IIFT: ANA Mosaic 1A EUROPattern
Instructions for the indirect immunofluorescence test

ORDER NO.	ANTIBODIES AGAINST	SUBSTRATE	SPECIES	FORMAT SLIDES x FIELDS
FC 1512-1005-1	cell nuclei (ANA global test)	HEp-20-10 cells liver (2 BIOCHIPs per field)	human monkey	10 x 05 (050)
FC 1512-1010-1				10 x 10 (100)
FC 1512-2005-1				20 x 05 (100)
FC 1512-2010-1				20 x 10 (200)
FC 1512-1050-1				10 x 50 (500)
FC 1512-2450-1				24 x 50 (1200)
FC 1512-12010-1				120 x 10 (1200)

Indication: This test kit is designed for the qualitative or semiquantitative in vitro determination of human antibodies of immunoglobulin class IgG against cell nuclei in patient samples for the diagnosis of many autoimmune diseases, particulary those of the rheumatic form.

Application: The indirect immunofluorescence test (IIFT) is the gold standard for the determination of antibodies against nuclear antigens (ANA, including cytoplasmic components). Antibodies against cell nuclei can be determined on numerous substrates. The BIOCHIP technology enables different substrates to be combined in one test field (multiplex test) and incubated with one patient serum. The substrate combination HEp-2 or HEp-20-10 cells with primate liver and/or monospecific dots (EUROPLUS: SS-A, SS-B, Jo-1, Scl70, nRNP/Sm, Sm, rib. P-proteins) allows results to be confirmed and differentiated already at the ANA screening stage and can yield additional findings (e.g. EMA).
HEp-20-10 cells have been optimised to form more mitotic stage cells. This facilitates the evaluation of ANA fluorescence patterns that require assessment of the mitotic stage.
The EUROPattern test systems have been developed specifically for the high-performance automation solution from EUROIMMUN AG.

Test principle: Substrate combinations of HEp-20-10 cells and primate liver are incubated with diluted patient sample. If the reaction is positive, 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 kit for 50 determinations (e.g. FC 1512-1005-1):

Description	Format	Symbol
1. Slides, each containing 5 x 2 BIOCHIPs coated with HEp-20-10 cells and primate liver	10 slides	SLIDE
2. FITC-labelled anti-human IgG (goat) with propidium iodide for EUROPattern, ready for use	1 x 1.5 ml	CONJUGATE PI
3. Positive control: autoantibodies against cell nuclei (ANA), for EUROPattern, human, ready for use	1 x 0.25 ml	POS CONTROL
4. Negative control: autoantibody-negative, human, ready for use	1 x 0.25 ml	NEG CONTROL
5. Salt for PBS pH 7.2	2 packs	PBS
6. Tween 20	2 x 2.0 ml	TWEEN 20
7. Mounting medium, ready for use	1 x 3.0 ml	GLYCEROL
8. Cover glasses (62 mm x 23 mm)	12 pieces	COVERGLASS
9. 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. FW 1512-1005-1) are provided together with cover glasses. Additional positive control (e.g., order no. CA 1570-0102-4) and negative control (e.g., order no. CA 1000-0102) 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 9999-0110 Reagent trays for slides containing up to 10 fields

Performing the test (reaction fields 5 x 5 mm)

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 **30 µl 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.

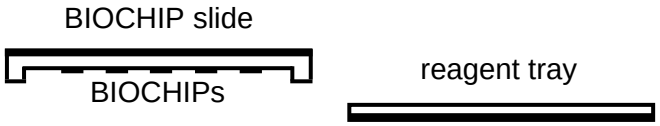
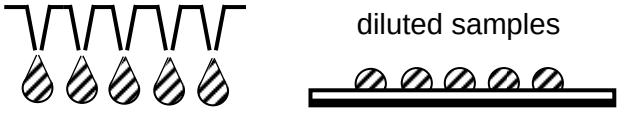
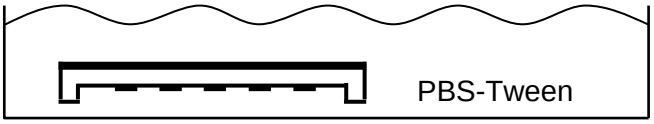
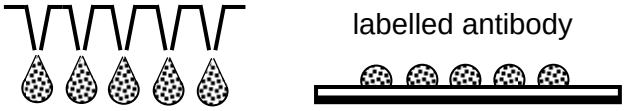
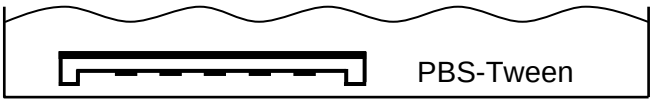
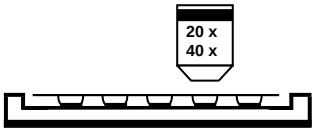
Pipette: Apply **25 µl of fluorescein labelled anti-human globulin** to each reaction field of a clean reagent tray. Add all droplets before continuing incubation. Use a stepper pipette. The labelled anti-human serum should be mixed thoroughly before use. To save time, conjugate can be pipetted onto 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 – drops of **max. 10 µl per reaction field**. 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.
General recommendation: objective 20x (tissue sections, infected and transfected cells), 40x (cell substrates).
EUROPattern: excitation filter: 450-490 nm, colour separator: 510 nm, blocking filter 515 nm.
Visual examination: excitation filter: 450-490 nm, colour separator: 510 nm, bandpass filter: 515-565 nm.
Light source: EUROIMMUN LED, EUROStar Bluelight.
Any software suggested result must be verified by trained laboratory professionals.

