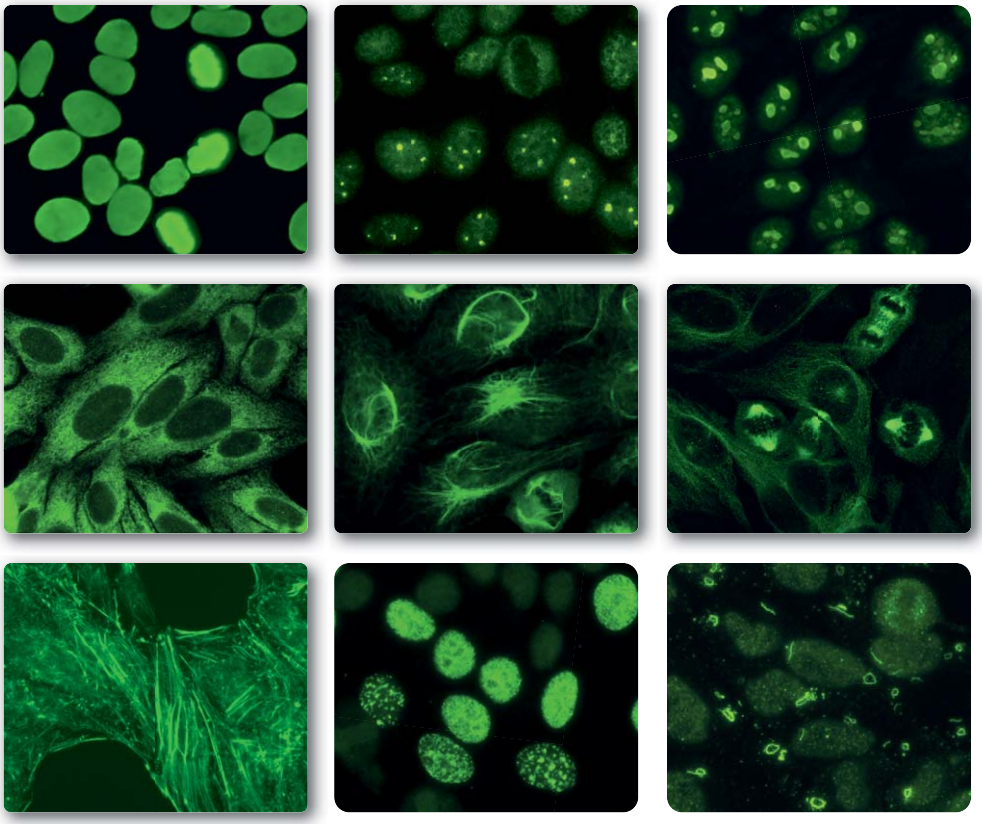
EUROIMMUN Medizinische Labordiagnostika AG

ANA diagnostics using indirect immunofluorescence



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Autoantibodies against cell nuclei (ANA)

Definition

Anti-nuclear autoantibodies are directed against antigens of the cell nucleus. These autoantigens are named after their biochemical characteristics (DNA, histones, ribonucleoproteins: RNP), the disease associated with the corresponding autoantibody (SS-A, SS-B: Sjögren's syndrome, antigens A and B; PM-Scl: polymyositis, progressive systemic sclerosis) or, occasionally, after the patient in whom the corresponding antibody was first detected (Sm, Ro, La).

More than 100 autoantigens are presented in HEp-2 cells.
The most important among them are:

Polynucleotides	Double-stranded DNA, single-stranded DNA, RNA
Histones	H1, H2A, H2B, H3, H4, H2A-H2B complex
Ribonucleoproteins	U1-(n)RNP, Sm, SS-A (Ro), SS-B (La)
Nucleolar antigens	U3-(n)RNP/fibrillarin, RNA polymerase I, PM-Scl (PM-1), 7-2-RNP (To), 4-6-S-RNA, NOR-90 (nucleolar organiser)
Centromeres	Kinetochore proteins
Other proteins	Topoisomerase I (Scl-70), PCNA (cyclin I), nuclear granules, Ku, Mi-2, lamins, lamin receptors

Analytics

Due to its high sensitivity and specificity, the indirect immunofluorescence test (IIFT) using human epithelial cells (HEp-2) and primate liver is the gold standard for the detection of anti-nuclear autoantibodies (ANA). The signal intensities of a positive and a negative sample differ significantly and microscopic evaluation allows an exact determination of how the indicator dye (usually fluorescein) is spread in the tissue or cells. Each bound autoantibody causes a typical fluorescence pattern, depending on the location of the corresponding autoantigen. If the analysis result is positive, test substrates with defined single antigens (ELISA, western blot, line blot) are used for further differentiation. Using mo-nospecific test methods alone is not sufficient for the determination of anti-nuclear autoantibodies since not all relevant antigens are available in their purified form. For verification of analysis results, monospecific tests should always be accompanied by IIFT.

Evaluation

Anti-nuclear autoantibodies (ANA) in patient serum are a characteristic finding in many diseases, in particular, but not exclusively, rheumatic diseases. In the foreground are the following:

Autoimmune disease	ANA prevalence (%)
Systemic lupus erythematosus (SLE, active)	95–100
Drug-induced lupus erythematosus	100
Mixed connective tissue disease (MCTD, Sharp syndr.)	100
Rheumatoid arthritis	20–40
Other rheumatic diseases	20–50
Progressive systemic sclerosis	85–95
Polymyositis/dermatomyositis	30–50
Sjögren's syndrome	70–80
Autoimmune hepatitis (AIH)	30–40
Ulcerative colitis	26

The detection of autoantibodies against cell nuclei 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 nuclear 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%, and it is lowest in rheumatoid arthritis at between 20% and 40%. Therefore, differential antibody diagnostics against nuclear antigens is indispensible for the diagnosis of individual rheumatic diseases and their differentiation from other autoimmune diseases.

Systemic lupus erythematosus

The determination of antibodies against double-stranded DNA (dsDNA) is considered the most important criterion for the diagnosis of systemic lupus erythematosus (SLE), also referred to as lupus erythematosus disseminatus (LED). Immune complexes consisting of dsDNA and corresponding autoantibodies cause tissue damage in the subcutis, kidneys and other organs. The antibody titer correlates with the activity of the disease. Antibodies against nucleosomes and

Sm are also considered to be pathognomonic for SLE. Antibodies against other polynucleotides, ribonucleotides, histones and further nuclear antigens can also be detected in this disease. In drug-induced lupus erythematosus with manifestations such as arthralgia, arthritis, exanthema, serositis, myalgia, heptomegalia and splenomegalia, antibodies against histones are constantly 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, hydantoines), antiarrythmics (e.g. procainamide, practolol), antihypertensives (e.g. reserpine, hydralazine), psychotropics (e.g. chlorpromazine), anti-thyroid drugs (e.g. thiouracil derivatives), anti-rheumatoid basis therapeutics (e.g. gold, D penicillamine) and other drugs such as contraceptives and allopurinol.

Autoantibodies in systemic lupus erythematosus (SLE)

Antigen	Prevalence (%)
Double-stranded DNA	60–90
Single-stranded DNA	70–95
Nucleosomes	50–70
RNA	50
RNA helicase A	6
Histones	50–80
U1-nRNP	15–40
Sm	5–40
SS-A (Ro)	20–60
SS-B (La)	10–20
PCNA-like	3
Ku	10
Ribosomal P proteins	10

Mixed connective tissue disease

High autoantibody titers against U1-nRNP are characteristic for mixed connective tissue disease. The antibody titer correlates with the activity of the disease.

Autoantibodies in mixed connective tissue disease (MCTD, Sharp syndr.)	
Antigen	**Prevalence (%)**
U1-nRNP	95–100
Single-stranded DNA	20–50

Rheumatoid arthritis

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

Autoantibodies in rheumatoid arthritis	
Antigen	**Prevalence (%)**
Histones	15–50
Single-stranded DNA	8
U1-nRNP	3

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 topoisomerase I (Scl-70) have only been observed in the diffuse form of the disease. Autoantibodies against centromeres are associated with the limited form of PSS.

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

Autoantibodies in progressive systemic sclerosis (diffuse form)	
Antigen	**Prevalence (%)**
Fibrillarin	5–10
PM-Scl (PM-1): (75 kDa / 100 kDa main antigen)	13 (10/7)

Autoantibodies in progressive systemic sclerosis (diffuse form)

Antigen	Prevalence (%)
Topoisomerase I (Scl-70)	25–75
RNA polymerase I	4
Ku, incl. overlap syndrome with PM/DM	25–50
7-2-RNP (To)	rare
NOR-90 (nucleolar organiser region)	rare

Polymyositis/dermatomyositis

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

Autoantibodies in polymyositis and dermatomyositis

Antigen	Prevalence (%)
PM-Scl (PM-1), incl. overlap syndrome with PSS	24–55
Jo-1 (histidyl-tRNA synthetase)	25–35
Mi-1	10
Mi-2	5–30
Ku, incl. overlap syndrome with PSS	25–50
Single-stranded DNA	40–50
SRP	5
TIF1-gamma	5
PL-7, PL-12 (aminoacyl-tRNA synthetases)	3–4

Sjögren's syndrome

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

Autoantibodies in primary Sjögren's syndrome

Antigen	Prevalence (%)
SS-A (Ro)	40–95
SS-B (La)	40–95

Autoantibodies in primary Sjögren's syndrome

Antigen	Prevalence (%)
Single-stranded DNA	13
(Salivary excretory ducts	40–60)

Primary biliary cholangitis (formerly: primary biliary cirrhosis)

In addition to antibodies against mitochondria, various autoantibodies against cell nuclei are associated with primary biliary cholangitis. Some of them can be considered pathognomonic. Furthermore, antibodies against SS-A and centromeres can also be frequently found in PBC. The presence of these two antibodies or antibodies against gp210 indicate an unfavourable prognosis.

