamente o dopo l'uso. P280 Indossare guanti/indumenti protettivi/Proteggere gli occhi/il viso. <b>Risposta:</b> P305+P351+P338 IN CASO DI CONTATTO CON GLI OCCHI: sciacquare accuratamente per parecchi minuti. Togliere le eventuali lenti a contatto se è agevole farlo. Continuare a sciacquare. P337+P313 Se l'irritazione degli occhi persiste, consultare un medico.
<b>Pittogramma</b>		
<b>AVVERTENZA</b>	<b>ATTENZIONE</b>	
<b>Indicazione di Pericolo</b>	H319 Provoca grave irritazione oculare.	



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El español



## AUTOSCREEN I/AUTOSCREEN II AUTOIMMUNE SCREENING TEST SYSTEM

Para uso diagnóstico *in vitro*.

AD AS148 (Rata)	48 Tests	AD AS196 (Rata)	96 Tests
AD AS348 (Ratón)	48 Tests	AD AS396 (Ratón)	96 Tests
AD AS248 (Rata)	48 Tests	AD AS296 (Rata)	96 Tests
AD AS448 (Ratón)	48 Tests	AD AS496 (Ratón)	96 Tests

### Aplicación:

La prueba de inmunofluorescencia indirecta se recomienda como prueba de criba para autoanticuerpos circulantes antinucleares (ANA), antimitocondriales (AMA), anti células parietales (PCA), anti músculo liso (AML) y antireticulina en suero del paciente.

### Principio:

La principal reacción de la prueba consiste en la unión de anticuerpos circulantes del suero del paciente a sus antígenos homólogos. Esto sucede durante el período de incubación en el que el suero recubre la superficie de antígeno. Tras un período de lavado para eliminar los anticuerpos humanos que no se han unido se procede a una reacción secundaria. El reactivo utilizado en la reacción secundaria es un conjugado de globulina humana marcados con fluoresceína. A continuación, la superficie de antígeno se aclara a fondo para eliminar el conjugado que no se ha unido y se examina en un microscopio de fluorescencia adecuado.

### Materiales suministrados:

Almacenamiento y estabilidad de los componentes

1. Rata o ratón (riñón/ estómago) almacenar a 2-8°C
2. Rata o ratón (hígado/ riñón/ estómago) almacenar a 2-8°C
3. Control positivo AMA almacenar a 2-8°C.
4. Control positivo homogéneo ANA almacenar a 2-8°C.
5. Control positivo de músculo liso AML almacenar a 2-8°C.
6. Control negativo universal (almacenar a 2-8°C.
7. Conjugado IgG (H y L) de FITC con azul de Evans (AD CGER2-AD CGER5), (almacenar a 2-8°C.
8. Sobre de tampón n.º AD PBS1 - tampón fosfato salino PBS, el tampón reconstituido no contiene conservantes y debe almacenarse a 2-8°C.
9. El medio de montaje FITC n.º AD TMM3 es estable cuando se almacena a 2-8°C.

### Otros materiales necesarios pero no suministrados:

Tubos de ensayo y gradilla, o sistema de micro-titulación  
Pipetas desechables  
Placa de tinción y pinzas para portaobjetos  
Cámara húmeda  
Matraz volumétrico (500 ml)  
Agua destilada  
Microscopio de fluorescencia  
Toallas de papel que no dejen pelusa

### Preparación del reactivo:

Sobre de tampón n.º AD PBS1. Rehidratar el tampón con 1 litro de agua destilada estéril.

### Toma de muestras:

Las muestras serológicas deben extraerse en condiciones asépticas. La hemólisis se evita separando rápidamente el suero del coágulo. Si se va a analizar en pocos días, el suero debe almacenarse a 2-8 °C. Puede conservarse de 3 a 6 meses a una temperatura de -20 °C o inferior. No conviene utilizar sueros lipémicos o fuertemente hemolíticos. Si las muestras se guardan a temperatura ambiente, es altamente recomendable añadir un conservante, como por ejemplo timerosal al 0,01% o azida sódica al 0,095%.

### Instrucciones del ensayo:

**Criba (screening):** diluya los sueros de prueba en proporción 1:20 en PBS.

**Titulaciones:** prepare diluciones de suero seriadas en proporción 2x a partir de 1:20 (es decir, 1:20, 1:40, 1:80, 1:160, etc.).