TITERPLANE Technique		
Pipette:	30 µl per field	
Incubate:	30 min	
Wash:	1 s flush 5 min cuvette	
Pipette:	25 µl per field	
Incubate:	30 min	
Wash:	1 s flush 5 min cuvette	
Mount:	max. 10 µl per field	
Evaluate:	EUROPattern microscopy with user verification	

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 between +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.

- **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

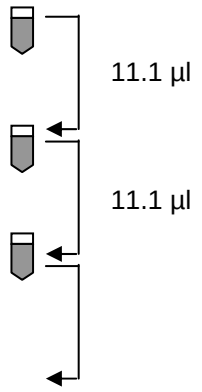
Samples: Human sera or EDTA, heparin or citrate plasma.

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. An additional 1:10 dilution can help to detect antibodies against aminoacyl synthetases in myositis patients more reliably (for instance add 11.1 µl sample to 100 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. An additional 1:10 dilution can help to detect antibodies against aminoacyl synthetases in myositis patients more reliably.

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
⋮	⋮



After every two dilution steps, a new pipette tip should be used to prevent carryover.

Evaluation

Magnification for HEp-20-10 cells: Eyepiece 10x, objective 20x (200x). This magnification deviates from our general recommendation given in the incubation protocol on page 2.

Fluorescence pattern (positive reaction): The evaluation of the fluorescence for HEp-20-10 is performed fully automatically using EUROPattern. The evaluation of the fluorescence for monkey liver is performed visually at the computer screen after automated image recording. 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-20-10) and primate liver is suitable. The cell nuclei show a distinct fluorescence, which is characterised by certain patterns. In the case of negative samples, the nuclei show no specific fluorescence. In each field evaluated, both interphase nuclei and mitotic cells of the HEp-20-10 cells as well as the liver should be examined, and this in several areas if possible. You can find an overview of the fluorescence patterns on the website on antinuclear antibodies (ANA) www.anapatterns.org (ICAP).

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:

ANA reactivity (IgG)	**Evaluation**
No reaction at 1:100	Negative. No antibodies against cell nuclei detectable in the patient samples
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.

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 1:10	1:100	1:1000	1:10000	Antibody titer
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
⋮	⋮	⋮	⋮	⋮

Some of the most important fluorescence patterns are the homogeneous and granular nuclear fluorescence as well as staining of the nucleoli and the centromeres (clearly identifiable especially in the mitotic cells). The relevant binding patterns frequently correspond with biochemically defined nuclear antigens:

Autoantigens of the cell nuclei		**Fluorescence pattern**
Polynucleotides	Double-stranded DNA Single-stranded DNA RNA	Homogeneous Homogeneous Partly homogeneous
Histones	H1, H2A, H2B, H3, H4, H2A-H2B complex	Homogeneous
Ribonucleoproteins of the nucleoplasm (ENA)	U1-nRNP Sm SS-A (Ro) SS-B (La)	Coarse granular, nulceoli negative Coarse granular, nulceoli negative Granular Granular
Antigens of the nucleolus	U3-nRNP/fibrillarin RNA polymerase I PM-Scl (PM-1) 7-2-RNP (To) 4-6-S-RNA	Nucleoli, granular Nucleoli, granular Nucleoli, homogeneous Nucleoli, homogeneous Nucleoli, homogeneous
Centromeres	Proteins of kinetochores	Typical granules
Other proteins	Scl-70 Cyclin (PCNA) Nuclear dots NOR-90 Ku Mi-1 Mi-2 Lamins	Almost homogeneous, accentuated nucleoli Granular, 50% 10 times brighter Nuclear dots Metaphase 1-2 granules Reticular Fine granular Fine granular Nuclear membrane