Autoantibodies in primary biliary cholangitis

Antigen	Prevalence (%)
AMA-M2	95
Nuclear dots	25–40
Nuclear membrane	20–40
SS-A	20
Centromeres	20–30

At times, antibodies against nuclear antigens are detectable in subjectively healthy individuals, with a prevalence of 5% and usually at a low titer (different immunoglobulin classes, but mainly IgM).

Anti-nuclear autoantibodies: The most important associated diseases

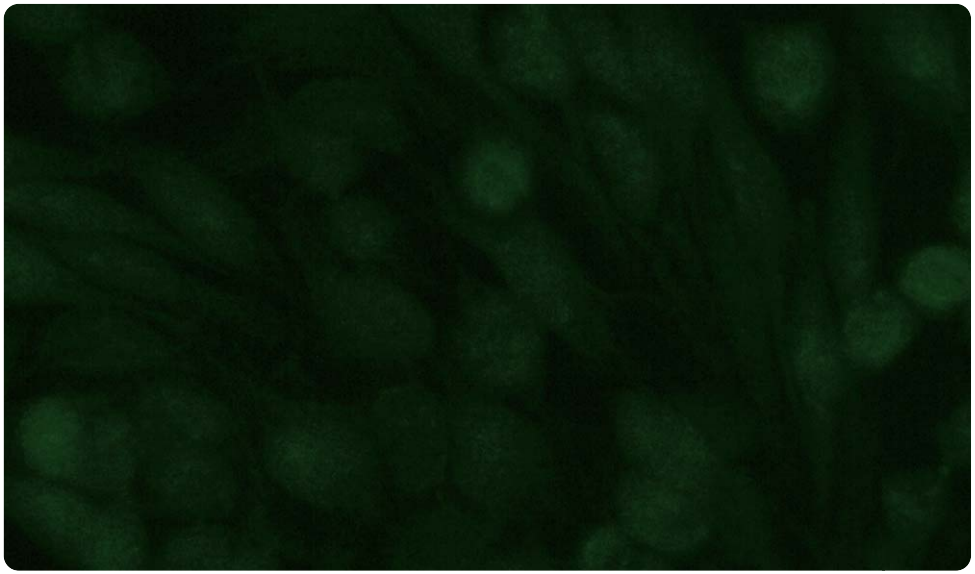
Antigen	Disease	Prevalence (%)
dsDNA	Systemic lupus erythematosus (SLE)	60–90
ssDNA	SLE	70–95
	Drug-induced SLE	60
	Mixed connective tissue disease	20–50
	Polymyositis/dermatomyositis	40–50
	Progressive systemic sclerosis (PSS), Sjögren's syndrome, rheum. arthritis	8–14
RNA	SLE	50
	PSS, Sjögren's syndrome	65

Anti-nuclear autoantibodies: The most important associated diseases

Antigen	Disease	Prevalence (%)
Histones	Drug-induced SLE	95
	SLE	50–80
	RA	15–50
U1-nRNP	MCTD (Sharp syndrome)	95–100
	SLE	15–40
	RA	3
Sm	SLE	5–40
SS-A (Ro)	Sjögren's syndrome	40–95
	SLE	20–60
	Neonatal lupus syndrome	100
SS-B (La)	Sjögren's syndrome	40–95
	SLE	10–20
Fibrillarin	PSS, diffuse	5–10
RNA polymerase I	PSS, diffuse	4
RNA helicase A	SLE	6
PM-Scl (PM-1)	Poly-/dermatomyositis/overlap syndr.	24–55
	PSS, diffuse	13
Centromeres	PSS, limited	80–95
Topoisomerase I	PSS, diffuse	25–75
PCNA-like	SLE	3
Ku	SLE	10
	Poly-/dermatomyositis, PSS	25–50
Mi-1, Mi-2	Dermatomyositis	5–30

Antibodies against cytoplasmic components of HEp-2 cells cannot always be clearly differentiated by their immunofluorescence pattern. Only a few cytoplasm-reactive antibodies can be assigned to a particular disease, e.g. antibodies against mitochondria in primary biliary cholangitis and antibodies against the proteins 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 ribosomes, Golgi apparatus, lysosomes and 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. When all these arguments are considered, the high immunological relevance and the resulting diagnostic value of anti-nuclear autoantibodies become evident.

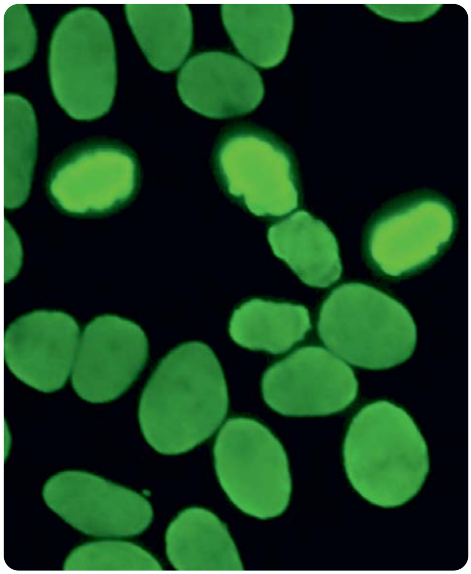
Autoantibodies negative (AC-0)



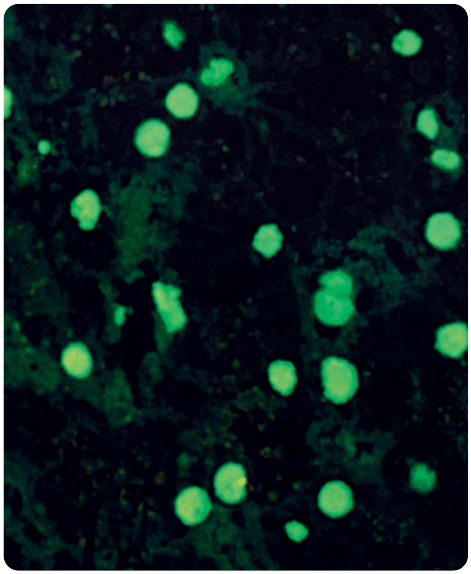
HEp-2 cells

HEp-2 cells show no specific fluorescence.

Autoantibodies against cell nuclei, homogeneous (AC-1)



HEp-2 cells



Primate liver

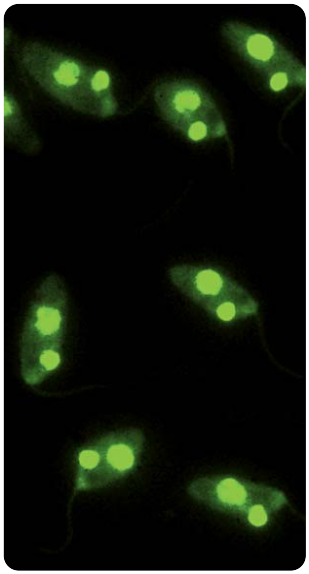
HEp-2 cells show a homogeneous fluorescence of the cell nuclei. The condensed chromosomes of mitotic cells are positive. The area surrounding the chromosomes is dark.

On the substrate **primate liver** a homogeneous, partly coarse to fine clumpy fluorescence of the cell nuclei can be observed.

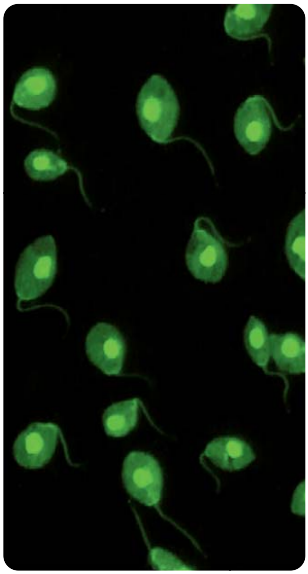
Known target antigens: dsDNA, ssDNA, nucleosomes and histones.

Clinical association: SLE, drug-induced SLE and juvenile idiopathic arthritis.