1. Una vez que el portaobjetos alcance la temperatura ambiente, rasgue el envoltorio tirando de la pestaña. Saque cuidadosamente el portaobjetos, evitando tocar las zonas de antígeno. El portaobjetos está listo para usar.
2. Ponga una gota del suero diluido (de 20 a 30  $\mu$ l) y de los controles en los pocillos de antígeno.
3. Coloque el portaobjetos con el suero del paciente y los controles en una cámara húmeda a temperatura ambiente (aproximadamente 20-24 °C) durante 30 minutos.
4. Retire el portaobjetos de la cámara húmeda. Utilice un frasco lavador para aclarar suavemente los restos de suero del portaobjetos procurando no dirigir el chorro directamente hacia el pocillo.
5. Lávelo en PBS durante cinco minutos. Repita la operación con PBS nuevo.
6. Coloque un secante sobre la poyata de laboratorio con la cara absorbente hacia arriba. Retire el portaobjetos del PBS, dele a vuelta de modo que la cara de tejido quede orientada hacia la cara absorbente del secante. Alinee los pocillos con los orificios del secante. Coloque el portaobjetos sobre el secante. No deje que el tejido se seque. Seque el dorso del portaobjetos con una toalla de papel que no deje pelusa. Al secarlo, aplique al portaobjetos la presión necesaria para absorber el tampón.
7. Ponga 1 gota (20-30  $\mu$ l) de conjugado en cada pocillo de antígeno. Repita los pasos 3 a 6.
8. Ponga 4 ó 5 gotas de medio de montaje en el portaobjetos.

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9. Coloque un cubreobjetos de 22 x 70 mm. Examine el portaobjetos con un microscopio de fluorescencia.  
Nota: para mantener la fluorescencia, guarde el portaobjetos montado en la nevera dentro una cámara húmeda y a oscuras.

### Control de calidad:

1. Para confirmar la reproducibilidad, sensibilidad y especificidad del ensayo es necesario incluir controles de suero positivo y negativo en los análisis todos los días.
2. El resultado del control de suero negativo debe ser poca (+) o ninguna fluorescencia. Si este control muestra una fluorescencia brillante, el problema puede estar en el control, el antígeno, el conjugado o en la técnica.
3. El resultado del control de suero positivo debe ser una fluorescencia brillante del orden de 3+ a 4+. Si este control muestra poca o ninguna fluorescencia, el problema puede estar en el control, el antígeno, el conjugado o en la técnica.
4. Además de los controles de suero positivo y negativo, debe analizarse un control de PBS para confirmar que el conjugado no tiñe el sustrato de antígeno de manera inespecífica. Si el antígeno presenta una fluorescencia brillante en el control de PBS, repita el análisis usando conjugado nuevo. Si el antígeno sigue presentando fluorescencia, el problema puede estar en el conjugado o en el antígeno.

### Interpretación del título:

**ANA:** El título es la dilución más alta de suero del paciente que muestra una fluorescencia débil (1+).

Menor de 1:20

Normal: descarta virtualmente la existencia de LES activo, siempre que el paciente no esté bajo terapia inmunosupresora o en remisión.

1:20 - 1:80

Prueba positiva, se da a menudo en la AR y otras enfermedades del tejido conectivo. Las muestras frescas deben probarse antes de dos semanas. Si el título aumenta, indica LES activo. Si no hay cambio en el título, puede haber otra enfermedad autoinmune en estado estático, un caso de LES tratado y controlado u otro proceso autoinmune.

1:160 o mayor

Sugiere claramente LES, aunque hay otras enfermedades autoinmunes y fármacos que pueden inducir estos títulos elevados.

**MA:** Cirrosis Biliar Primaria (PBC) es una colestasis intra hepática crónica que se encuentra más frecuentemente en la mujer que en el hombre con una incidencia mayor en el grupo de edades entre 30 y 60 años. El diagnóstico de la PBC está basada en observaciones clínicas, hallazgos histológicos en biopsias de hígado, por incremento de la actividad de la fosfatasa alcalina, niveles de IgM elevados y presencia de anticuerpos mitocondriales. El resultado positivo se observa como una fluorescencia granular en el citoplasma de los túbulos renales. La fluorescencia está limitada al citoplasma del epitelio tubular distal y proximal. La fluorescencia de otros antígenos celulares así como el núcleo, músculo liso, ó fluorescencia no-granular limitada a la porción central (lumen) de los túbulos proximales no se informaran como MA positivos.

El título es la dilución más alta del suero de paciente que presenta una débil (1+) fluorescencia del epitelio renal tubular.