If each sample is investigated with a substrate combination of HEp-20-10 cells and primate liver (order no. FC 1512-1005-1) and comparison of the fluorescence patterns allows cell nuclei antibodies to be further differentiated: antibodies against U1-nRNP, Sm, dsDNA, histones and nuclear dots react with HEp-20-10 cells and liver with the same intensity, whereas antibodies against SS-A, SS-B, centromeres and cyclin show a much weaker fluorescence pattern on liver tissue.
Antibodies against Ku show only a granular fluorescence on the HEp-20-10 cells, whereas a coarse granular, perinuclear fluorescence can be seen on primate liver, which can be clearly differentiated from other fluorescence patterns. Only with the combination of HEp-20-10 cells and primate liver it is possible to determine, among other things, if antibodies against centromeres and nuclear dots are present in the same sample.

Limitations of the procedure

1. A diagnosis should not be made 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 antinuclear antibodies by indirect immunofluorescence, patient sera are nowadays predominantly tested using a combination of two substrates: human epithelial cells (HEp-2/HEp-20-10) and frozen sections of primate liver. These cells show a broad spectrum of human nuclear antigens. The cell line HEp-20-10, which is of identical genetic origin as the HEp-2 cell line, shows an increased amount of cells in the mitotic phase compared to the conventional cell line. By comparing fluorescence patterns on HEp-20-10 cells and liver, pre-differentiation of a large number of antibodies is possible. The primate liver completes the spectrum of detectable autoantibodies. Important are antibodies against ribosomal P-proteins, liver cell membrane (LMA), bile ducts, endothelial cells, endomysium and granulocytes (cANCA, pANCA).

If the immunofluorescence test is positive, it can be followed up by various monospecific analyses (e.g. enzyme immunoassays) in order to identify the specific mitochondria antigens against which the antibodies are directed.

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
HEp-20-10 cells (human)	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.
Liver (monkey)	No deviation	No deviation	No deviation

Cross reactivity: 11 characterised samples were incubated which did not produce any unexpected fluorescence pattern (CDC 1 to CDC 11). The results showed no cross reactivity with these sera.

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

Reference range: Titer 1: < 100

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
HEp-20-10 cells (human)	ANA	IgG	10%	1:100	n = 200
Liver (monkey)			4.5%		

Specificity and sensitivity:

Substrate	Ig class	Reference (number and origin of samples)	Specificity	Sensitivity
HEp-20-10 cells (human): ANA	IgG	IIFT: HEp-2 (n = 413, Germany)	96%	100%
Liver (monkey): ANA	IgG	IIFT: HEp-2 (n = 254, Germany)	91%	87%

IIFT: HEp-20-10 EUROPattern (software validation)

Substrate	Ig class	Reference (number of samples, origin of samples)	Agreement
Nuclear fluorescence	IgG with PI	IIFT: HEp-20-10 EUROPattern (manual evaluation) (analysis of a patient cohort of n = 61 characterised patient samples and a control cohort of n = 95 samples, origin: Europe)	89% agreement between automatic and manual evaluation
Cytoplasmic fluorescence			94% agreement between automatic and manual evaluation

Clinical significance

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's syndrome)	100%
Rheumatoid arthritis	20% - 40%
Other rheumatic diseases	20% - 50%
Progressive systemic sclerosis	85% - 95%
Polymyositis and dermatomyositis	30% - 50%
Sjögren's syndrome	70% - 80%
Chronic active hepatitis	30% - 40%
Ulcerative colitis	26%

Systemic lupus erythematosus

For systemic lupus erythematosus (SLE), the determination of antibodies against double-stranded DNA is considered to be one of the most important criteria for diagnosis. Immune complexes of double-stranded DNA and the corresponding autoantibodies cause tissue damage in the subcutis, the kidneys and other organs. The antibody titer correlates with the clinical activity of the disease. Autoantibodies against nucleosomes occur independently of anti-dsDNA antibodies; 18% of SLE sera react exclusively with nucleosomes and not with dsDNA. Antibodies against nucleosomes generally indicate a severe course of SLE. They occur at a prevalence of 9% to 89% in cases without lupus nephritis and at a prevalence of 18% to 86% in cases with lupus nephritis. Furthermore, antibodies against Sm are also considered to be pathognomonic for SLE. In addition, antibodies against other polynucleotides, ribonucleotides, histones and other nuclear antigens can be found.

In drug-induced lupus erythematosus with manifestations such as arthralgia, arthritis, exanthema, serositis, myalgia, heptomegalia and splenomegalia, antibodies against histones are observed. This reversible form of SLE can be induced by antibiotics (e.g. penicillin, streptomycin, tetracyclines), chemotherapeutic agents (e.g. INH, sulfonamides), anticonvulsants (e.g. phenytoin, hydantoins), antiarrythmics (e.g. procainamide, practolol), antihypertensives (e.g. reserpine, hydralazine), psychotropics (e.g. chlorpromazine), antithyroid drugs (e.g. thiouracil derivates), antirheumatoid basic therapeutics (e.g. gold, D-penicillamine) and other drugs such as contraceptives and allopurinol.

