EUROPLUS LKS Mosaic
Instructions for the indirect immunfluorescence test

ORDER NO.	ANTIBODIES AGAINST	SUBSTRATE	SPECIES	FORMAT SLIDES x FIELDS
FA 1620-1 to FA 1620-5 (see page 16)	mitochondria (AMA) smooth muscles (ASMA) mitochondria (AMA) smooth muscles (ASMA) cell nuclei (ANA) M2 antigen	kidney stomach kidney stomach HEp-2 cells M2 BIOCHIPs	rat rat mouse mouse human ----	10 x 03 (030) 10 x 05 (050) 10 x 10 (100) 20 x 05 (100) 20 x 10 (200)

Indication: This test kit is designed for the qualitative or semiquantitative in vitro determination of human antibodies of immunoglobulin class IgG in patient samples for the diagnosis of primary biliary cirrhosis (PBC), autoimmune hepatitis (AIH), systemic lupus erythematosus (SLE) and autoimmune rheumatoid diseases.

Application: Antibodies against ANA, AMA and ASMA play an important part in the diagnosis of AIH and PBC. With the combination of the IIFT substrates HEp-2, kidney and stomach the relevant antibodies can be detected simultaneously. BIOCHIPs with purified M2 antigen supplement this diagnostic approach.

Test principle: Combinations of substrates are incubated with a diluted serum sample. If a positive reaction is obtained, specific antibodies of classes IgA, IgG and IgM attach to the antigens. In a second step, the attached antibodies are stained with fluorescein-labelled anti-human antibodies and made visible with a fluorescence microscope.

Contents of a test system for 50 determinations (e.g. FA 1620-1005-1):

Description	Format	Symbol
1. Slides, with a mosaic of BIOCHIPs (specifications see page 16)	10 slides	SLIDE
2. Fluorescein-labelled anti-human IgG (goat), ready for use	1 x 1.5 ml	CONJUGATE
3. Positive control: autoantibodies with titer information against mitochondria (AMA-M2), human, ready for use	1 x 0.25 ml	POS CONTROL
4. Positive control: autoantibodies against smooth muscles (ASMA), human, ready for use	1 x 0.1 ml	POS CONTROL
5. Negative control: autoantibody-negative, human, ready for use	1 x 0.1 ml	NEG CONTROL
6. Salt for PBS pH 7.2	2 packs	PBS
7. Tween 20	2 x 2.0 ml	TWEEN 20
8. Mounting medium, ready for use	1 x 3.0 ml	GLYCEROL
9. Cover glasses (62 mm x 23 mm)	12 pieces	COVERGLASS
10. Instruction booklet	1 booklet	---

LOT	Lot description		Storage temperature
IVD	In vitro diagnostic medical device	CE	Unopened usable until

Single slides (e.g., EUROIMMUN order no. FB 1620-1005-1) are provided together with cover glasses. Additional positive control (e.g., order no. CA 1622-0102-3) and negative control (e.g., order no. CA 1000-0101) can be ordered.
Performance of the test requires reagent trays TRAY, which are not provided in the test kits. They are available from EUROIMMUN under the following order no.:

- ZZ 9999-0105 Reagent trays for slides containing up to 5 fields (9 x 7 mm)
- ZZ 9999-0110 Reagent trays for slides containing up to 10 fields (5 x 5 mm)

Performing the test

The **TITERPLANE Technique** was developed by EUROIMMUN in order to standardise immunological analyses: Samples or labelled antibodies are applied to the reaction fields of a reagent tray. The BIOCHIP slides are then placed into the recesses of the reagent tray, where all BIOCHIPs of the slide come into contact with the fluids, and the individual reactions commence simultaneously. Position and height of the droplets are exactly defined by the geometry of the system. As the fluids are confined to a closed space, there is no need to use a conventional “humidity chamber”. It is possible to incubate any number of samples next to each other and simultaneously under identical conditions.

Prepare: The preparation of the reagents and of the serum and plasma samples is described on **page 4** of this test instruction.

Pipette: Apply a defined volume (see scheme next page) **of diluted sample** to each reaction field of the reagent tray, avoiding air bubbles. Transfer all samples to be tested before starting the incubation (up to 200 droplets). Use a polystyrene pipetting template.