Menos de 1:20

Normal, negativo

1:20 - 1:80

Positivo, Sugiere enfermedad hepática. Repetir con muestra fresca en dos semanas.

1:160 ó mayor

Presumiblemente cirrosis biliar primaria.

El rango de títulos en PBC es desde 1:10 a 1:6000 con un 50% de los pacientes con PBC que presentan títulos de 1:2000 a 1:6000. Los títulos de MA no presentan cambios con el tiempo ó la terapia y no sirven para monitorizar la respuesta de la terapia.

**SMA:** ACH es una enfermedad crónica del hígado afectando principalmente a las mujeres jóvenes pero también afecta a ambos sexos a todas las edades. Es caracterizada en biopsias hepáticas por deterioro de la función hepática debido por necrosis de las células del parénquima hepático en áreas linfocíticas y de filtración celular plasmática. Se considera como resultado positivo unas manchas brillantes difusas citoplasmáticas en las capas de músculo liso de la mucosa muscularis que se encuentra en el estómago de rata ó ratón. La fluorescencia puede estar también presente en la paredes capilares de las capas gástricas y rodeando arterias y venas. La fluorescencia de otros antígenos celulares así como el núcleo, células parietales ó tejido conjuntivo no deberían de informarse como positivos de SMA.

El título es la dilución más alta del suero de paciente que presenta una débil (1+) fluorescencia del epitelio renal tubular.

Menos de 1:20

Normal, negativo

1:20 - 1:80

Positivo, Sugiere enfermedad hepática. Repetir con muestra fresca en dos semanas.

1:160 ó mayor

Presumiblemente hepatitis crónica activa.


### Resultados:

**ANA:** los resultados positivos se observan en forma de uno de los cuatro patrones básicos de tinción vistos individualmente o en diversas combinaciones. Los patrones característicos se ven mejor cuando se observan bajo objetivos de alto aumento en seco.

1. Homogéneo (difuso) - Se observa una fluorescencia uniforme, finamente difusa en todo el núcleo.
2. Periférico (en el borde, filamentosos) - La fluorescencia de la membrana nuclear es más intensa que la del área central.
3. Moteado - Numerosas "motas" pequeñas de fluorescencia por todo el núcleo.
4. Nucleolar - Los nucleolos se tiñen uniformemente y aparecen como de 1 a 5 grandes áreas esféricas de fluorescencia dispersas por el núcleo.

**Precauciones:**

1. Todos los componentes humanos han sido probados mediante radioinmunoensayo para (HBsAg) y HTLVIII/LAV con un método aprobado por la FDA, y han dado resultados negativos (no repetidamente reactivos). No obstante, esto no garantiza la ausencia de HBsAg o HTLVIII/LAV. Todos los componentes humanos deben manipularse con las debidas precauciones.
2. Los controles y el conjugado contienen sodium azide (0.095%).
3. No utilice ningún componente que haya sobrepasado la fecha de caducidad.
4. Para garantizar la validez de los resultados, siga las instrucciones del procedimiento exactamente como aparecen aquí.
5. Para uso diagnóstico in vitro.
6. Sujete los portaobjetos por los bordes, ya que la presión directa sobre los pocillos puede estropear el antígeno.
7. Una vez iniciado el procedimiento, no deje que el antígeno de los pocillos se seque. Esto podría dar falsos negativos o producir artefactos innecesarios.
8. Use distintas puntas de pipeta para cada una de las muestras y reactivos para evitar la contaminación cruzada.
9. Los reactivos deben inspeccionarse en busca de evidencias de contaminación bacteriana o micótica.
10. No reutilizar portaobjetos.

<b>Componente</b>	AD PBS1 PBS Sobre de tampón AD TMM3 medio de montaje	<b>Consejos de prudencia</b> <b>Prevención:</b> P264 Lavarse o concienzudamente tras la manipulación. P280 Llevar guantes/prendas/gafas/máscara de protección.
<b>Pictograma</b>		<b>Respuesta:</b> P305+P351+P338 EN CASO DE CONTACTO CON LOS OJOS: Aclarar cuidadosamente con agua durante varios minutos. Quitar las lentes de contacto, si lleva y resulta fácil. Seguir aclarando.
<b>Palabra Clave</b>	<b>ATENCIÓN</b>	P337+P313 Si persiste la irritación ocular: Consultar a un médico.
<b>Indicación de Peligro</b>	H319 Provoca irritación ocular grave.	