Autoantibodies in systemic lupus erythematosus	
Antigen	Prevalence
Double-stranded DNA	60% - 90%
Single-stranded DNA	70% - 95%
RNA	50%
RNA helicase A	6%
Histones	50% - 80%
Nucleosomes, dependent on SLE severity either without or with lupus nephritis	9% - 89% or 18% - 86%
U1-nRNP	15% - 40%
Sm	5% - 40%
SS-A (Ro)	20% - 60%
SS-B (La)	10% - 20%
Cyclin (PCNA)	3%
Ku	10%
Ribosomal P-proteins	10%
(Hsp-90: Heat shock protein, 90 kDA	50%)
(Cardiolipin	40% - 60%)

Sharp's syndrome

High autoantibody titers against U1-nRNP are characteristic for Sharp's syndrome (MCTD = mixed connective tissue disease). The antibody titer correlates with the clinical activity of the disease.

Autoantibodies in Sharp's syndrome (Mixed connective tissue disease = MCTD)	
Antigen	Prevalence
U1-nRNP	95% - 100%
Single-stranded DNA	20% - 50%

Rheumatoid arthritis

In rheumatoid arthritis, antibodies against histones can be observed in more than half of all cases, while antibodies against U1-nRNP are rarely found. Antibodies against RANA ("rheumatoid arthritis nuclear antigen") cannot be detected with HEp-2 cells.

Cell nuclei antibodies in rheumatoid arthritis	
Antigen	Prevalence
Histones	15% - 50%
Single-stranded DNA	8%
U1-nRNP	3%
(RANA	90% - 95%)

Progressive systemic sclerosis

Progressive systemic sclerosis (PSS; scleroderma) can manifest itself in two forms, which cannot always be clearly differentiated. Until now, antibodies against fibrillarin, RNA-polymerase I and Scl-70 have only been noticed in the diffuse form. Autoantibodies against centromeres are associated with the limited form of progressive systemic sclerosis.

Autoantibodies in progressive systemic sclerosis (diffuse form)	
Antigen	Prevalence
Fibrillarin	5% - 10%
PM-Scl (PM-1), including overlap syndrome	50% - 70%
Scl-70	25% - 75%
RNA polymerase I	4%
7-2-RNP (To)	rare
NOR-90 (nucleolus organiser region)	rare

Autoantibodies in progressive systemic sclerosis (limited form)	
Antigen	Prevalence
Centromeres	80% - 95%

Polymyositis/dermatomyositis

Autoantibodies against PM-Scl occur in polymyositis and dermatomyositis, but other nuclear antibodies (against Mi-1, Mi-2, Ku) and antibodies against Jo-1 can also be identified in these diseases.

Autoantibodies in polymyositis and dermatomyositis	
Antigen	Prevalence
PM-Scl (PM-1), including overlap syndrome	50% - 70%
Jo-1 (histidyl-tRNA synthetase)	25% - 35%
Mi-1	10%
Mi-2	5%
Ku	50%
Single-stranded DNA	40% - 50%
PL-7 (threonyl-tRNA synthetase)	4%
PL-12 (alanyl-tRNA synthetase)	3%

Sjögren's syndrome

In Sjögren's syndrome (primary Sjögren's syndrome), antibodies against SS-A and SS-B are present, mainly in combination with one another. In addition, autoantibodies against the secretory ducts of the salivary gland are found in 40 to 60% of all cases.

Autoantibodies in primary Sjögren´s syndrome	
Antigen	Prevalence
SS-A (Ro)	40% - 95%
SS-B (La)	40% - 95%
Single-stranded DNA	13%
(RANA	70%)
(Salivary gland excretory ducts	40% - 60%)
(Rheumatoid factors	60% - 80%)

Autoantibodies against nuclear antigens occur in many other diseases, such **as primary biliary liver cirrhosis** ("Nuclear Dots", SS-A) and **chronic active autoimmune hepatitis** (SS-A, lamins). At times, antibodies against nuclear antigens are detectable in subjectively healthy individuals, usually at a low titer (various immunoglobulin classes, mainly IgM).