Incubate: Start reactions by fitting the BIOCHIP slides into the corresponding recesses of the reagent tray. Ensure that each sample makes contact with its BIOCHIP and that the individual samples do not come into contact with each other.
Incubate for **30 min** at room temperature (+18°C to +25°C).

Wash: Rinse the BIOCHIP slides with a flush of PBS-Tween using a beaker and immerse them immediately afterwards in a cuvette containing PBS-Tween for at least **5 min**. Shake with a rotary shaker if available. Wash max. 16 slides, then replace PBS-Tween with new buffer.

Pipette: Apply a defined volume (see scheme next page) **of fluorescein-labelled anti-human globulin** to each reaction field of a clean reagent tray. Add all droplets before continuing incubation. Use a stepper pipette. The labelled anti-human serum should be mixed thoroughly before use. To save time, conjugate can be pipetted onto separate reagent trays during the incubation with the diluted sample.

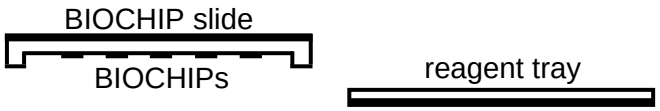
Incubate: Remove one BIOCHIP slide from cuvette. Within five seconds blot only the back and the long sides with a paper towel and immediately put the BIOCHIP slide into the recesses of the reagent tray. Do not dry the areas between the reaction fields. Check for correct contact between the BIOCHIPs and liquids. Then continue with the next BIOCHIP slide. From now on, protect the slides from direct sunlight.
Incubate for **30 min** at room temperature (+18°C to +25°C).

Wash: Fill cuvette with new PBS-Tween. Rinse the BIOCHIP slides with a flush of PBS-Tween using a beaker and put them into the cuvette filled with the new PBS-Tween for at least **5 min**. Shake with a rotary shaker if available. Wash max. 16 slides, then replace PBS-Tween with new buffer.

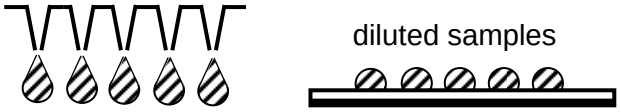
Mount: Place mounting medium onto a cover glass – volume per reaction field see scheme next page. Use a polystyrene mounting tray. Remove one BIOCHIP slide from PBS-Tween and dry the back and all four sides with a paper towel. Put the BIOCHIP slide, with the BIOCHIPs facing downwards, onto the prepared cover glass. Check immediately that the cover glass is properly fitted into the recesses of the slide. Correct the position if necessary.

Evaluate: Read the fluorescence with the microscope.
General recommendation: objective 20x (tissue sections, infected and transfected cells), 40x (cell substrates).
Excitation filter: 450-490 nm, colour separator: 510 nm, blocking filter: 515 nm.
Light source: mercury vapour lamp, 100 W, EUROIMMUN LED, EUROStar Bluelight.

TITERPLANE Technique

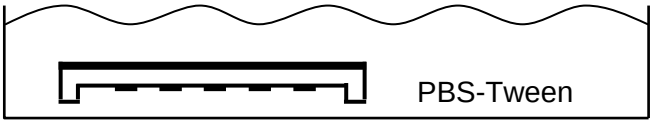


Pipette:	30 µl per field (5 x 5 mm) 70 µl per field (9 x 7 mm)



Incubate:	30 min

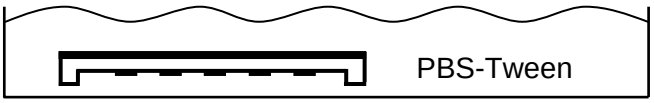
Wash:	1 s flush 5 min cuvette



Pipette:	25 µl per field (5 x 5 mm) 65 µl per field (9 x 7 mm)

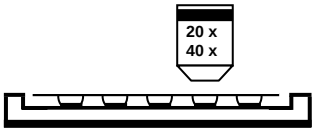
Incubate:	30 min

Wash:	1 s flush 5 min cuvette



Mount:	max. 10 µl per field (5x5 mm) max. 20 µl per field (9x7 mm)

Evaluate:	fluorescence microscopy



Automated Incubation: The test kit can be incubated by using automated devices, e.g. IF Sprinter, Sprinter XL, EUROLabLiquidHandler or others. The incubation and washing conditions programmed should be the same as described in the manual procedure. The test settings for EUROIMMUN devices are validated in combination with the kit. Any other combination has to be validated by the user. For details please refer to the device manual.