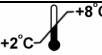


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<b>EC</b>	<b>REP</b>
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	Manufactured by Prodotto da Fabricado por Fabriqué par hergestellt von
<b>REF</b>	Catalog number Numero di catalogo Número de Catálogo Numéro de catalogue Katalognummer
<b>LOT</b>	Lot Lotto Lote Lot Charge
<b>EC</b> <b>REP</b>	EC Authorized Representative Rappresentante Autorizzato CE Representante Autorizado CE CE Représentant autorisé EG autorisierter Bevollmächtigter
	EC Declaration of Conformity Dichiarazione di Conformità CE Declaración de Conformidad CE CE Déclaration de Conformité EG Konformitätserklärung
	Number of tests Numero di test Número de determinaciones Nombre de tests Anzahl der Teste
	See instructions for use Vedere le istruzioni per l'uso Consultar la instrucciones de uso Voir instructions Gebrauchsanweisung beachten
	Expiration date Data di scadenza Caducidad Date d'expiration Haltbarkeitsdatum
	Store at 2-8°C / 35-46°F Conservare a 2-8°C/35-46°F Almacenar a 2-8°C / 35-46°F Conservar à 2-8°C Bei 2-8°C / 35-46°F lagern
	Caution Attenzione Precaución Précautions Achtung
	Potential biological risk Potenziale rischio biologico Riesgo potencial biológico Risque biologique potentiel Potentielle biologische Gefährdung
<b>RFU</b>	Ready for use Pronto all'uso Listo para su uso Prêt à l'emploi Gebrauchsfertig
<b>IVD</b>	For in vitro diagnostic use Per uso diagnostico <i>in vitro</i> Para uso solo in vitro Usage in vitro Für in-vitro diagnostische Verwendung
<b>RUO</b>	For research use only Solo per ricerca Para uso solo en investigación Pour recherche Nur für Forschungszwecke
<b>IUO</b>	For investigational use only Solo per uso investigativo Para uso solo en investigación Pour investigation Nur für Forschungszwecke
<b>IFA/DFA</b> <b>PBS</b>	Phosphate Buffered Saline Tampone salino fosfato Fosfato Salino Tamponado Tampon phosphate salin PBS Phosphatgepufferte Salzlösung
<b>SOR</b>	Sorbent Assorbent Sorbente Absorbant Sorbens

<b>SLIDE</b>	Tissue Substrate Slide Vetrini con substrate di tessuto Porto objetos de Sustrato de Tejido Lame portant le substrat tissulaire Gewebe substrat-Objektträger
<b>MM</b>	Mounting Medium Mezzo di montaggio Medio de Montaje Liquide de montage Eindeckmedium
<b>10x</b>	Concentration Concentrazione Concentración Concentration Konzentration
<b>ENS</b>	Enhancement solution Soluzione di rinforzo Solución de realce Solution d'amplification Verstärkungslösung
<b>WASHB</b>	Wash Buffer Tamponi di lavaggio Tampon de lavado Tampon de lavage Waschpuffer
<b>MPS</b> <b>12x8</b>	Microplate Strips Strip per Micropietra Tiras de micro placa Microplaque Mikrotiterplattenstreifen
<b>CONJ</b>	Conjugate Coniugato Conjugado Conjugué Konjugat
<b>SUB</b>	Substrate Substrato Sustrato Substrat Substrat
<b>STOP</b>	Stop Solution Soluzione bloccante Solución de Parada Solution d'arrêt Stopplösung
<b>CAL X</b>	Calibrator(s) Calibratore (i) Calibrador (s) Calibrateur(s) Kalibrator(en)
<b>CONTROL -</b>	Negative Control Controllo Negativo Control Negativo Contrôle négatif Negative Kontrolle
<b>CONTROL +</b>	Positive Control Controllo Positivo Control Positivo Contrôle positif Positive Kontrolle
<b>CONJ</b> <b>CNS</b>	Counterstain Colorante di contrasto Contraste Contre-colorant Gegenfärbung
<b>CS</b>	Coverslip Coprioggetto Cubre portaobjetos Lamelles couvre-objet Deckgläser
<b>CONJ</b> <b>+</b>	Positive Conjugate Coniugato Positivo Conjugado Positivo Conjugué Positif Positivekonjugat
<b>CONJ</b> <b>-</b>	Negative Conjugate Coniugato Negativo Conjugado Negativo Conjugué Négatif Negativkonjugat
<b>DIL</b>	Sample Diluent Diluente del campione Diluyente de muestra Tampon de dilution Probenverdünnungslösung