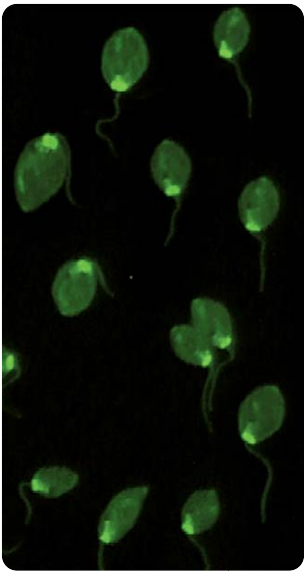
Autoantibodies against dsDNA



AAb against dsDNA pos. (kinetoplast)



AAb against dsDNA neg. (cell nucleus)

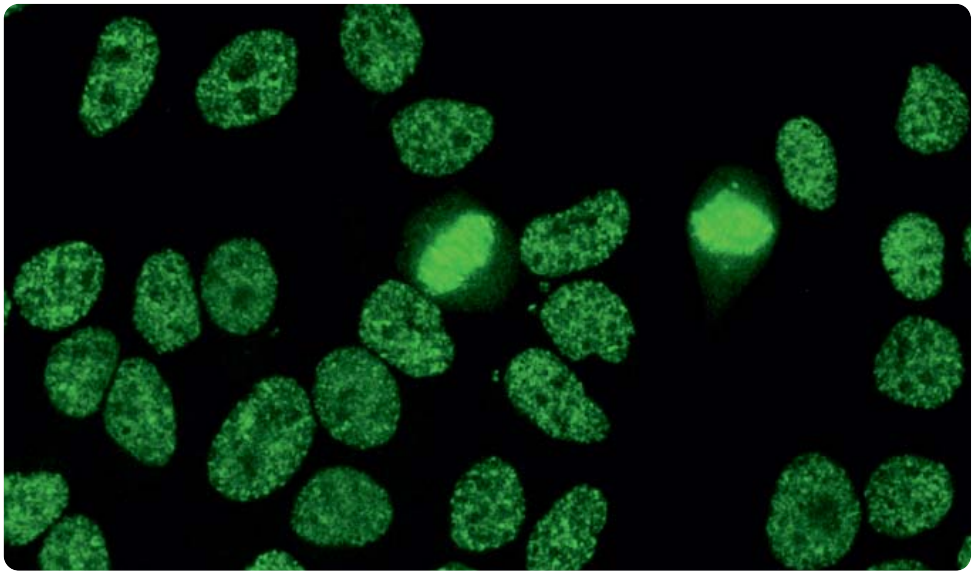


AAb against dsDNA neg. (basal body)

The standard substrate for the immunofluorescence test is the haemoflagellate **Crithidia luciliae**. It possesses a dsDNA-containing giant mitochondrion (kinetoplast) which, apart from dsDNA, essentially displays no antigens which occur also in the cell nucleus. Antibodies which react with the kinetoplast are therefore directed exclusively against dsDNA. With C. luciliae they produce a homogenous, partly edge-accentuated fluorescence of the kinetoplast. Any reaction in the cell nucleus is not evaluated; fluorescence in the basal body of the flagellum is without significance. Antibodies to ssDNA cannot stain the kinetoplast.

Clinical association: Autoantibodies against dsDNA are found exclusively in SLE and in 60–90 % of cases, depending on the method of investigation and the disease activity.

Autoantibodies against cell nuclei, DFS pattern (AC-2)



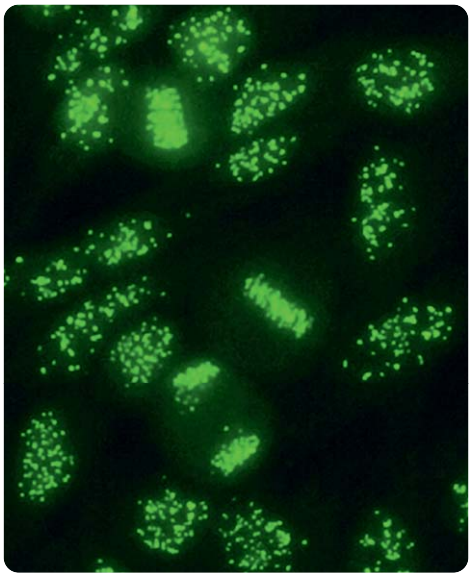
HEp-2 cells

On the substrate **HEp-2 cells** autoantibodies against the DFS70 antigen (and possibly other antigens) depict a uniformly distributed dense fine speckled fluorescence with granular staining of the condensed chromosomes.

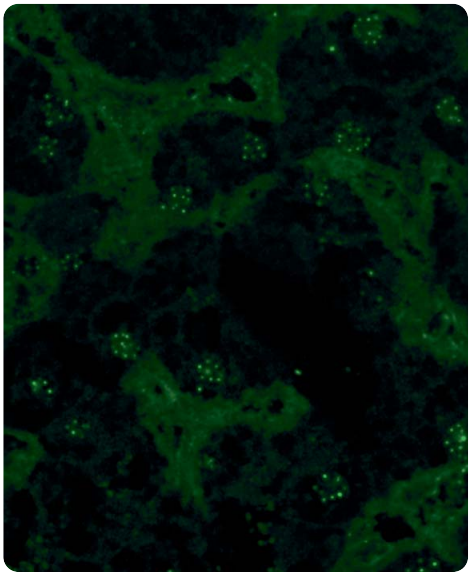
Known target antigen: DFS70.

Clinical association: Autoantibodies against DFS70 have been found in patients with different diseases (amongst others atopic dermatitis, asthma and interstitial cystitis) and in healthy blood donors. Due to their low prevalence in systemic autoimmune rheumatic diseases it had been discussed whether the detection of these autoantibodies can be used as an exclusion criterion. It has recently been shown, however, that anti-DFS70 antibodies also occur in autoimmune rheumatic diseases with a prevalence of up to 11%. The clinical association remains unclear.

Autoantibodies against centromeres (AC-3)



HEp-2 cells



Primate liver

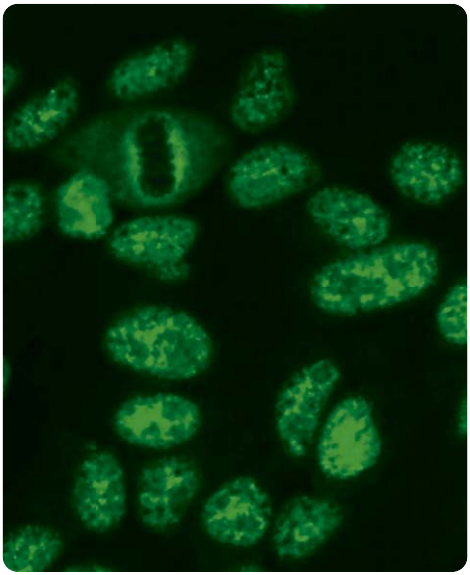
HEp-2 cells show a very specific fluorescence pattern, which is characterised by fine, evenly sized granules (generally 46 or 92 centromeres per cell nucleus). The granules in interphase cells are spread evenly over the nucleus, while in mitotic cells they are arranged either ribbon-like on the equatorial plane (metaphase) or in two parallel ribbons approaching the centrioles (anaphase).

On tissue sections of **primate liver** 10 to 20 granules, which are spread over the cell nucleus, can be seen. The fluorescence of these granules is significantly weaker than the HEp-2 cell staining and is therefore easy to miss. Mitotic cells are only rarely detected on liver substrate.

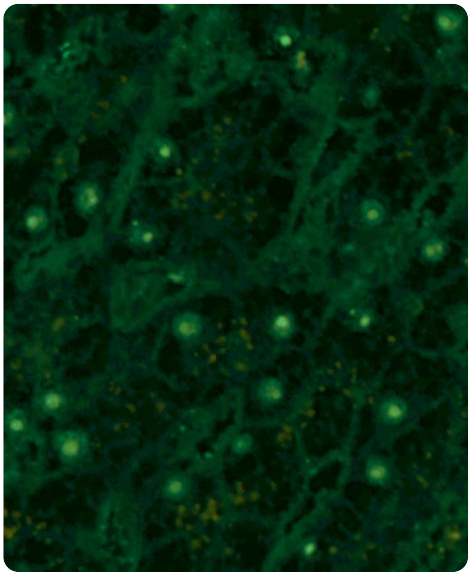
Known target antigens: CENP-A and -B.

Clinical association: With a high specificity and a prevalence of 80–95%, antibodies against centromeres are pathognomonic for the limited form of progressive systemic sclerosis. In the limited form the extremities are favoured and the inner organs less affected.

Autoantibodies against nucleoplasm, fine speckled (AC-4)



HEp-2 cells



Primate liver

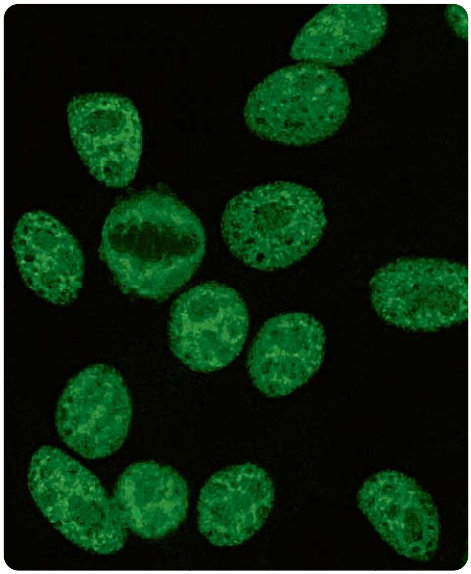
HEp-2 cells show a fine speckled fluorescence of the cell nuclei in the interphase. The nucleoli are also reactive, but they are slightly silhouetted against the nucleoplasm. In some samples they do not react at all. Mitotic cells show a speckled fluorescence, with the chromosomes excluded.