Preparation and stability of reagents

Note: After initial opening, the reagents are stable until the expiry date when stored at +2°C and +8°C and protected from contamination, unless stated otherwise below.

- **Slides:** Ready for use. Remove the protective cover only when the slides have reached room temperature (+18°C up to +25°C; condensed water can damage the substrate). Do not touch the BIOCHIPs. If the protective cover is damaged, the slide must not be used for diagnostics.

- **Fluorescein-labelled secondary antibody (FITC):** Ready for use. Before using for the first time, mix thoroughly. The conjugate is sensitive to light. Protect from sunlight.

- **Positive and negative controls:** Ready for use. Before using for the first time, mix thoroughly. Positive control with titer information: The label contains the target value and the substrate used to determine the target value. The lower tolerance limit is one titer level below the target value, the upper tolerance limit lies two titer levels above the target value. The control is to be diluted with PBS-Tween. Diluted controls must be incubated within one working day.

- **PBS-Tween:** 1 pack of “Salt for PBS” should be dissolved in 1 liter of distilled water (optimal: aqua pro infusione, aqua ad injectabilia) and mixed with 2 ml of Tween 20 (stir for 20 min until homogeneous). The prepared PBS-Tween can be stored at +2°C to +8°C, generally for 1 week. PBS-Tween should not be used if the solution becomes cloudy or contamination appears.

- **Mounting medium:** Ready for use.

- **Reagent trays:** Reaction fields of the reagent tray must be hydrophilic and surrounding area hydrophobic. If necessary, leave in 2% Deconex 11 universal (EUROIMMUN order number: ZZ 9912-0101) for 12 hours. Afterwards rinse generously with water and dry. Cleaning: Rub reagent trays with 5% Extran MA 01 (EUROIMMUN order number: ZZ 9911-0130) and rinse with plenty of water. To disinfect: Spray reagent trays generously with Mikrozid AF (EUROIMMUN order number: ZZ 9921-0125), turn over and leave for 5 minutes. Afterwards, rinse generously with water and dry.

Storage and stability: The slides and the reagents should be stored at a temperature between +2°C and +8°C. Unopened, all test kit components are stable until the indicated expiry date.

Waste disposal: Patient samples, controls and slides are to be handled as potentially infectious materials. All reagents are to be disposed of in accordance with official disposal regulations.

Warning: The BIOCHIPs coated with antigen substrates have been treated with a disinfecting fixing agent. Neither HBsAg nor antibodies against HIV-1, HIV-2, and HCV could be detected in the control sera using appropriate ELISA or indirect immunofluorescence tests. **Nevertheless, all test system components should be handled as potentially infectious materials.** Some of the reagents also contain the agent sodium azide in a non-declarable concentration. Avoid skin contact.

Preparation and stability of serum and plasma samples

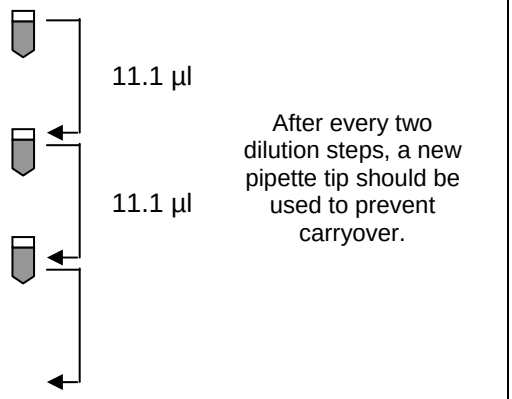
Samples: Human sera or EDTA, heparin or citrate plasma.

Stability: The patient samples to be investigated can generally be stored up to 14 days at a temperature between +2°C and +8°C. Diluted samples must be incubated within one working day.

Recommended sample dilution for qualitative evaluation: The sample to be investigated is diluted 1:100 in PBS-Tween. For example, dilute 10.1 µl sample in 1000 µl PBS-Tween and mix thoroughly, e.g., vortex for 4 seconds.

Recommended sample dilution for semiquantitative evaluation: The dilution of samples to be investigated is performed using PBS-Tween. Add 100 µl of PBS-Tween to each tube and mix with 11.1 µl of the next highest concentration, e.g., vortex for 2 seconds. EUROIMMUN recommends incubating samples from a dilution of 1:100.