Autoantibodies against cell nuclei: Important associated diseases		
Antigen	Disease	Prevalence
Double-stranded DNA	Systemic lupus erythematosus (SLE)	60% - 90%
Single-stranded DNA	Systemic lupus erythematosus (SLE) Drug-induced erythematosus Mixed connective tissue disease (MCTD or Sharp's syndrome) Polymyositis/dermatomyositis Scleroderma, Sjörgren's syndrome, rheum. arthritis	70% - 95% 60% 20% - 50% 40% - 50% 8% - 14%
RNA	Systemic lupus erythematosus (SLE) Scleroderma, Sjörgren's syndrome	50% 65%
Histones	Drug-induced lupus erythematosus Systemic lupus erythematosus (SLE) Rheumatoid arthritis	95% 58% - 80% 15% - 50%
U1-nRNP	Mixed connective tissue disease (MCTD, Sharp's syndrome) Systemic lupus erythematosus (SLE) Rheumatoid arthritis	95% - 100% 15% - 40% 3%
Sm	Systemic lupus erythematosus (SLE)	5% - 40%
Nucleosomes	Systemic lupus erythematosis (SLE) without lupus nephritis with lupus nephritis	 9% - 89% 18% - 86%
SS-A (Ro)	Sjögren's syndrome Systemic lupus erythematosus (SLE) Neonatal lupus syndrome	40% - 95% 20% - 60% 100%
SS-B (La)	Sjögren's syndrome Systemic lupus erythematosus (SLE)	40% - 95% 10% - 20%
Fibrillarin	Progressive systemic sclerosis, diffuse form	5% - 10%
RNA polymerase I	Progressive systemic sclerosis, diffuse form	4%
PM-Scl (PM-1)	Polymyositis/dermatomyositis/overlap syndrome Progressive systemic sclerosis, diffuse form	50% - 70% 5% - 10%
Centromeres	Progressive systemic sclerosis, limited form	80% - 95%
Scl-70	Progressive systemic sclerosis, diffuse form	25% - 75%
Cyclin (PCNA)	Systemic lupus erythematosus (SLE)	3%
Ku	Systemic lupus erythematosus (SLE) Poly-/dermatomyositis, progressive systemic sclerosis	10% 30% - 55%
Mi-1, Mi-2	Dermatomyositis	5% - 10%

Antibodies against components of the cytoplasm of HEp-2 cells cannot always be clearly differentiated. Only a few cytoplasm-reactive antibodies can be assigned to a particular disease, e.g. antibodies against mitochondria in primary biliary liver cirrhosis and against Jo-1, PL-7 and PL-12 in polymyositis and dermatomyositis. Further rare antibodies found in polymyositis are those directed against OJ, EJ and signal recognition particles (SRP). Other cytoplasmic antibodies – against Golgi apparatus, lysosomes, and some cytoskeletal components, such as vimentin and cytokeratins – are of minor clinical significance. The diagnostic value of mitosis-associated antigens has also not yet been finally clarified. All these arguments show the high immunological relevance and the resulting diagnostic value of autoantibodies against cell nuclei (ANA).

Literature references

1. Alba P, Bento L, Cuadrado MJ, Karim Y, Tungekar MF, Abbs I, Khamashta MA, D'Cruz D, Hughes GR. **Anti-dsDNA, anti-Sm antibodies, and the lupus anticoagulant: significant factors associated with lupus nephritis.** Ann Rheum Dis 62 (2003) 556-560.
2. Brouwer R, Hengstman GJ, Vree Egberts W, Ehrfeld H, Bozic B, Ghirardello A, Grondal G, Hietarinta M, Isenberg D, Kalden JR, Lundberg I, Moutsopoulos H, Roux-Lombard P, Vencovsky J, Wikman A, Seelig HP, van Engelen BG, van Venrooij WJ. **Autoantibody profiles in the sera of European patients with myositis.** Ann Rheum Dis 60 (2001) 116-123.
3. EUROIMMUN AG. Meyer W, Scheper T, Janssen A, Torkler S, Schlumberger W, Stöcker W. **EUROLINE Myositis Profile: A newly developed line immunoassay for the detection of myositis specific antibodies.** In: Conrad K et al. (Hrsg.). From Etiopathogenesis to the Prediction of Autoimmune Diseases: Relevance of Autoantibodies. Pabst Science Publishers 5 (2007) 612-613.
4. EUROIMMUN AG. Schlumberger W, Dähnrich C, Frahm S, Siegemund M, Meyer W, Suer W, Stöcker W. **Diagnostic relevance of autoantibodies against nucleosomes.** Autoimmunity Reviews 1 (2002) 32.
5. EUROIMMUN AG. Stöcker W, Schlumberger W, Krüger C. **Alle Beiträge zum Thema Autoimmundiagnostik.** In: Gressner A, Arndt T (Hrsg.) Lexikon der Medizinischen Laboratoriumsdiagnostik. 2. Auflage. Springer Medizin Verlag, Heidelberg (2012).
6. EUROIMMUN AG. Stöcker W, Teegen B, Meyer W, Müller-Kunert E, Proost S, Schlumberger W, Sonnenberg K. **Differenzierte Autoantikörper-Diagnostik mit BIOCHIP-Mosaiken.** In: Conrad, K. (Hrsg.): **Autoantikörper.** Pabst-Verlag (1998) 78-99.
7. EUROIMMUN AG. Suer W, Dähnrich C, Schlumberger W, Stöcker W. **Autoantibodies in SLE but not in scleroderma react with protein-stripped nucleosomes.** J Autoimmun 22 (2004) 325-334.
8. Frank MB, McCubbin V, Trieu E, Wu Y, Isenberg DA, Targoff IN. **The association of anti-Ro52 autoantibodies with myositis and scleroderma autoantibodies.** Journal of Autoimmunity 12 (1999) 137-142.
9. Fritsch C, Hoebeke J, Dali H, Ricchiuti V, Isenberg DA, Meyer O, Muller S. **52-kDa Ro/SSA epitopes preferentially recognized by antibodies from mothers of children with neonatal lupus and congenital heart block.** Arthritis Res Ther 8 (2006) 4-24.
10. Hanke K, Brückner CS, Dähnrich* C, Huscher D, Komorowski* L, Meyer* W, Janssen* A, Backhaus M, Becker M, Kill A, Egerer K, Burmester GR, Hiepe F, Schlumberger* W, Riemekasten G. (*EUROIMMUN AG). **Antibodies against PM/Scl-75 and PM/Scl-100 are independent markers for different subsets of systemic sclerosis patients.** Arthritis Res Ther 11 (2009) R22.
11. Hartung K, Seelig HP. **Laboratory diagnostics of systemic autoimmune diseases. Part 1. Collagenoses.** [Article in German] Z Rheumatol 65 (2006) 709-724.