On tissue sections of **primate liver** there is no speckled reaction in the hepatocyte nuclei, but the nucleoli show a smooth fluorescence in samples with a high antibody titer.

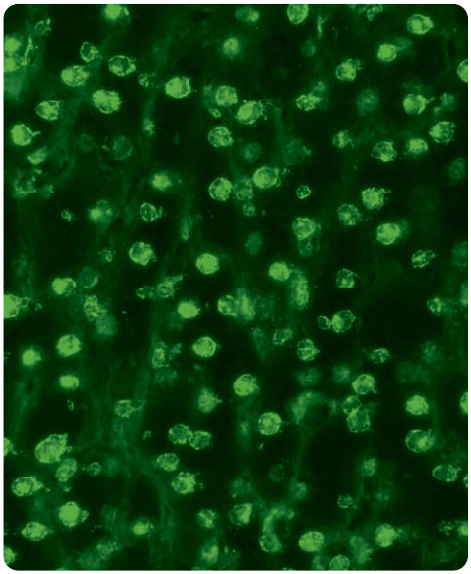
Known target antigens: SS-A and SS-B.

Clinical association: Sjögren's syndrome, SLE and neonatal LE.

Autoantibodies against Ku (AC-4)



HEp-2 cells



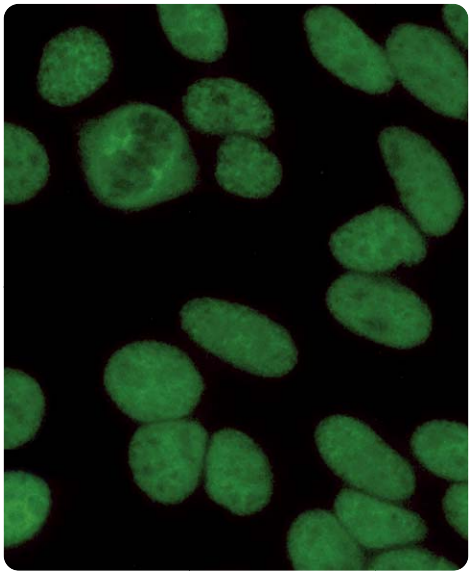
Primate liver

In the indirect immunofluorescence test with **HEp-2 cells**, antibodies against Ku exhibit a fine speckled fluorescence of the cell nuclei and the nucleoli are positive in parts. There is hardly any difference noticeable to antibodies against SS-A, SS-B, Sm and RNP.

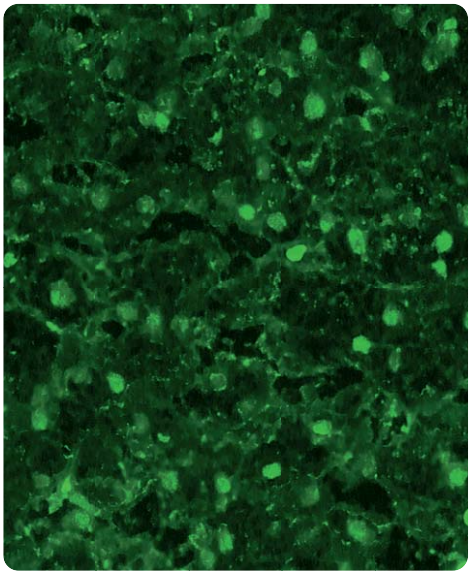
However, if **primate liver** sections are incubated in parallel, possibly in the same field, a typical clumpy-speckled staining of the cell nuclei is found, which is an almost certain proof of antibodies to Ku.

Clinical association: Autoantibodies against Ku occur with the following prevalences: 24–55% in overlap syndrome of poly-/dermatomyositis and progressive systemic sclerosis (often accompanied by primary pulmonary hypertension), 5–10% in various forms of myositis, 10% in systemic lupus erythematosus and up to 5% in progressive systemic sclerosis.

Autoantibodies against Mi-2 (AC-4)



HEp-2 cells



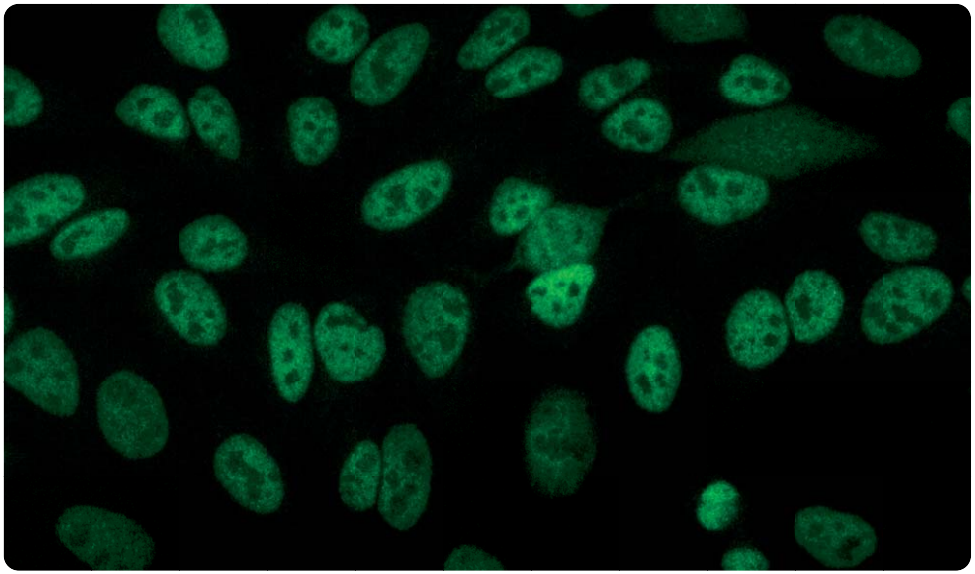
Primate liver

Autoantibodies to Mi-2 show a fine-speckled fluorescence of the cell nuclei in the indirect immunofluorescence test with **HEp-2 cells**. The nucleoli are partly unaffected.

With **primate liver**, autoantibodies against Mi-2 depict a fine speckled fluorescence of the hepatocyte nuclei.

Clinical association: Antibodies against Mi-2 are highly specific markers for dermatomyositis with nail fold hypertrophy. They are found in 5–30% of patients with dermatomyositis and in 8–12% of patients with idiopathic myositis.

Autoantibodies against TIF1-gamma (AC-4)

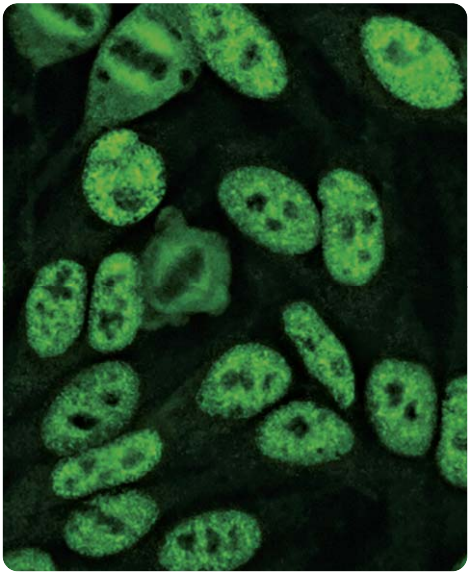


HEp-2 cells

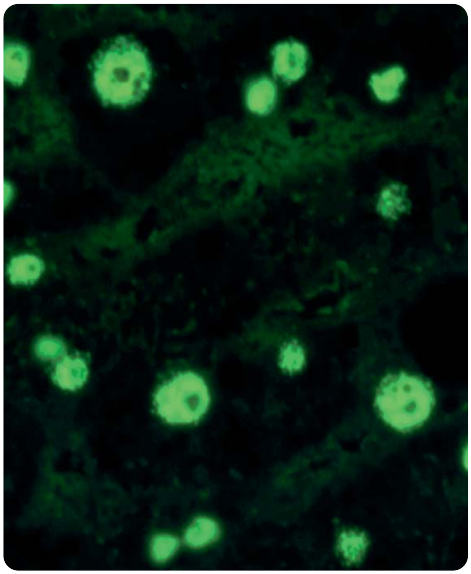
Autoantibodies against TIF1-gamma cause a fine speckled fluorescence on **HEp-2 cells**, which is distributed over the whole cell nucleus but leaves the nucleoli free. Mitotic cells also exhibit a fine speckled fluorescence, but the chromosomes are spared.

Clinical association: Antibodies against TIF1-gamma can be detected with a prevalence of 5% in patients with dermatomyositis. In particular, they are specific for cancer-associated (paraneoplastic) (dermato)myositis (CAM).

