1. Intended Use

AESKUSLIDES ANA/AMA/ASMA/APCA is a indirect immunofluorescence assay to detect autoantibodies against mitochondrial - / smooth muscle - antigen / circulating parietal cells in human serum.

2. Clinical Application and Principle of the assay

Autoimmune diseases are caused by a disorder of the cellular and / or humoral immunological reaction. These reactions which normally occur against external influences may under certain circumstances turn against the body itself and thereby cause various diseases.

ANA The presence of anti-nucleär-Antibodies maybe detected in every provided tissue by positive nucleär fluorescence.

AMA Anti - mitochondrial antibodies (AMA) predominantly react with the inner membrane of the mitochondria (rich in phospholipids). AMA mostly appear with diseases such as primary biliary cirrhosis, pseudo - LE syndrome and various forms of chronic aggressive hepatitis. High titre AMA results are mainly found with non - suppurating gallbladder infections or primary biliary cirrhosis (positive results at about 90%). In these cases antibodies appear before the clinical symptoms and will hardly be influenced by therapy during the course of the disease.
Low antibody titres are observed with scleroderma, Sjögren syndrome, rheumatoid arthritis and other autoimmune diseases.

**ASMA** Antibodies against smooth, unstriated muscle occur in various liver diseases, for example acute and chronic hepatitis, primary biliary cirrhosis, and other forms of liver cirrhosis. Furthermore, the detection of ASMA supports the diagnosis of SLE, infectious mononucleosis, breast and ovarian carcinoma and malignant melanomas.

**APCA** Circulating antibodies against the structures of the parietal cell of the gastric mucosa are generally due to pernicious anemia. They may, however, also be detected with other diseases of the stomach (chronic atrophic gastritis, gastric ulcer), diseases of the thyroid (Hashimoto’s thyroiditis, myxedema) and more rarely with hypoferric anemia, diabetes mellitus and in older patients.

**Antigen Characterisation** Antigens substrat: mouse stomach, - kidney, -liver

**Cross-reactivity** Cross-reactivities are unknown.

The detection of antibodies is based on the principle of indirect immunofluorescence assay (IIFA). Glass microscope slides are coated with tissue sections or cells (HEp-2-cells (ANA), Granulocytes (ANCA) or Crithidia luciliae (nDNA)). If the patient’s serum contains specific antibodies they will bind during the first incubation. After removing unbound material by washing steps, bound antibodies are detected by Fluorescein conjugated anti-human immunoglobulins during the second incubation. A specific green fluorescent staining of antigen-antibody-complex can be visualized with the aid of a fluorescent microscope.

### 3. Kit Contents

**To be reconstituted:**

- **50 x Washing Buffer**
  - Dilute the concentrated washing buffer 1:50 in distilled water (e.g.: 20 ml + 980 ml)
  - Containing: PBS, sodium azide (preservative)

- **10 x Sample Buffer**
  - Dilute the concentrated sample buffer 1:10 in distilled water (e.g.: 20 ml + 180 ml)
  - Containing: PBS, BSA, sodium azide (preservative)
**Ready to use:**

Glass microscope slides 10 x 5 wells, coated with tissue sections

- **Positive Control** 0.5 ml
  Containing: Human serum (diluted), sodium azide (preservative)

- **Negative Control** 0.5 ml
  Containing: Human serum (diluted), sodium azide (preservative)

- **Fluorescein (FITC) conjugated anti-human IgAGM(H+L Chain)** 2.0 ml

- **Evans Blue 2%** 3.0 ml

- **Mounting Medium** 12 ml

**Materials required but not provided:**

- Distilled water,
- Test tubes for sample dilution,
- Measuring flask,
- Volumetric pipette,
- Timer,
- Fluorescence microscope with FITC system, (490 nm excitation filter, 510 nm barrier filter)
- Incubator tray,
- Staining dish,
- Pipetting tips,
- Cover slips (24x60 mm).

**4. Storage and Shelf Life**

Store all reagents at 2°C - 8°C / 35 - 46°F, protected from intense light. The expiration date of each component is indicated on the respective vial label. Do not use reagents beyond the expiration date.

Store all reagents and the slides at 2-8°C/35-46°F, in their original containers. Once prepared, reconstituted solutions are stable for 1 month at 4°C/39°F, at least. **Reagents and the slides shall be used within the expiry date indicated on each component, only.**
5. Precautions of Use

5.1 Health hazard data

*This product is for in vitro diagnostic use only.* Thus, only staff trained and specially advised in methods of in vitro diagnostics may perform the kit. Although this product is not considered particularly toxic or dangerous in conditions of normal use, refer to the following for maximum safety:

**Recommendations and precautions**

This kit contains potentially hazardous components. Though kit reagents are not classified being irritant to eyes and skin we recommend to avoid contact with eyes and skin and wear disposable gloves. All human source material used for some reagents of this kit (controls e.g.) has been tested by approved methods and found negative for HBsAg, Hepatitis C and HIV. However, no test can guarantee the absence of viral agents in such material completely. Thus handle kit controls and patient samples as if capable of transmitting infectious diseases and according to national requirements.