Dilution	Dilution scheme
1:10	100 µl PBS-Tween + 11.1 µl undiluted sample
1:100	100 µl PBS-Tween + 11.1 µl 1:10 diluted sample
1:1000	100 µl PBS-Tween + 11.1 µl 1:100 diluted sample
⋮	⋮



Evaluation

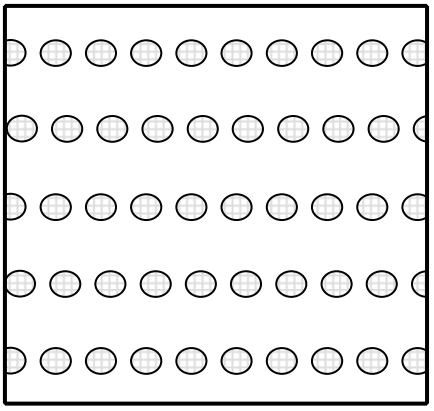
Fluorescence pattern (positive reaction): Antibodies against **mitochondria (AMA)** can be determined using various histological substrates and HEp-2 cells. For the targeted identification of antibodies against **mitochondria (AMA)**, frozen sections of **kidney (rat)** are used as standard substrate. The cytoplasm of the proximal and distal tubule cells shows a granular, basally emphasised fluorescence. The glomeruli are only weakly stained by AMA. The pattern shown is essentially the same as that obtained for the positive control serum. Any fluorescence of the luminal tubule sections (brush border) is not taken into account.

With frozen sections of stomach (rat) antibodies against **smooth muscles (ASMA)** show a distinct cytoplasmic fluorescence of the tunica muscularis as well as the lamina muscularis mucosa and the interglandular contractile fibrils of the tunica mucosa. The pattern shown is essentially the same as that obtained for the positive control serum. In the case of negative samples, the contractile elements show no staining. Any fluorescence of the luminal tubule sections (brush border) is not taken into account.

Antibodies against **nuclear antigens (ANA)** can be found on numerous substrates. For the targeted determination and differentiation of antinuclear antibodies, a substrate consisting of human epithelial cells (HEp-2) is used. The cell nuclei show a distinct fluorescence, which is characterised by certain patterns. In each field evaluated, both – interphase nuclei cells and mitotic cells of Hep-2 cells – should be examined, and this in several areas if possible.
In the case of negative samples, the nuclei show no specific fluorescence.

M2 BIOCHIP
M2 was applied in microscopic fine droplets onto the BIOCHIP. If **M2-specific antibodies** exist in the serum, the circular areas show a green fluorescence in front of the dark background.

In case of a **negative reaction** the entire M2-BIOCHIP is dark, the described circular areas can hardly be detected. For a secure discrimination between positive and negative results the positive and negative **control** as well as various normal sera must be compared with the patient samples.



M2-coated BIOCHIP

For each incubation a positive and negative control should be used as a reference.

If the positive control shows no specific fluorescence pattern or the negative control shows a clear specific fluorescence, the results are not to be used and the test is to be repeated.

A large range of fluorescence images can be found on the EUROIMMUN website (www.euroimmun.com).

Recommended qualitative evaluation:

AMA reactivity (IgG)	**Evaluation**
No reaction at 1:100	Negative. No antibodies against mitochondria detected in the patient sample.
Positive reaction at 1:100	Positive. Indication of various diseases, e.g. primary biliary cirrhosis (PBC) and others (see clinical significance).

ASMA reactivity (IgG)	**Evaluation**
No reaction at 1: 100	Negative. No antibodies against smooth muscles detected in the patient sample.
Positive reaction 1:100	Positive. Indication of chronic-active hepatitis, viral hepatitis, infectious mononucleosis and others.

ANA reactivity (IgG)	**Evaluation**
No reaction at 1:100	Negative. No antibodies against cell nuclei detectable in the patient sample.
Positive reaction at 1:100	Trace. For IF types: pattern homogeneous, centromeres, nuclear dots, Jo-1, typical patterns of SS-A/SS-B, Sm/RNP possible indication of various rheumatic and other diseases.
Positive reaction at 1:320	Positive. Indication of various rheumatic and other diseases.

