Instructions For Use

Valid as of 21.02.2023

AKLIDES® AMA

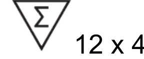
4117

Indirect immunofluorescence assay
for the determination of IgG antibodies in
human serum against mitochondrial antigens

Medipan GmbH
Ludwig-Erhard-Ring 3
15827 Blankenfelde-Mahlow OT Dahlewitz
Germany

Phone: +49 33708 4417 0
Fax: +49 33708 4417 25

info@medipan.de
www.medipan.de



1 Intended Purpose

The AKLIDES® AMA is an immunofluorescence assay (IFA) for the qualitative determination of IgG antibodies in human serum against mitochondrial antigens on tissue sections of rat kidney.

The AKLIDES® AMA is intended as an aid in the diagnosis of diagnosis of primary biliary cirrhosis (PBC) in conjunction with other clinical and laboratory findings.

The immunoassay is designed for manual processing and automated imaging using the AKLIDES® or akiron® system.

The immunoassay is designed for professional *in vitro* diagnostic use.

2 Diagnostic Relevance

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.

Anti-mitochondrial antibodies (AMA) predominantly react with the inner membrane of the mitochondria (rich in phosholipids). AMA mostly appear with diseases such as primary biliary cirrhosis, pseudo-LE syndrome and various forms of chronic aggressive hepatitis. High titer 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 titers are observed with scleroderma, Sjögren syndrome, rheumatoid arthritis and other autoimmune diseases.

Mitochondria are components of different cells; detection of AMA by indirect immunofluorescence is therefore possible on several tissues. Rat kidney is used as reference substrate showing a characteristic pattern: a fine granular fluorescence in the cytoplasm of the kidney tubules. Distal tubules have a higher content of mitochondria and therefore display a more intensive fluorescence pattern in contrast to the proximal tubules.

3 Test Principle

The IFA (indirect immunofluorescence assay) is an immuno-assay for the determination of specific antibodies. Tissue sections or cells containing the antigen of interest are immobilized on slides. If specific antibodies are present in the patient´s sample, they bind to the antigen in the tissue. A secondary antibody conjugated with fluorescein-isothiocyanat (FITC) detects the generated immune complex. The slides are examined using a fluorescence microscope. A specific fluorescent staining pattern demonstrates the presence of specific antibodies in the patient´s sample.

4 Test Components

Component	Description
Substrate slide **A** **SLIDE 4** 12 pieces	12 slides (ready-to-use), 4 wells per slide, each well coated with cryostat sections of rat kidney
Negative control **CI** **CONTROL | -** 1 x 2.0 mL	Dilution of human serum (ready-to-use; contains sodium azide)
Positive control **CII** **CONTROL | +** 1 x 2.0 mL	Dilution of human serum (ready-to-use; contains sodium azide)
PBS buffer **B** **WASHB** 2 x 10 g	PBS buffer (dry substance for 2 x 1000 mL)
Conjugate IgG **D** **CONJ** 1 x 5.0 mL	Polyclonal anti-human IgG antibody conjugated to FITC (ready-to-use; contains sodium azide)
Mounting medium **G** **MOUNT** 1 x 3.0 mL	PBS buffered solution with glycerol and antifading reagent (ready-to-use; contains DAPI and sodium azide)
Blotting templates **F** **TEMPL** 12 pieces	Blotting templates
Coverslips **H** **COVER** 12 pieces	Coverslips
QC Certificate 1 piece	-
Instructions for Use 1 piece	-

5 Materials required but not provided

- Common laboratory equipment
- Precision pipettes (5 – 1000 µL), and disposable tips
- Graduated cylinders (100 – 1000 mL)
- Sample tubes for the preparation of dilutions and racks
- Vortex mixer or other rotators
- Moist chamber
- Plastic squeeze wash bottle
- Coplin jars or staining dishes with slide racks
- AKLIDES® or akiron® system or equivalents for imaging
- Adsorbent paper or paper towel
- Distilled or de-ionized water

6 Storage and Stability

Upon receipt, all test components must be stored at 2 °C to 8 °C, preferably in the original kit box. If stored properly in their original containers, all components are stable until their expiry date. After opening, all kit components are stable for at least 2 months, if stored properly.

7 General Information

This product is for *in vitro* diagnostic use only. The instructions for use must be carefully read before use. They are valid only for the present product with the given composition and must be strictly followed to ensure reliable test results. Deviations can lead to erroneous test results. Components must not be exchanged by test reagents of different lots or of other manufacturers.

Contamination of reagents must be avoided by use of aseptic techniques when removing aliquots from the vials. After use, reagent vials must be tightly closed with their corresponding caps.

Cross-contamination of samples or reagents can lead to inconsistent test results and must be avoided by use of consistent pipetting techniques.

Exposure of reagents to strong light must be avoided throughout the entire test procedure and storage.

Insufficient washing will result in poor precision and elevated measurement signals. After each washing step any residual fluid has to be removed completely.