5.2 General directions for use

1. Do not pipette by mouth. Do not smoke, eat or drink when manipulating the kit.
2. Do not mix or substitute reagents from different lot numbers. This may lead to variations in the results.
3. Keep all flasks sealed after use to avoid bacterial contamination.
4. Always pipette all solutions with new sterile pipetting tips.
5. Never expose components to higher temperature than 37°C / 98,6°F.
6. Never let the slide wells dry out during the whole procedure.
7. Never freeze the slides.

*Each laboratory should establish its own in house controls upon its own techniques, controls, equipment and patient population according to their own established procedures.*

*A definite clinical diagnosis should not be based on the results of the performed test only, but should be made by the physician after all clinical and laboratory findings have been evaluated.*
6. Sample Collection, Handling and Storage

**Preparation of samples**

Use preferentially freshly collected serum samples. Blood withdrawal must follow national requirements. Collect blood samples aseptically. Lipemic, icteric, hemolyzed or microbially contaminated specimens may cause interference. Sera with particles should be cleared by low speed centrifugation (<1000 x g). Blood samples should be collected in clean, dry and empty tubes. After separation, the serum samples should be used immediately, respectively stored tightly closed at 2-8°C/35-46°F up to three days, or frozen at -20°C/ -4°F for longer periods. Avoid repeated freezing and thawing.

7. Assay Procedure

**7.1 Preparation prior to pipetting**

Allow all components to reach room temperature (20 - 26°C / 64 - 78,8°F) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test.

1. Preparation of the sample buffer and the washing buffer:
   Dilute the concentrated sample buffer and washing buffer 1:10 respectively 1:50 with distilled water.

2. Dilution of samples:
   Dilute patient sera (for screening titer see paragraph 10) with 1x sample buffer.
   Controls are ready to use!

3. Prepare a protocol:
   We recommend the use of the provided test protocol on page 11.

**7.2 Test procedure**

1. Remove required slide(s) from pouch(es), mark them. Do not touch the wells. Do not allow the slides to dry out.

2. **First incubation**
   Pipette a adequate volume of each diluted serum and controls (ready to use) into the appropriate wells, avoid direct contact of pipette with slide surface.
Make sure that each well is completely covered with a corresponding serum. It is important to use as much testmaterial as necessary to cover the well completely. But avoid a running between the wells because this may cause incorrect results. Incubate slide(s) 30 minutes at room temperature in a moist incubator tray.

3. **Washing**
   After incubation remove slides from incubator tray and rinse briefly with washing buffer using a pipette. Do not squirt buffer directly on the wells.

   **NOTE:** To avoid cross contamination on slide, direct washing buffer stream along midline of slide.

   Wash slide(s) 15 minutes with washing buffer in a slide staining dish. For optimal results it is necessary to change the buffer solution after 5 minutes for three times. Lift slide(s) from staining dish and carefully remove excess washing buffer.

   **NOTE:** It is important that slide wells do not dry out during the procedure or damage to the substrate may occur. Please do not blot or dry the slide in any manner or allow slide to sit without fluorescent antibody reagent for longer than some seconds.

4. **Second incubation**
   After the washing procedure return slide immediately to incubator tray and cover each well with 30µl of FITC-conjugate. Incubate slide(s) 30 minutes at room temperature in the dark.

5. **Washing**
   After incubation remove slides from incubator tray and rinse briefly with washing buffer using a pipette. Do not squirt buffer directly on the wells. Wash slide(s) 10 minutes with washing buffer in a slide staining dish. For optimal results it is necessary to change the buffer solution after 5 minutes for two times.

6. **Optional counterstain:**
   Dilute counterstain (Evans Blue)1:3000 in washing buffer and mix well. Tilt counterstain into the staining dish and incubate the slides for 3-5 minutes in it. Evans Blue covers unspecific background fluorescence.
Remove slide(s) after max. 5 minutes and rinse briefly with washing buffer.
Remove excess washing buffer.
Please do not blot or dry the slide in any manner.

7. **Mounting**
Add an adequate volume of mounting medium along midline of each slide.
Carefully place coverslip in position, avoiding air bubbles.