Autoantibodies against nucleoplasm, coarse speckled (AC-5)



HEp-2 cells



Primate liver

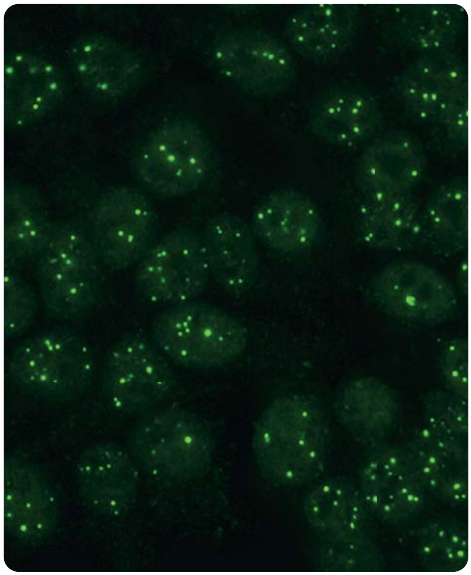
HEp-2 cells generally show a coarse speckled, sometimes medium to fine speckled fluorescence, which is spread over the entire cell nucleus, leaving the nucleoli free. In mitotic cells the condensed chromosomes are dark, while the periphery shows an almost homogeneous, smooth fluorescence.

Tissue sections of **primate liver** also show a speckled fluorescence. The nucleoli do not react. The antibodies react with primate liver to the same extent as with HEp-2 cells.

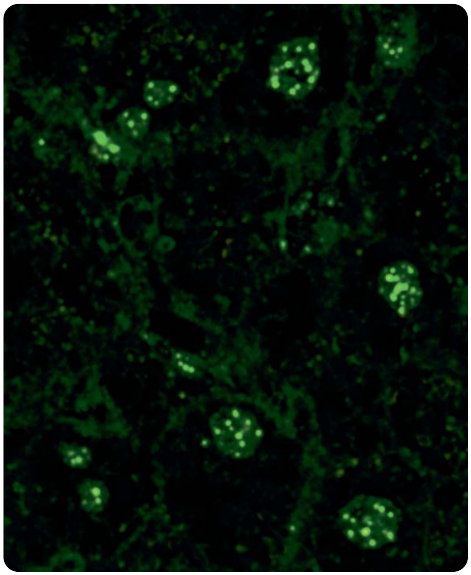
Known target antigens: hnRNP, U1-nRNP, Sm and RNA polymerase III.

Clinical association: SLE and mixed connective tissue disease.

Autoantibodies against nuclear dots (AC-6)



HEp-2 cells



Primate liver

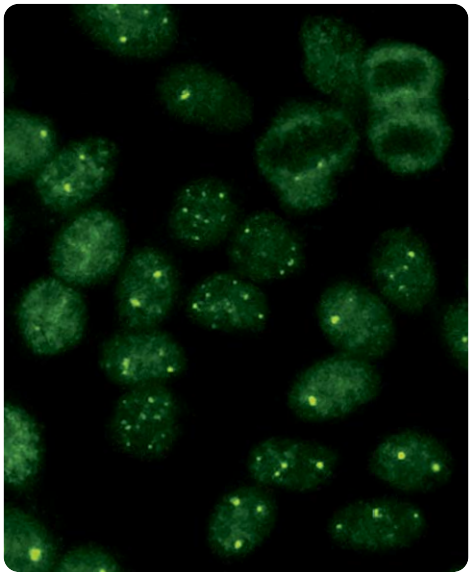
In immunofluorescence using **HEp-2 cells**, 6–20 differently sized granules which are spread over the cell nucleus (nuclear dots) can be seen in the nuclei during interphase. The cytoplasm is dark if antibodies against mitochondria, which are associated with primary biliary cholangitis, are not present at the same time. In mitotic cells the nuclear dots are dissolved. Outside the (unstained) chromosomes only isolated granules fluoresce.

Antibodies against nuclear dots react with **primate liver** to the same extent as with HEp-2 cells. If both substrates are used in parallel, these antibodies can even be identified if antibodies against centromeres are present at the same time. This can occasionally be observed in cases of primary biliary cholangitis.

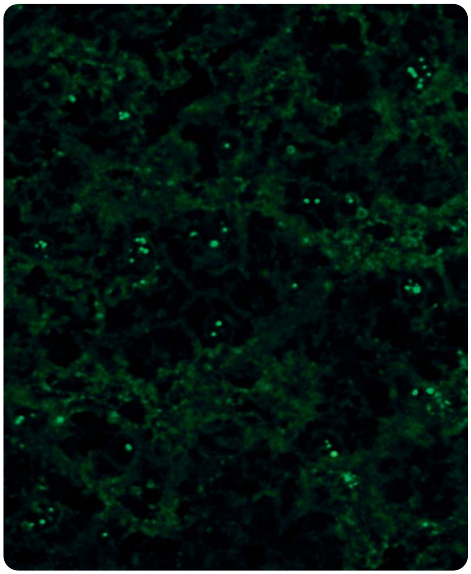
Known target antigens: Sp100, Sp140, PML, SUMO and MJ/NXP-2.

Clinical association: Autoantibodies against nuclear dots occur in 25–40% of patients with primary biliary cholangitis. The pattern is also an indicator for rheumatic diseases.

Autoantibodies against few nuclear dots (AC-7)



HEp-2 cells



Primate liver

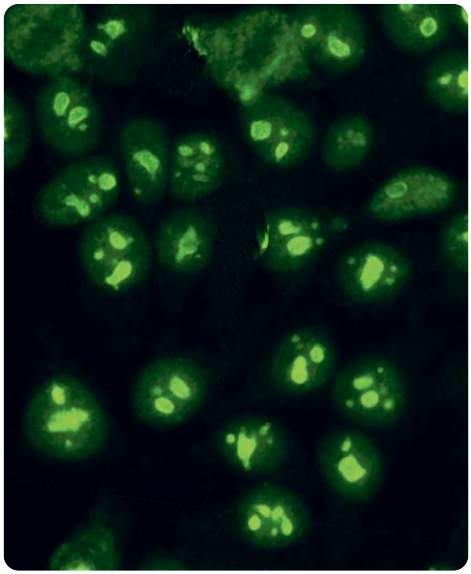
In the immunofluorescence on HEp-2 cells, the pattern few nuclear dots merely shows 1–6 dots per cell nucleus – frequently near the nucleoli. In the late S/G2 phase of the cell cycle, the cells show relatively many dots (4–6). The metaphase chromatin is usually negative. These nucleolar dots are denominated Cajal bodies (formerly: coiled bodies).

On the **primate liver** tissue section the dots manifest slightly augmented in comparison with the HEp-2 cells. The liver may, however, also show a negative reaction.

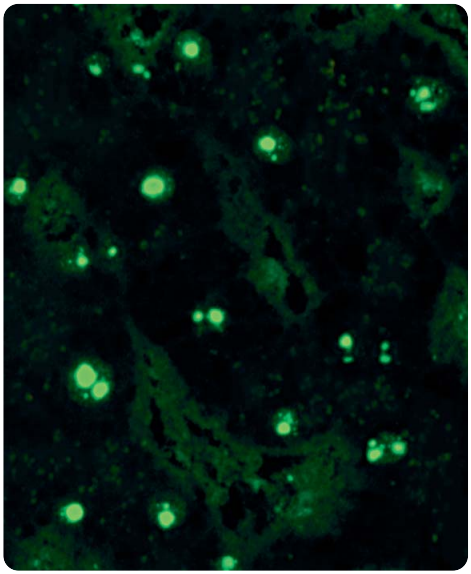
Known target antigens: p80-coilin and SMN (survival of motor neuron).

Clinical association: Sjögren's syndrome and SLE.

Autoantibodies against PM-Scl (AC-8)



HEp-2 cells



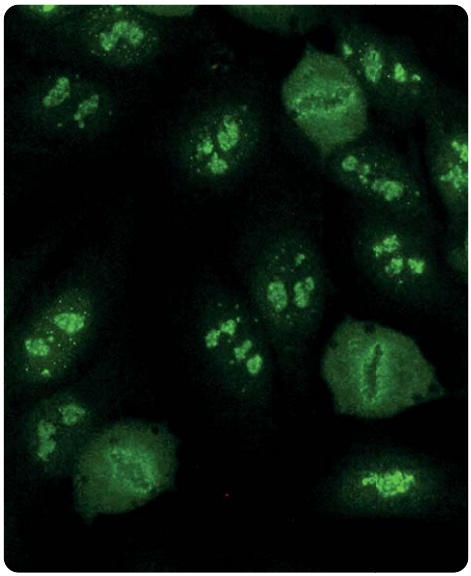
Primate liver

In the immunofluorescence test with **HEp-2 cells**, autoantibodies against PM-Scl exhibit a homogeneous fluorescence of the nucleoli with a simultaneous weaker, fine-speckled reaction of the nucleoplasm. The condensed chromosomes of the mitotic cells are unaffected; a fine, speckled fluorescence is shown outside of the chromosomes.