8 Preparation

8.1 Preparation of Reagents

All components including the slides must be brought to room temperature (RT: 18 °C to 25 °C) before use for at least 30 min. All liquid components must be mixed gently to ensure homogeneity.

8.1.1 Slides

The slides are individually sealed with a desiccant in aluminum bags. Remove slides carefully from the bags directly before use.

8.1.2 Controls

The positive and the negative controls are ready-to-use and must not be diluted any further. Controls must be used in each test run. Laboratories can also validate their own control samples and use them alternatively.

8.1.3 PBS Buffer

A PBS buffer mixture must be dissolved in 1.0 L distilled water by stirring or shaking before use. The resulting washing solution should have a pH of 7.4 ± 0.2 and is stable for four weeks when stored at 2 °C to 8 °C. Do not use if the pH changes, the solution turns cloudy, or if precipitates form.

8.1.4 Conjugate

The conjugate is ready-to-use. Exposure to strong light should be avoided.

8.1.5 Mounting Medium

The mounting medium is ready-to-use.

8.2 Preparation of Samples

8.2.1 Sample Material

The use of freshly collected serum from blood taken by venipuncture is recommended. The use of icteric, lipemic, hemolytic or bacterially contaminated samples should be avoided. Insoluble substances must be removed from the sample by centrifugation. Samples must not be thermally inactivated.

8.2.2 Sample Dilution

The samples must be diluted 1:20 (e. g. 10 µL + 190 µL) with PBS buffer and mixed thoroughly. Building of foam should be avoided.

8.2.3 Sample Storage

Samples may be kept at 2 °C to 8 °C up to three days. Long-term storage requires -20 °C. Repeated freezing and thawing should be avoided. For multiple use, samples should be aliquoted and kept at -20 °C.

9 Test Performance

9.1 Procedure

A sufficient number of slides must be prepared. Do not touch the wells and do not allow the substrate slides to dry during the entire test procedure. The indicated incubation times and temperatures must be adhered to and significant time shifts during pipetting samples and reagents must be avoided.

Step	Description
1. Addition of, controls and diluted samples	Add 50 µL ready-to-use controls and 50 µL of diluted samples per well. Make sure each well is completely covered
2. Incubation	Incubate for 30 min. at RT in a moist chamber
3. Wash cycle	Aspirate the liquid from each well or rinse gently with PBS wash solution using a squeeze wash bottle. Do not focus the PBS stream directly onto the wells. To prevent cross contaminations avoid rinsing from one well across other wells. For multi row slides run PBS stream from the midline of the slide successively along both rows to the edge. Wash 2 x 5 min in changing PBS solution in a Coplin jars or staining dishes, agitate gently at entry and prior to removal. Remove the slides from the Coplin jars and carefully dry the slide by tapping the edge of the slide onto absorbent towel, carefully dry the slide around the wells using a blotting template to remove residual droplets.
4. Addition of conjugate	Add 50 µL ready-to-use conjugate to each well. Make sure each well is completely covered.
5. Incubation	Incubate for 30 min. at RT in moist chamber protected from direct light.
6. Wash cycle	Repeat entire wash cycle from 3.
7. Addition of mounting medium	Apply 1 drop per well ready-to-use mounting medium
8. Coverage	Carefully place one coverslip onto the slide allowing the mounting medium to form a uniform layer between coverslip and slide. Avoid air bubbles. Wipe off excess mounting medium from the edges and the bottom of the slide using absorbent paper.
9. Analysis	Analyze the slide using the AKLIDES® system with 490 nm excitation and 520 nm emission wavelengths within 24 hours.

9.2 Automation

Automated processing of the immunoassay must be performed analogous to manual use and validated by the user.

10 Test Evaluation

10.1 Metrological Traceability

The immunoassay is calibrated using internal samples as references.

10.2 Evaluation

The AKLIDES® or akiron® system provides images for qualitative evaluation by the operator.

A serum dilution is considered negative for AMA if the tissue lacks the specific fluorescence pattern in the kidney tubular cells.

A serum dilution is considered positive for AMA if a clearly discernable granular cytoplasmatic fluorescence in the tubular cells of the kidney is visible. The distal tubules are richer in mitochondria and therefore display a more intensive fluorescence in contrast to the proximal tubules.

10.3 Criteria of Validity

Test runs are only valid if the following criteria of validity are fulfilled:

- The negative control must be evaluated negative.
- The positive control must be evaluated positive.

If these criteria are not met, the test is not valid and must be repeated.

10.4 Troubleshooting

In case of an invalid test run, the expiry dates and storage conditions, incubation times and temperatures, and precise calibration of all instruments used should be verified. If no reason for an invalid test run could be identified, please contact the supplier or manufacturer of the product. A troubleshooting guide is available upon request.

10.5 Reference Ranges

The reference ranges are indicated below:

	Interpretation
Antibody titer < 1:20	negative
Antibody titer ≥ 1:20	positive

As a result of different seroprevalences in individual regions, each laboratory should verify the reference ranges by own analysis and adapt, if necessary.