8. Read slide(s) immediately at 400 - 800 x total magnification with a fluorescent microscope.(490 nm excitation filter, 510 nm barrier filter)

### 7.3 Work flow

**For the test procedure see paragraph 9.**

- Pipette each diluted serum and controls into the appropriate wells.
- Place a small volume of deionized or distilled water in an incubator tray and place slide(s) on supports in the incubator tray.
- Incubate slide(s) 30 minutes at room temperature (20 - 26°C / 64 - 78.8°F).
- Remove slides from incubator tray and rinse briefly with washing buffer using a pipette. Wash slides 5 minutes for three times in a staining dish.
- Cover each well with 30µl of FITC - conjugate inside the incubator tray.
- Incubate slide(s) 30 minutes at room temperature (20 - 26°C / 64 - 78.8°F) in the dark.
- Remove slides from incubator tray and rinse briefly with washing buffer using a pipette. Wash slides 5 minutes for two times in a staining dish.
- Dilute counterstain (Evans Blue) 1:3000 in washing buffer.
- Incubate slides with diluted counterstain 3-5 minutes.
- Remove slide(s) after max. 5 minutes and rinse briefly with washing buffer,
- Add mounting medium along midline of each slide
- Read slide(s) immediately at 400 - 800 x total magnification with a fluorescent microscope (490 nm excitation filter, 510 nm barrier filter).

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<td>Buffer squirted directly on the substrate in the well</td>
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<td>- Serum dried in the well, fluorescence stronger at the edge</td>
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<td>Background fluorescence</td>
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10. Interpretation

EMA

Screening titre 1:5

The endomysium is the supporting structure that surrounds the combination of smooth and stiatted muscle fibres that are found in the middle of the third of oesophagus. It contains collagen and reticulin together with the endomysial target which has yet to be characterised.

Gliadin is the ethanol-soluble fraction of gluten which is the inflammatory antigen in coeliac disease. Antibodies are associated with coeliac disease and dermatitis herpetiformius.

AMA

Screening titre 1:20

Granular fluorescense of the mitochondria is seen in the cell cytoplasm of the gastric cells, kupffer cells, hepatocytes and the renal tubular cells.

ANA/AMA/ASMA/APCA

Screening titre 1:20

The combined tissue section allows the differentiation of various antibodies within one test area and may thus be applied as a diagnostic test for the following autoimmune antibodies. (In case of diverse antibodies it is advisable to look for further diagnostic identification). The evaluation should always be performed with the positive and negative controls.

ANA: The presence of anti-nucleär-Antibodies maybe detected in every provided tissue by positive nuclear fluorescence.

AMA: The presence of anti - mitochondrial antibodies displays a fine granular cytoplasmatic fluorescence of the renal tubules. The distal tubules are richer in mitochondria and therefore display a more intense fluorescence in contrast to the proximal tubules.

ASMA: The presence of ASMA is indicated by a fluorescence of the smooth muscle fibres of the blood vessels of kidney and stomach, of muscularis mucosa, tunica muscularis ventriculi as well as the interglandular contractile fibrillae of the stomach mucosa.
**APCA:** Finely granular fluorescence of the parietal cells in the gastric mucous membrane indicate APCA. Since AMA also react with parietal cells, anti-mitochondrial antibodies (renal tubules) should be excluded in the APCA assessment.

**AMA:** 1:20-1:80 (e.g. 10µl Serum + 790µl sample buffer)
A positive reaction is found in several liver diseases

>1:160 (e.g. 10µl Serum + 1590µl sample buffer)
Indicates biliary cirrhosis. AMA titres remain constant over a long period of time, and despite therapy so that the determination of titre as a measure of therapy control is not useful.

**ASMA:** 1:20-1:80 (e.g. 10µl Serum + 790µl sample buffer)
A positive reaction is found in several liver diseases, viral hepatitis and primary biliary cirrhosis. However the titres here may fall below the determination border. Low titres may be observed in patients with gallbladder infections, alcoholic cirrhosis, SLE and in 2% of the normal, healthy population.

>1:160 (e.g. 10 µl Serum + 1590 µl sample buffer)
Chronic active hepatitis is indicated. In contrast to viral hepatitis the titres fall only slightly and may persist for several years. Patients with infectious mononucleosis may also show high ASMA titres.

**APCA:** The APCA titre provides no information about the disease state of the patient. The antibody determination should be evaluated together with the measurement of Intrinsic factor and / or histopathology results.

The appropriate end titer is that in which the patient serum shows a simple positive fluorescence. Weak fluorescence of the cell nuclei with titres between 1:20 and 1:80 or vagueness with respect to the clinical results should be checked by way of monitoring control. In such a case the samples should be collected about every 3 weeks and similarly tested.

Examples for dilution:

1:40 20µL Serum + 780µL Samplebuffer
1:80 10µL Serum + 790µL Samplebuffer (respectively 1:2 of the „1:40“Dilution)
1:160 10µL Serum + 1590µL Samplebuffer
1:320  5µL Serum + 1595µL Samplebuffer
1:640  2.5µL Serum + 1597.5µL Samplebuffer