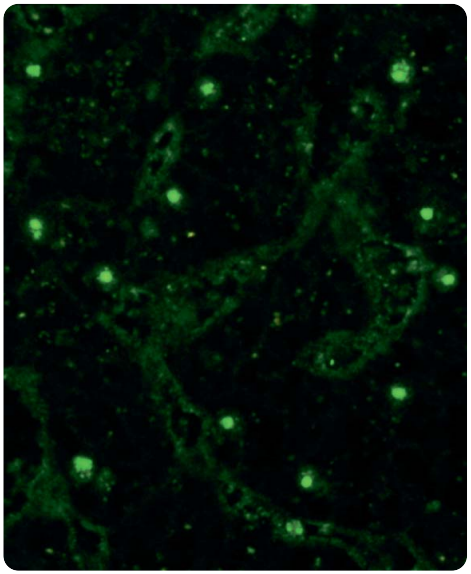
A homogeneous fluorescence of the nucleoli also appears on frozen sections of **primate liver**, as well as a very weak, fine-speckled to reticular staining of the cell nucleus.

Clinical association: PM-Scl antibodies can be detected in 24–55% of patients with polymyositis/systemic sclerosis overlap syndrome. Here, the autoanti-bodies are usually directed against both main antigens: PM-Scl75 and PM-Scl100. If progressive systemic sclerosis is exclusively present, antibodies to PM-Scl75 show a prevalence of 10%, and antibodies to PM-Scl100 a prevalence of 7%. Using test systems which detect only anti-PM-Scl100, some patients with pro-gressive systemic sclerosis remain unidentified.

Autoantibodies against U3-nRNP/fibrillarin (AC-9)



HEp-2 cells



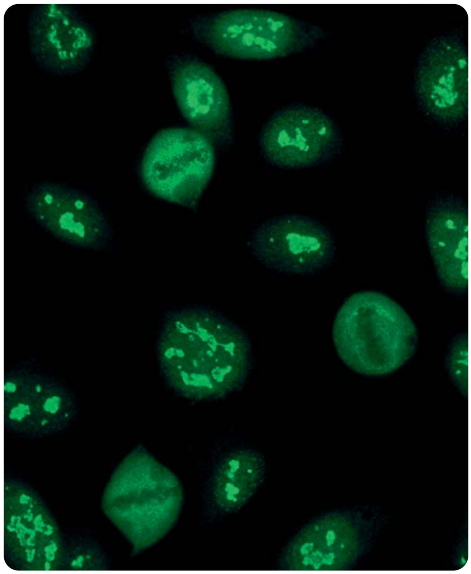
Primate liver

On **HEp-2 cells** interphase cells show a speckled fluorescence of the nucleoli. Mitotic cells show a coronary perichromosomal fluorescence.

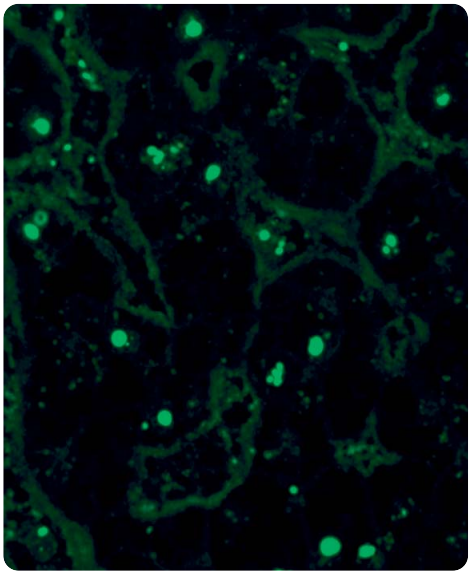
The substrate **primate liver** depicts a homogeneous fluorescence of the cell nuclei.

Clinical association: Antibodies against fibrillarin have so far been observed only in progressive systemic sclerosis (diffuse form). The prevalence is 5–10%.

Autoantibodies against RNA polymerase I (AC-10)



HEp-2 cells



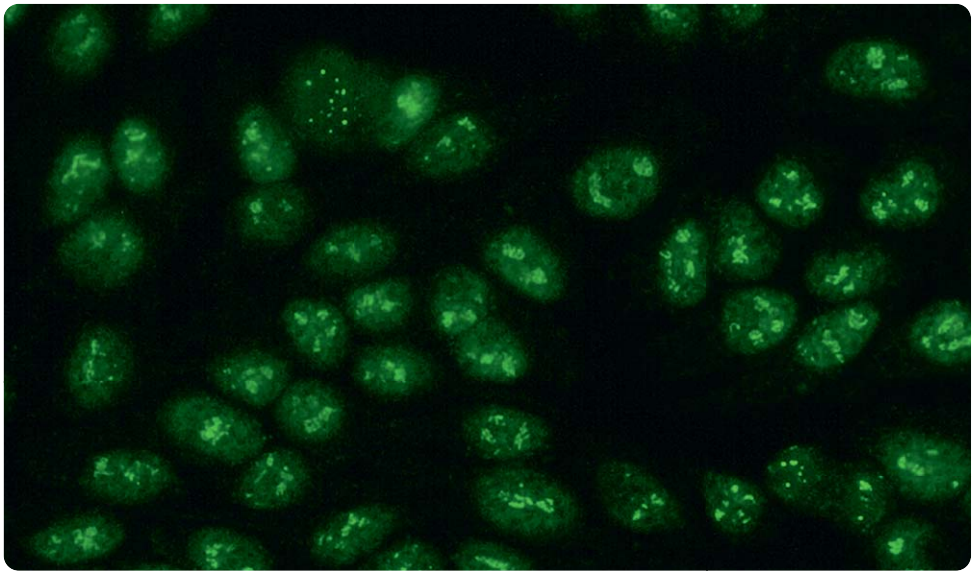
Primate liver

HEp-2 cells show a granular fluorescence of the nucleoli. The nucleoplasm is almost dark. In mitotic cells the region of condensed chromosomes is not stained. Outside of the chromosomes a fine granular to smooth fluorescence can be seen. If autoantibodies against NOR-90 occur in parallel, one to several dots fluoresce on mitotic cells.

On **primate liver** tissue sections, the nucleoli show a positive reaction.

Clinical association: Antibodies against RNA polymerase I have so far only been detected in progressive systemic sclerosis (diffuse form). The prevalence amounts to 4%.

Autoantibodies against NOR-90 (AC-10)

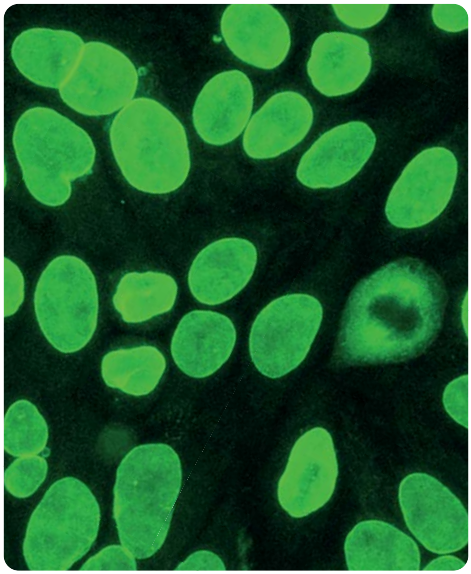


HEp-2 cells

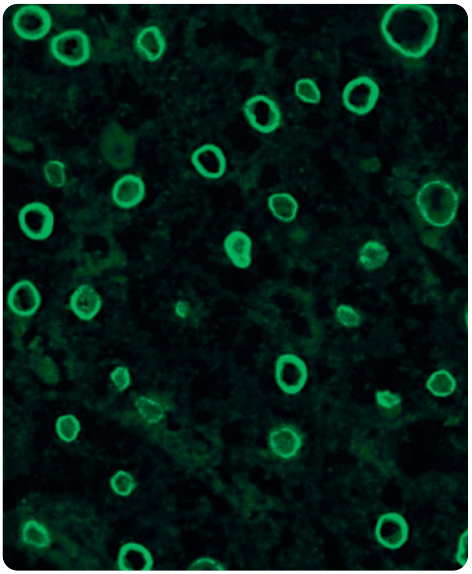
On **HEp-2 cells** in the metaphase, one to few little dots fluoresce within the condensed chromosome material. They correspond to the nucleolus organisator (NOR). The cytoplasm of mitotic cells may be weakly positive. Interphase cells show a granular fluorescence of the nuceloli.

Clinical association: Progressive systemic sclerosis (diffuse form).

Autoantibodies against nucl. membrane (AC-11/AC-12)



HEp-2 cells



Primate liver

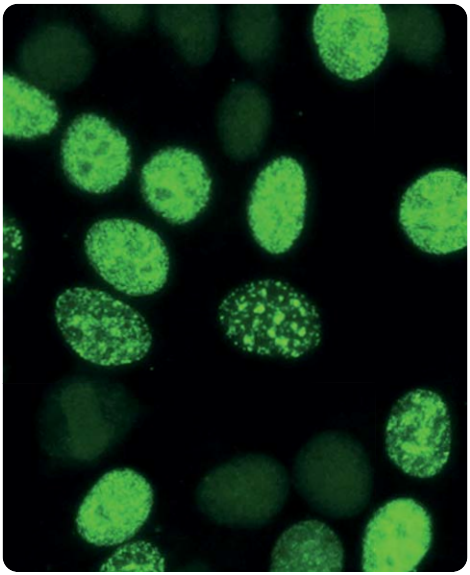
On **HEp-2 cells** the interphase cells show a homogeneous fluorescence of the cell nuclei, with the rims of the nuclei accentuated. The chromosomes of mitotic cells are dark.