12. Haugbro K, Nossent JC, Winkler T, Figenschau Y, Rekvig OP. **Anti-dsDNA antibodies and disease classification in antinuclear antibody positive patients: the role of analytical diversity.** Ann Rheum Dis 63 (2004) 386-394.

13. Herold M, Klotz W, Demel U, Endler G, Forster E, Griesmacher A, Hofmann J, Hübner C, Steiner G, Wagner S, Sack U, Conrad K. **Internationaler Konsens zur ANA-Bestimmung – was ändert sich im deutschen Sprachraum?** J Lab Med 39 (2015) 145-152.

14. Lyons R, Narain S, Nichols C, Satoh M, Reeves WH. **Effective use of autoantibody tests in the diagnosis of systemic autoimmune disease.** Ann N Y Acad Sci 1050 (2005) 217-228.

15. Mahler M, Kessenbrock K, Szmyrka M, Takasaki Y, Garcia-De La Torre I, Shoenfeld Y, Hiepe F, Shun-le C, von Mühlen CA, Locht H, Höpfl P, Wiik A, Reeves W, Fritzler MJ. **International multicenter evaluation of autoantibodies to ribosomal P proteins.** Clin Vaccine Immunol 13 (2006) 77-83.

16. Mahler M, Raijmakers R, Dähnrich* C, Blüthner M, Fritzler MJ. (*EUROIMMUN AG). **Clinical evaluation of autoantibodies to a novel PM/Scl peptide antigen.** Arthritis Res Ther 7 (2005) 704-713.

17. Phan TG, Wong RC, Adelstein S. **Autoantibodies to extractable nuclear antigens: making detection and interpretation more meaningful.** Clin Diagn Lab Immunol 9 (2002) 1-7.

18. Pradhan VD, Patwardhan MM, Ghosh K. **Anti-nucleosome antibodies as a disease marker in systemic lupus erythematosus and its correlation with disease activity and other autoantibodies.** Indian J Dermatol Venereol Leprol 76 (2010) 145-149.

19. Stinton LM, Barr SG, Tibbles LA, Yilmaz S, Sar A, Benedikttson H, Fritzler MJ. **Autoantibodies in lupus nephritis patients requiring renal transplantation.** Lupus 16 (2007) 394-400.

20. Stöcker* W, Rateike* M, Morrin* M, Voigt* J, Rentzsch* K, Krause* C, Ens* K, Fauer* H, Barth E, Feirer* C, Wuttig* D, Fechner* K, Kloth* S, Martinetz T. (*EUROIMMUN AG). **Computer-Aided immunofluorescence microscopy (CAIFM) in the diagnosis of autoimmune and infectious diseases.** Scientific presentation at the 10th Dresden Symposium on Autoantibodies, Dresden, Germany, 2011.