10.6 Interpretation of Test Results

A positive test result indicates the presence of specific antibodies. A negative result indicates the absence of specific antibodies, but does not exclude the possibility of an autoimmune reaction. In case of a borderline test result, a reliable evaluation is not possible.

The detection of antibodies largely depends on the tissue section used. Antibodies to other parts of the tissue section could lead to a respective fluorescence pattern (e.g. cell nuclei, smooth muscle). These patterns are to be judged negative in relation to AMA but can indicate other autoimmune diseases and should be confirmed using the reference tissue respectively.

10.7 Limitations of the Method

Samples and wash solutions contaminated with bacteria or fungi could cause unspecific staining of the cell culture substrate.

Proteolytic enzymes in patient samples could result in damage or loss of the tissue sections fixed on the slide.

The interpretation of test results must always be considered in combination with the clinical picture of the patient. The diagnosis should not be based on the results of a sole diagnostic method. All clinical and laboratory findings should be evaluated to state a diagnosis. For confirmation, further investigations should be carried out.

11 Performance Characteristics

11.1 Analytical Performance Characteristics

11.1.1 Analytical Sensitivity and Specificity

No cross-reactivities of antibodies against similar antigens have been observed.

11.1.2 Precision

The precision of test results was assessed by the determination of the intra- and interassay variation by the analysis of multiple samples with different antibody activities. No differences in the qualitative evaluation have been detected.

11.2 Diagnostic Performance Characteristics

11.2.1 Diagnostic Sensitivity and Specificity

Sensitivity and specificity were assessed by the analysis of samples from patients with Primary biliary cirrhosis and unselected blood donors.

	Diagnostic Performance
Sensitivity	> 90 %
Specificity	> 95 %

12 Warnings and Precautions

The product is designed exclusively for *in vitro* diagnostic use by qualified, authorized and trained personnel. All test components and human samples should be handled with care as potentially hazardous. Good laboratory practices (GLP) and all relevant regulations should be adhered to.

In case the product is damaged or product information including labelling is wrong or incorrect, please contact the manufacturer or supplier.

This product contains preparations of human and / or animal origin. Any material derived from human body fluids or organs used for the preparation of components were tested and found negative for HBsAg (Hepatitis B-Virus-surface Antigen) and anti-HIV as well as anti-HCV antibodies. However, all components and all patient samples should be handled as potentially hazardous in accordance with national laws and appropriate guidelines on biological safety.

As the product contains potentially hazardous materials, the following precautions should be followed: Do not smoke, eat or drink while handling kit material or samples. Avoid direct contact to kit material or samples by wearing protective gloves laboratory coat and safety glasses. Never pipette material by mouth. Wipe up spills promptly and wash the affected surface thoroughly with a decontaminant. Wash hands thoroughly after use.

Some of the reagents contain sodium azide (< 0.1 %) as a preservative and must not be swallowed or allowed to come into contact with skin or mucosa. The possible formation of heavy metal azides in the drainage has to be prevented by sufficient rinsing with water.

Some of the reagents contain DAPI (5 µg/mL) as a staining agent and must not be swallowed or allowed to come into contact with skin or mucosa.

The information in the safety data sheet on possible hazards, first aid measures, measures in the event of the unintentional release of large quantities, handling and storage, personal protective equipment, information on disposal as well as information on toxicology must be observed.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the member state in which the user and/or the patient is established.

13 Disposal

For decontamination and disposal the recommendations of the CDC as well as the relevant local and national environmental

guidelines and regulations should be adhered to. Samples, potentially contaminated materials and infectious waste must be decontaminated, e.g. by autoclaving for 20 min. at 121 °C.

14 References

- Humbel RL: Autoanticorps et Maladies Auto-Immunes, Elsevier 2nd edition, 1997.
- Jones DE: Autoantigens in primary biliary cirrhosis. J. Clin. Pathol. 2000, 53, 813 – 21.
- Lyerla HC, Forrester FT: The Immunofluorescence (IF) test. In: Immunofluorescence methods in virology, USDHHS, Georgia, 1979, 71 – 81.

15 Symbols

Symbol	Meaning
	Manufacturer
CE	CE marking of conformity
IVD	*In vitro* diagnostic medical device
REF	Catalogue number
UDI	Unique device identifier
LOT	Batch code
	Temperature limit
	Use-by date
	Consult instructions for use
Σ	Contains sufficient for <n> tests
	Do not re-use
	Caution
	Biological risk
	Keep away from sunlight
SLIDE x	Slide with x wells
CONTROL -	Negative control
CONTROL +	Positive control
CONJ	Conjugate
WASHB	Wash buffer
MOUNT	Mounting medium
TEMPL	Blotting templates
COVER	Coverslip

16 Changes

Changes in current Instructions for Use	
Current Version	010/02.2023
Summary of Changes	Editorial changes in Chapter 8.1.4.