Anti-M2 reactivity (IgG)	**Result**
No reaction at 1:100	Negative. No antibodies against M2 detected in the patient samples.
Positive reaction at 1:100	Positive. Upon appropiate symptoms shown proof of primary biliary liver cirrhosis (PBC).

Recommended semiquantitative evaluation: The titer is defined as the sample dilution factor for which specific fluorescence is just identifiable. This should be compared with the reaction obtained using an equivalently diluted negative serum.

Antibody titers can be determined according to the following table from the fluorescence of the different sample dilutions.

Fluorescence at				Antibody titer
1:10	1:100	1:1000	1:10000	
weak	negative	negative	negative	1:10
moderate	negative	negative	negative	1:32
strong	weak	negative	negative	1:100
strong	moderate	negative	negative	1:320
strong	strong	weak	negative	1:1000
strong	strong	moderate	negative	1:3200
strong	strong	strong	weak	1:10000
⋮	⋮	⋮	⋮	⋮

Limitations of the procedure

1. A diagnosis should not be made based on a single test result. The clinical symptoms of the patient should always be taken into account along with the serological results by the physician.
2. Adaptation of this assay for use with automated sample processors and other liquid handling devices, in whole or in part, may yield differences in test results from those obtained using the manual procedure. It is the responsibility of each laboratory to validate that their automated procedure yields test results within acceptable limits.
3. Mishandling of slides during the staining procedure, especially allowing slides to dry between steps, may result in a “washed out" pattern appearance and/or a high level of background staining.
4. Coplin jars used for slide washing should be free from all residues. Use of coplin jars containing residue may cause staining artefacts.
5. The light source, filters and optical unit of the fluorescence microscope can influence the sensitivity of the assay. Using traditional mercury vapour lamp systems, the performance of the microscope depends on correct maintenance, especially alignment of the lamp and replacement of the lamp after the recommended period of time. The EUROIMMUN fluorescence microscopes with LED Bluelight as the light source offer many advantages. Contact EUROIMMUN for details.

Test characteristics

Antigen: For the detection of autoantibodies against mitochondria (AMA) by indirect immunofluorescence **kidney** (rat) is used as a standard substrate. Using a BIOCHIP combination with **stomach** (rat) enables the specific analysis for antibodies against smooth muscles (ASMA) at the same time.
Mitochondria contain many different biochemically definable antigens which are of significance for autoimmune diseases. Correspondingly, nine AMA types are today differentiated (M1 - M9).

For the determination of antinuclear antibodies (ANA) by means of indirect immunofluorescence, human epithelial cells **(HEp-2)** are preferred nowadays. HEp-2 cells show a wide spectrum of human nuclear antigens, and assessment of the fluorescence pattern makes the predifferentiation of a large number of antibodies possible.

The **M2 antigen** is a component of three biochemically related multi-enzyme complexes of the inner mitochondria membrane. These catalyse the oxidative decarboxylation of pyruvate, of alpha-ketoglutarates and of the branched-chain alpha-keto acids. In this respect, M2 is contained in at least four different proteins: in E2 of the alpha-ketoglutarate dehydrogenase complex (50 kDa), E2 of the branched-chain alpha-keto acid dehydrogenase complex (50 kDa), in protein X of the pyruvate dehydrogenase complex (52 kDa) and in E2 of the pyruvate dehydrogenase complex (74 kDa). The E2 enzymes catalyses the transfer of an acyl group to coenzyme A, protein X is a subunit of the pyruvate dehydrogenase complex, the function of which is unknown.

Measurement range: The dilution starting point for this measurement system is 1:100. Samples can be further diluted by a factor of 10 so that the dilution series is 1:1000, 1:10000 etc. There is no upper limit to the measurement range.

Reproducibility: The intensity of the specific fluorescence as a numeric value is called fluorescence intensity level by EUROIMMUN. These values can reach from “0” (no specific fluorescence) to “5” (extremely strong specific fluorescence).