21. Tan EM, Chan EK, Sullivan KF, Rubin RL. **Antinuclear antibodies (ANAs): diagnostically specific immune markers and clues toward the understanding of systemic autoimmunity.** Clin Immunol Immunopathol 47 (1988) 121-141.

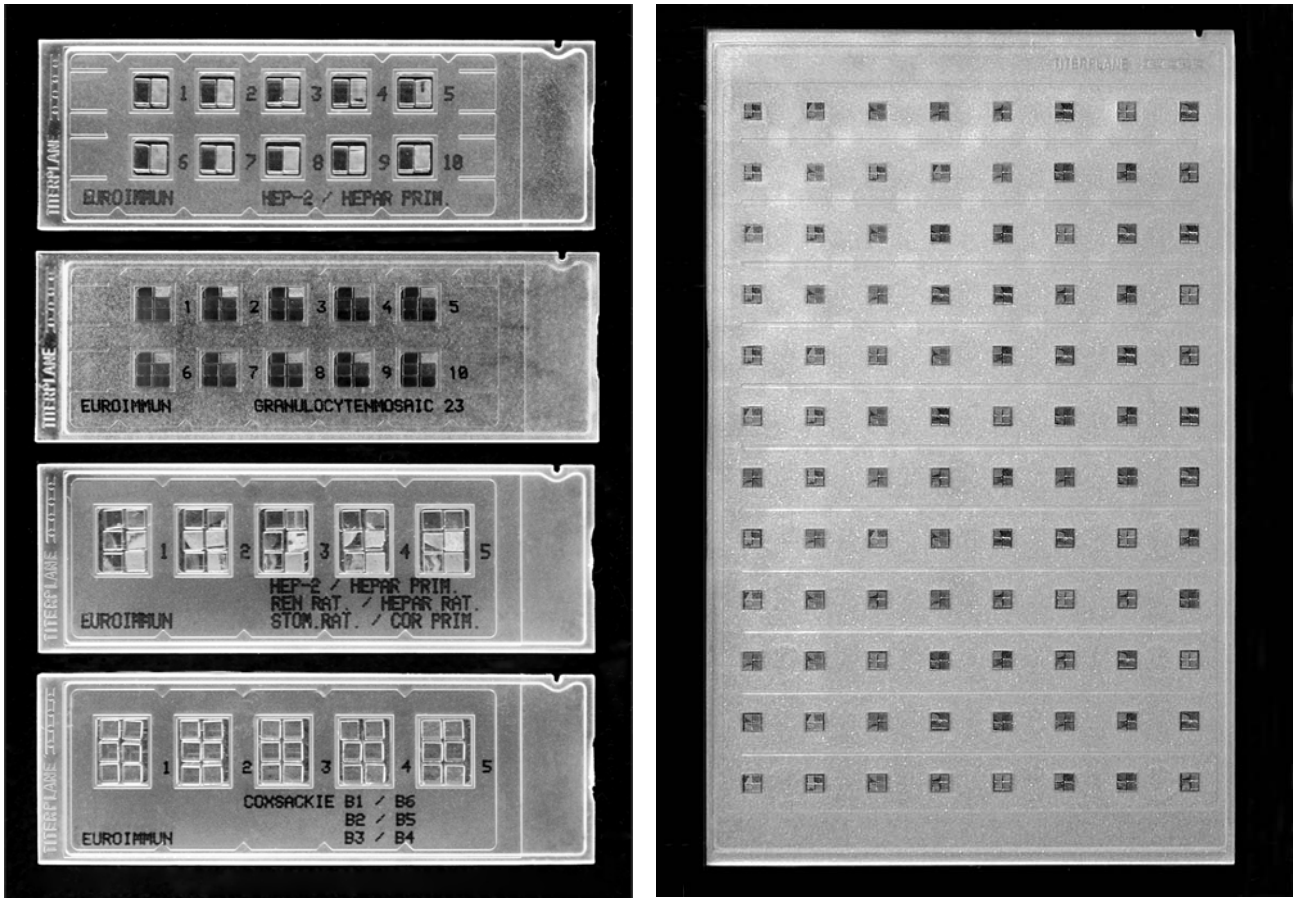
22. Uibel S, Hanke K, Dähnrich* C, Brückner* C, Egerer K, Hiepe F, Schlumberger* W, Riemekasten G. (*EUROIMMUN AG). **Anti-Scl70 antibody levels correlate with skin and organ fibrosis in patients with systemic sclerosis – analysis from the Charité SSc cohort.** In: Conrad K et al. (Hrsg). **From Etiopathogenesis to the Prediction of Autoimmune Diseases: Relevance of Autoantibodies.** Pabst Science Publishers 5: (2007) 475-476.