On tissue sections of **primate liver** a characteristic linear fluorescence of the nuclear membrane can be seen.

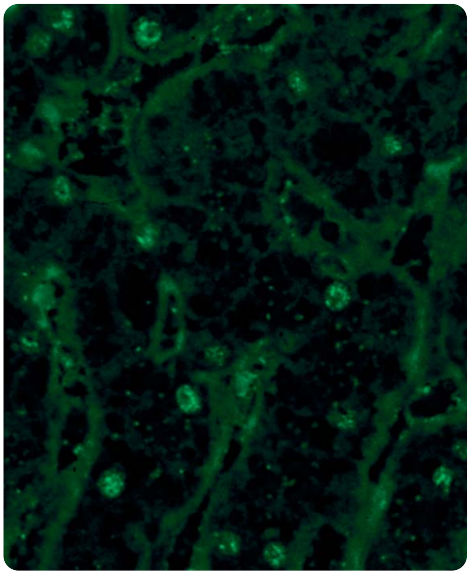
Known target antigens: gp210, lamin A, lamin B and C, and lamin B receptor.

Clinical association: Antibodies against nuclear membrane occur in primary biliary cholangitis (PBC).

Autoantibodies against PCNA (AC-13)



HEp-2 cells



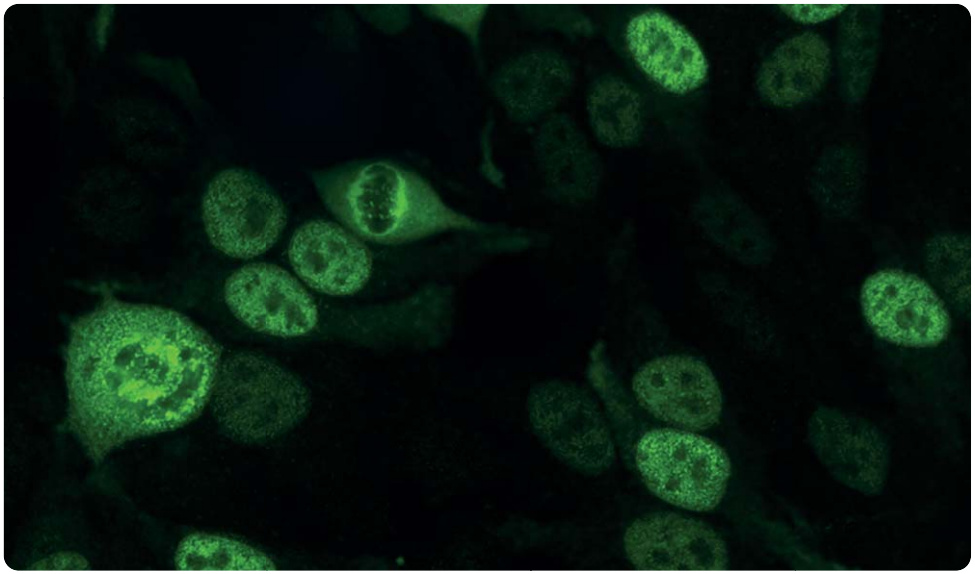
Primate liver

Autoantibodies against PCNA show a cell cycle-dependent fluorescence pattern with **HEp-2 cells**. Half of the cell nuclei of all interphase cells exhibit a bright, fine speckled basic fluorescence with the nucleoli being unaffected. The same fluorescence pattern is found with the other half, but the intensity is lower by a factor of 10. The area of the condensed chromosomes is not stained in the mitosis; the surrounding area of the chromosomes shows only a weak, fine speckled fluorescence, corresponding to the darker nuclei of the interphase cells in pattern and intensity.

The reaction with **primate liver** is largely negative.

Clinical association: PCNA antibodies are specific for SLE. The prevalence, however, is only 3%.

Autoantibodies against CENP-F (Cyclin II – Mitosin) (AC-14)



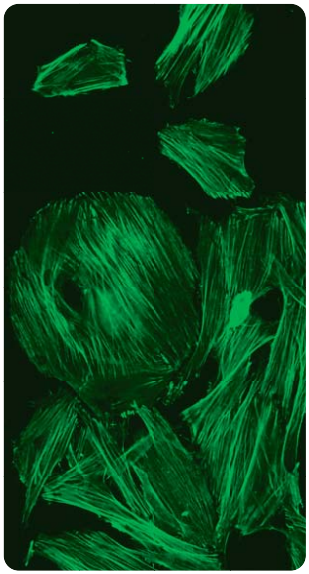
HEp-2 cells

On **HEp-2 cells,** autoantibodies against CENP-F show a fine to coarse speckled fluorescence of the cell nuclei. The staining intensity varies strongly, G2-phase nuclei show the strongest fluorescence while G1-phase nuclei react with much weaker intensity or not at all. Apart from this, the mitotic cells fluoresce especially strongly (with the exception of the chromosome region), smooth to fine speckled. The centromeres are exclusively positive in the prometa- and metaphase and then show many small and mat aligned dots. In prometaphase cells, the nuclear membrane is often slightly stained. During ana- and telophase, sometimes an intensive fluorescence of the midbody occurs. The cytoplasm of mitotic cells is diffusely stained.

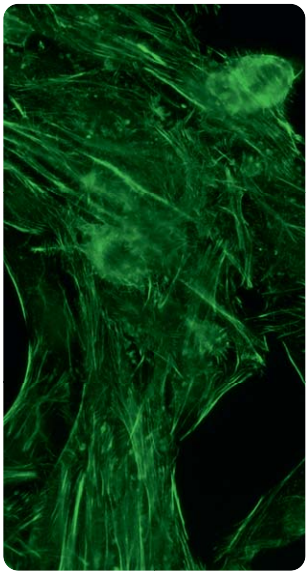
The **primate liver** does not show any specific reaction.

Clinical association: In 50% of the patients who display antibodies against CENP-F, a malign underlying disease is present. Different tumours must be taken into consideration.

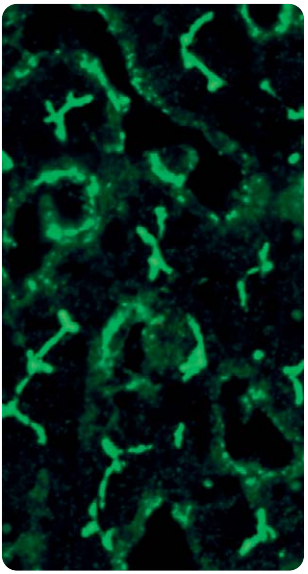
Autoantibodies against F-actin (AC-15)



VSM47



HEp-2 cells



Primate liver

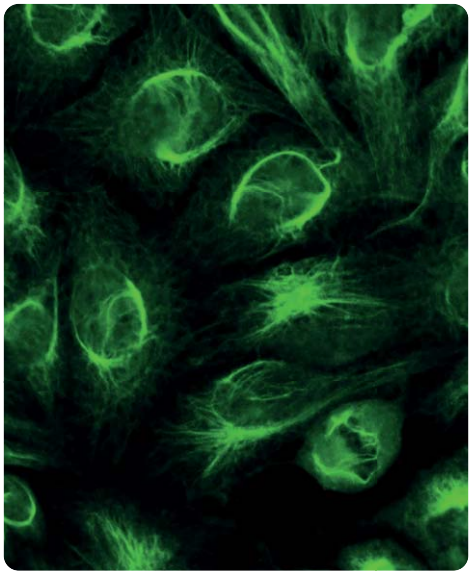
Autoantibodies against F-actin cause a microfilamentous fluorescence pattern using the **cell line VSM47** (vascular smooth muscle).

On **HEp-2 cells** individual or several bunched fibre structures fluoresce. Theyare located primarily in the cytoplasm, but can also stretch over the cell nuclei.

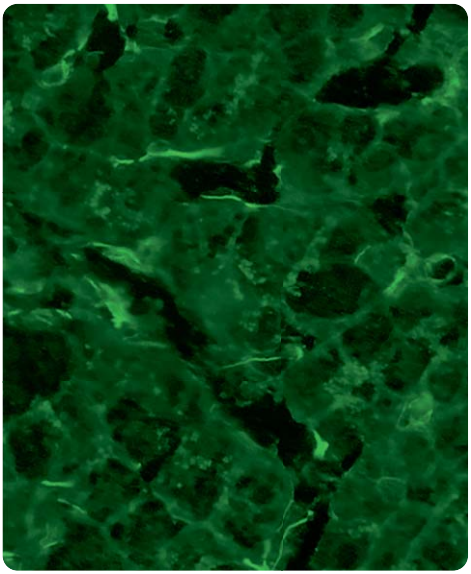
On tissue sections of **primate liver** there is a strong reaction of the bile canaliculi.