Reproducibility	**Inter-lot**	**Intra-assay**	**Inter-assay**
Minimum requirement	3 lots x 3 samples x 1 run x single determination: max. ± 1 intensity level	1 lot x 3 samples x 1 run x tenfold determination: max. ± 1 intensity level	1 lot x 3 samples x 2 runs x double determination: max. ± 1 intensity level
Stomach (rat) ASMA	No deviation	No deviation	No deviation
Kidney (rat) AMA/LKM	Maximum deviation ± 1 intensity level	No deviation	No deviation
Stomach (mouse) Anti-GMA	Maximum deviation ± 1 intensity level	Is assured since inter-lot reproducibility was investigated with more than 10 lots.	Is assured since inter-lot reproducibility was investigated with more than 10 lots.
M2 BIOCHIPs Anti-M2			
Kidney (mouse) Anti-LKM/AMA			
HEp-2 cells (human) ANA	No deviation	Maximum deviation ± 1 intensity level	Maximum deviation ± 1 intensity level

Cross reactivity:

Antibodies	**Cross reactivity**
AMA, ASMA	The 11 incubated samples (CDC 1 to CDC 11) produced only the expected fluorescence patterns. No cross reactivity was detected.
ANA	The 11 incubated samples (CDC 1 to CDC 11) produced only the expected fluorescence patterns. No cross reactivity was detected.

Interference: Haemolytic, lipaemic and icteric samples showed no influences on analysis results.

Reference range: Titer: 1: <100 (AMA, anti-LKM, ASMA, ANA, anti-M2)

The following antibody prevalences were determined using a panel of samples from healthy blood donors (origin: Germany):

Substrate	Antibodies against	Conjugate	Prevalence	Cut-off	Number of samples
Kidney (rat)	AMA	IgG IgAGM	1%	1:100	n = 300
Stomach (rat)	ASMA	IgG IgAGM	4%	1:100	n = 300
Kidney (mouse)	LKM	IgG IgAGM	0%	1:100	n = 200
Stomach (mouse)	ASMA	IgG IgAGM	3%	1:100	n = 200
HEp-2 cells (human)	ANA	IgG	12.5%	1:100	n = 200
M2-BIOCHIPs	M2	IgG IgAGM	1.5% 1.1%	1:100	n = 205 n = 88

Specificity and Sensitivity:

Substrate	Ig class	Reference (number of samples, origin of samples)	Specificity	Sensitivity
Kidney (rat): AMA	IgG	Reference centres (n = 33, Germany)	100%	100%
	IgAGM	(n = 38, Germany)	100%	100%
		AMA-M2 ELISA: n = 103, Germany	100%	98%
Kidney (rat): Anti-LKM	IgG, IgAGM	Reference centres (n = 32, Germany) (n = 37, Germany)	100%	100%
Stomach (rat): ASMA	IgG, IgAGM	Reference centres (n = 31, Germany)	100%	100%
Kidney (mouse): Anti-LKM	IgG, IgAGM	Reference centres (n = 31, origin: Germany) (n = 32, origin: Germany)	100%	100%
Kidney (mouse): AMA	IgG, IgAGM	Reference centres (n = 31, origin: Germany) (n = 32, origin: Germany)	100%	100%
Stomach (mouse): Anti-ASMA	IgG, IgAGM	Reference centres (n = 29, origin: Germany)	100%	100%
HEp-2 cells (human): ANA	IgG	ELISA + Blot (n = 128, Germany)	100%	100%
M2-BIOCHIPs: Anti-M2	IgAGM	(n = 103, Anti-M2 ELISA, origin: Germany)	93%	98%
		(n = 33, origin: Germany)	100%	89%
	IgG	(n = 33, origin: Germany)	100%	78%

Clinical significance

AMA

The AMA IIFT is used for the serological determination of autoantibodies against mitochondria (AMA). AMA are particularly associated with primary biliary cirrhosis (PBC). As immunological markers for this severe autoimmune disease, these antibodies have a high diagnostic value. The determination of AMA can be performed qualitatively or quantitatively.

PBC is a chronic non-suppurative destructive cholangitis with progressive inflammatory destruction of the small biliary ducts and liver cirrhosis in the final stage. In 80% to 90% of cases the patients are female, mainly between 20 and 60 years of age. In rare cases, the disease also affects children. In Germany the prevalence is around 3 to 4 cases per 100,000 inhabitants. Demographic differences (Caucasians, Africans, etc.) are minimal.

The first symptoms of PBC, such as fatigue or exhaustion and severe itching, are rather uncharacteristic. These symptoms can precede an increase in liver test results and PBC diagnosis for months or years. Finally, as a result of cholestasis, skin changes in the form of xanthelasma of the inner corner of the eye, vitamin A, D, E and K deficiency, steatorrhoea and possibly osteoporosis develop. Liver biopsy is not required but can aid diagnosis to determine the disease activity and the stage of PBC.