23. van Venrooij WJ, Charles P, Maini RN. **The consensus workshops for the detection of autoantibodies to intracellular antigens in rheumatic diseases.** J Immunol Methods 5 (1991) 181-189.

24. Varga J. **Systemic sclerosis: an update.** Bull NYU Hosp Jt Dis 66 (2008) 198-202.

25. Voigt* J, Krause* C, Rohwäder* E, Saschenbrecker* S, Hahn* M, Danckwardt* M, Feirer* C, Ens* K, Fechner* K, Barth E, Martinetz T, Stöcker* W. (*EUROIMMUN AG). **Automated Indirect Immunofluorescence Evaluation of Antinuclear Autoantibodies on HEp-2 Cells.** Clinical and Developmental Immunology (2012). doi:10.1155/2012/651058.

BIOCHIP Mosaic®



Slides for indirect immunofluorescence can be produced with single substrates or BIOCHIP Mosaics® from up to 60 different substrates according to your individual requirements: adrenal gland, bladder (rat), brain stem, cartilage*, cerebellum, cerebrum, colon, Crithidia luciliae sensitive, eye, F-actin (VSM47), granulocytes (fixed with EOH, HCHO or MOH), heart muscle, HEp-2 cells, HEp-20-10 cells, hippocampus, HUVEC, hypothalamus*, inner ear (rat)*, intestinal goblet cells, jejunum, kidney (primate, rat, mouse), lacrimal gland*, lactoferrin-specific granulocytes, lip*, lipocytes*, liver (primate, rat, mouse), lung*, lymphocytes, mamma*, monocytes*, mouth mucosa*, oesophagus (primate, rat), optic nerve (AQP-4, NMO IgG), ovary, pancreas, parathyroid gland, parotid gland, peripheral nerve, placenta*, pituitary gland, pons*, prostate*, salt-split skin, skeletal muscle, spermatozoa, spinal cord, stomach (primate, rat, mouse), substantia nigra*, testis, thrombocytes, thymus*, thyroid gland, tongue, umbilical cord, vesicula seminalis* etc. Adenovirus, Bartonella henselae, B. quintana, Bordetella parapertussis, B. pertussis, Borrelia afzelii, B. burgdorferi (strains CH, USA), B. garinii, Campylobacter coli*, C. jejuni, Candida albicans, C. glabrata*, C. krusei*, C. parapsilosis*, C. tropicalis*, Chikungunya virus, Chlamydia pneumoniae, C. trachomatis, C. psittaci, CMV, Coxsackievirus (A7, A9, A16, A24, B1 to B6), Dengue virus type 1 to 4, EBV-CA, EBNA, EBV-EA, Echinococcus granulosus, ECHO virus, hantavirus, Haemophilus influenzae*, Helicobacter pylori, HHV-6, HSV-1, HSV-2, Influenza virus A (strains H3N2, H1N1, H5N1), Influenza virus B, Japanese encephalitis virus, Klebsiella pneumoniae*, Legionella bozemanii*, L. dumoffii*, L. gormanii*, L. jordanis*, L. longbeachae, L. micdadei*, L. pneumophila (serotypes 1 to 14), Leishmania donovani, Listeria monocytogenes (strains 1/2a, 4b)*, measles virus, mumps virus, M. pneumoniae, Parainfluenza virus type 1 to 4, Rift valley fever virus, RSV, rubella virus, Saccharomyces cerevisiae, sandfly fever virus, SARS-CoV, Schistosoma mansoni, Sindbis virus*, TBE virus, TO.R.C.H. profile, Toxoplasma gondii, Treponema pallidum, T. phagedaenis, usutu virus*, VZV, West Nile virus, Yellow fever virus, Yersinia enterocolitica (O:3, O:4, O:6 and O:9)*. **EUROPLUS:** HEp-2/liver + RNP/Sm, Sm, SS-A, SS-B, Scl-70, rib. P-proteins, Jo-1; granulocytes + MPO, PR3; primate stomach (parietal cells) + intrinsic factor; primate liver (endomysium) + gliadin (GAF-3X); rat kidney + AMA M2; thyroid gland + thyroglobulin; renal glomeruli and tubules + GBM; BP180 (NC16A-4X); Borrelia burgdorferi and afzelii + OspC and VlsE; EBV-CA + gp125 + p19; Plasmodium falciparum (HRP-2, MSP-2). **Transfected cells:** AQP-4, BP230gc, collagen VII, desmoglein 1 + 3, envoplakin*, GABABR, GAD65, glutamate receptors (type NMDA, AMPA*), glycine receptors*, PLA2R, rPAg 1 + 2 (pancreas antigen 1 + 2), VGKC (CASPR2, LGI1), Crimean Congo fever virus (GPC, N).

* Currently not available as IVD in the European Union.