Stage I:	Inflammatory reaction of the so-called portal fields
Stage II:	Additional necroses
Stage III:	Formation of connective tissue-like septums
Stage IV:	Cirrhotic liver tissue changes (in around 6% of cases increased risk of hepatocellular carcinoma)

In the final stage of PBC (decompensated cirrhosis) only liver transplantation will save the patient's life. In around 75% of cases the transplant patients recover fully from PBC. Some patients, however, suffer a PBC relapse after transplantation, but only with a very slow disease course.

Beside the clinical diagnosis of PBC ultrasound can give further information. During the early stage of PBC the liver is generally normal, but can look like a fatty liver or become larger in later stages. In the final stage of cirrhosis the by then hardened liver becomes smaller and shows a knobbed or corrugated surface.

Since clinical symptoms and imaging procedures do not permit a reliable diagnosis of PBC, serological diagnostics play an important role. Antibodies against mitochondria (AMA) can be detected by IIFT using different histological substrates and HEp-2 cells; tissue section of rat kidney being the standard substrate. With the AMA IIFT autoantibodies against AMA as a reliable indicator of PBC can be successfully determined with high specificity and sensitivity.

The diagnostic value of AMA detection can be further increased by performing a second analysis using IIFT Mosaic, ELISA or Westernblot. In the second step the AMA types are differentiated. M2, M4, M8 and M9 together are highly specific for PBC, with a prevalence of almost 100%. With an AMA screening test followed by a confirmation test for AMA differentiation the greatest possible diagnostic security is achieved.

Nine AMA types (M1 - M9) are of (differential) diagnostic value. Their associated diseases and the prevalences are specified in the following table:

Antibodies against	Associated disease	Prevalence
M1	Syphilis (indication of activity)	100%
	Systemic lupus erythematosus	50%
	Progressive systemic sclerosis, Sjögren's syndrome, Sharp syndrome, rheumatoid arthritis	5 - 15%
M2	Primary biliary cirrhosis (high titer)	up to 96%
	Other chronic liver diseases	30%
	Progressive systemic sclerosis	7 - 25%
M3	Pseudolupus syndrome	100%
M4	Primary biliary cirrhosis	up to 55%
M5	Unspecified connective tissue diseases	rare
M6	Hepatitis (iproniazide-induced)	100%
M7	Acute myocarditis	60%
	Cardiomyopathies	30%
M8	Primary biliary cirrhosis	up to 55%
M9	Primary biliary cirrhosis	37 - 82%
	Other forms of hepatitis	3 - 10%

Note: With a negative AMA result and continued suspicion of PBC, the additional determination of autoantibodies against nuclear granules (nuclear dots, SP100) is recommended, since these are also pathognomonically relevant. These autoantibodies are optimally determined using indirect immunofluorescence with the substrate combination HEp2 cells/primate liver.

AIH (with subtypes AIH type 1 and AIH type 2)

Like most autoimmune diseases AIH is characterised by a female predominance (>75%). The prevalence is given as 10 to 20 cases per 100,000 persons. In a study in Norway the incidence was determined as 19 cases per 1,000,000 inhabitants per year. A connection with other autoimmune syndromes is observed in around 50% of cases (overlap syndrome AIH with e.g. autoimmune thyroiditis or ulcerative colitis).

The disease often progresses to liver cirrhosis. Hepatocellular carcinoma can also develop. Despite suitable medication therapy, which is successful in up to 90% of patients, for around 10% of patients the last therapeutic option is a liver transplant. In 20% to 40% of affected individuals the AIH recurs even after transplantation (de novo AIH). The 5-year survival rate after transplantation is around 80% to 90%.

Up to 90% of AIH patients show pathological titers of at least one AAb. Due to the low prevalence of the highly specific AAb against SLA/LP (soluble liver antigen/liver pancreas antigen), which amounts to 15% to 30% in Europe and North America and around 7% in Japan, the investigation of other AAb is indispensable in suspected cases of AIH: These include in particular:

- AAb against cell nuclei (ANA), especially those that produce a homogeneous pattern
- AAb against smooth muscle (ASMA, important target antigen F-actin)
- AAb against dsDNA
- AAb against liver-kidney microsomes (LKM-1; target antigen cytochrome P450 IID6)
- AAb against cytosolic liver antigen type 1 (LC-1; target antigen formiminotransferase cyclodeaminase, FTCD)
- AAb against granulocytes (P-ANCA, perinuclear antineutrophil cytoplasmic antibody)

AAb against F-actin show the highest sensitivity (30 to 40%) of all AIH-associated AAb. They can only be determined with high specificity for AIH by IIFT. The specificity for AIH of SLA/LP AAb is 100%, for LC1 AAb it is near to 100%. AAb against LKM-1 can occur in viral hepatitis as well as in AIH. For patients who are positive for anti-SLA/LP and anti-Ro-52, a higher AIH activity with a more severe disease course is to be expected.

ASMA

Autoantibodies against smooth muscles (ASMA) occur in various liver diseases (autoimmune hepatitis, liver cirrhosis). Their determination is of particular significance for the diagnosis of autoimmune (lupoid) chronic active hepatitis. ASMA can also be found in infectious mononucleosis and other virus infections, in systemic lupus erythematosus, in breast and ovarian carcinomas as well as in malignant melanomas, but play no diagnostic role in these diseases. In the case of viral hepatitis the titer generally falls off again rapidly.

High concentrations of antibodies against smooth muscles are an indication of autoimmune hepatitis (AIH), the prevalence being 70%. The IgG and IgM titers can correlate with the disease activity. AIH occurs mostly in women, and half of the cases develop before the age of 30. In 40% of patients, the disease commences with acute hepatitis. Liver biopsies show a necrosis of the parenchymal cells with lymphocyte and plasma cell infiltration.

With the help of autoantibodies and various virus parameters, autoimmune hepatitis can be divided into various etiologic subgroups. In the case of AIH, autoantibodies against cell nuclei, double-stranded DNA and granulocyte cytoplasm (pANCA) can frequently also be found along with antibodies against smooth muscles.

Low ASMA titers are also detected in patients with primary biliary liver cirrhosis (50%), alcohol-related liver cirrhosis, obstruction of the biliary ducts and in about 2% of apparently healthy persons.

ANA

The detection of autoantibodies against cell nuclei (ANA) is an important diagnostic indicator in many autoimmune diseases. Antibodies against nuclear antigens are directed against various cell nuclear components (biochemical substances in the cell nucleus). These encompass nucleic acids, cell nucleus proteins and ribonucleoproteins. They are a characteristic finding in many diseases, in particular rheumatic diseases. The frequency (prevalence) of anti-nuclear antibodies in inflammatory rheumatic diseases is between 20% and 100%, the lowest occurring in rheumatoid arthritis at between 20% and 40%. Therefore, differential antibody diagnostics against nuclear antigens is indispensible for the identification of individual rheumatic diseases and their differentiation from other autoimmune diseases:

Autoimmune disease	**Prevalence ANA**
Systemic lupus erythematosus (SLE)	80% - 100%
Drug-induced erythematosus	100%
Mixed connective tissue disease (MCTD, Sharp syndrome) Rheumatoid arthritis Other rheumatic diseases	100% 20% - 40% 20% - 50%
Progressive systemic sclerosis Polymyositis and dermatomyositis Sjögren’s syndrome	85% - 95% 30% - 50% 70% - 80%
Autoimmune hepatitis	30% - 40%
Ulcerative colitis	26%

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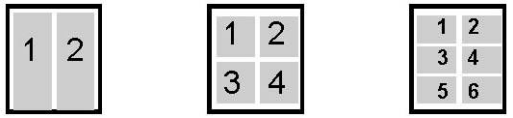

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		1	2	3	4	5	6	
FA 1620-####-1	IIFT Mosaic: Kidney (Rat)/Stomach (Rat)	Kidney, rat	Stomach, rat					5x5
FA 1620-####-2	IIFT Mosaic: Kidney (Mouse)/Stomach (Mouse)	Kidney, mouse	Stomach, mouse					5x5
FA 1620-####-3	EUROPLUS Kidney (Rat)/M2	Kidney, rat	M2 BIOCHIPs					5x5
FA 1620-####-5	EUROPLUS HEp-2/Stomach/ Kidney/M2	HEp-2 cells	Stomach, rat	Kidney, rat	M2 BIOCHIPs			5x5