Clinical association: The determination of autoantibodies against F-actin is of particular significance for the diagnosis of AIH (prevalence around 40–90 %), the exclusion of a combined liver disease (overlap syndrome) and for delimitation of AIH against alcohol- or drug-induced cirrhosis and other forms of chronic liver inflammation, such as virus-induced hepatitis and primary sclerosing cholangitis (PBC).

Autoantibodies against tropomyosin (AC-16)



HEp-2 cells



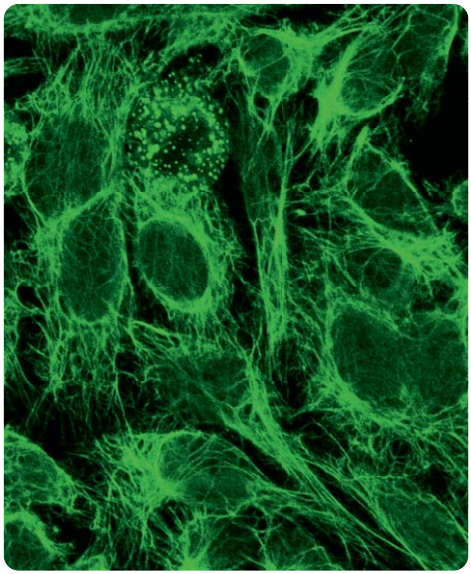
Primate liver

On **HEp-2 cells**, autoantibodies against tropomyosin cause a pattern of fibre slings.

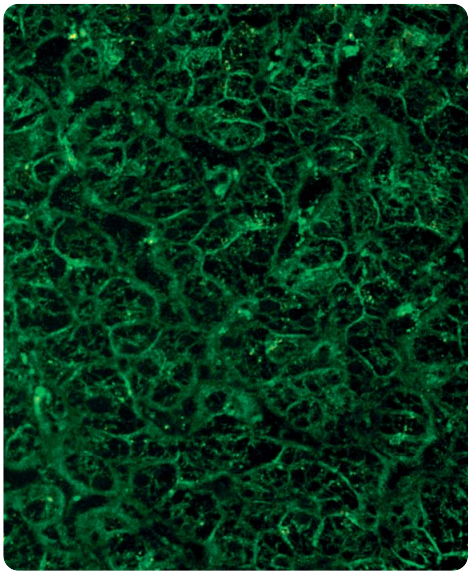
The **primate liver** shows a fibrillar pattern in the parenchyma.

Clinical association: About the diagnostc relevance of autoantibodies against tropomyosin is as little known as about that of the even more rare autoantibodies against cytokeratin, vimentin and others. They are considered to be associated with various inflammatory reactions and infections.

Autoantibodies against vimentin (AC-16)



HEp-2 cells



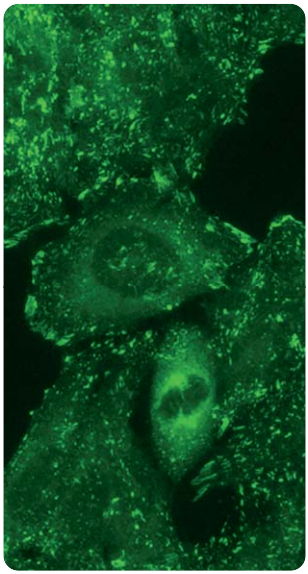
Primate liver

Antibodies against vimentin cause staining of a fine net of fibres in the cytoplasm of **HEp-2 cells**. The net is particularly dense near the cell nuclei. In mitotic cells numerous round fluorescing droplets can be seen outside the dark chromosomes. These are probably condensed vimentin.

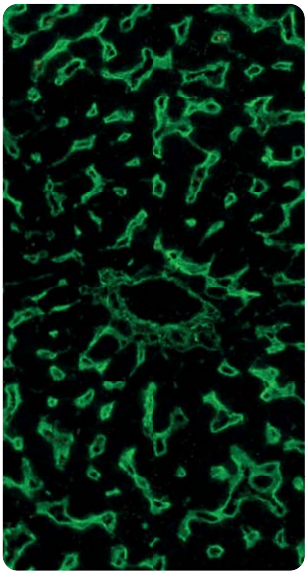
On tissue sections of **primate liver** there is an unspecific fluorescence.

Clinical association: The diagnostic relevance of autoantibodies against vimentin remains as unclear as that of the much rarer autoantibodies against cytokeratin, tropomyosin, etc. They are considered to be associated with different inflammatory reactions and infections.

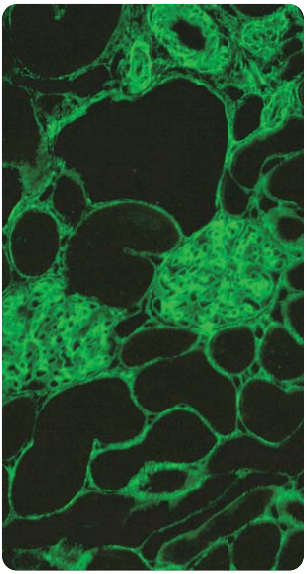
Autoantibodies against vinculin (AC-17)



HEp-2 cells



Primate liver



Rat kidney

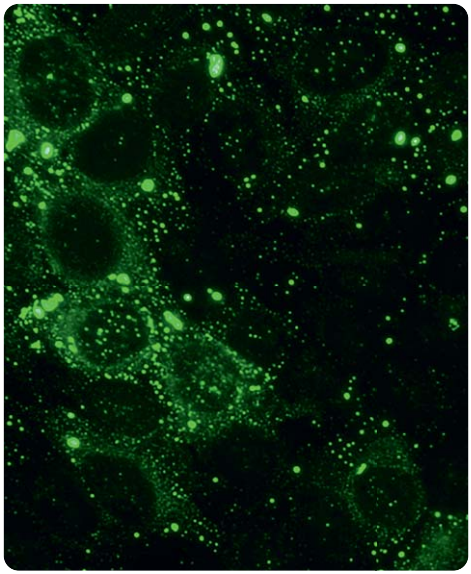
In the cytoplasm of **HEp-2 cells**, antibodies against vinculin lead to an increased staining of short sections regularly spread along the stress fibers of the cytoskeleton.

The **primate liver** shows a fluorescence of the basal boundary surface of endothelium and stroma in the sinusoids.

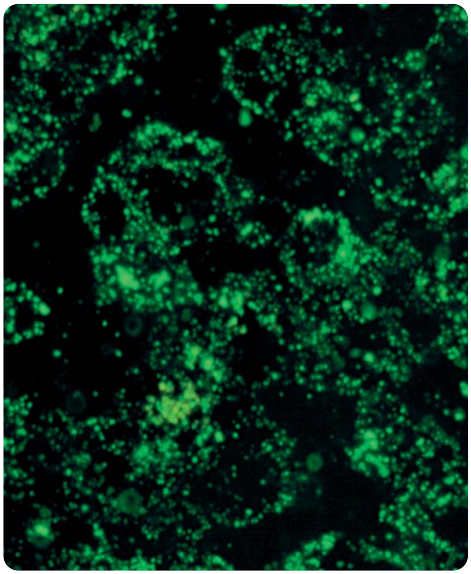
The glomeruli and tubuli of **rat kidney** show a filamentous fluorescence.

Clinical association: Autoantibodies agianst vinculin are very rare and are associated with Myasthenia gravis, ulcerative colitis and Crohn's disease.

Autoantibodies against lysosomes (AC-18)



HEp-2 cells



Primate liver

On **HEp-2 cells** antibodies against lysosomes show a fine to medium or coarse droplet-shaped fluorescence of the cytoplasm.

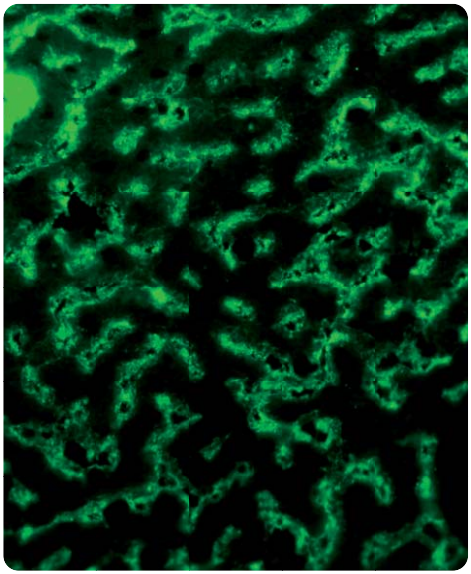
On frozen tissue sections of **primate liver** there is an unspecific fluorescence.

Known target antigens: GWB proteins (e.g. GW182, Su/Ago2).

Clinical association: PBC and neurological diseases. Autoantibodies against lysosomes are sometimes also detected in healthy persons.

Autoantibodies against PL-7 and PL-12 (AC-19)

HEp-2 cells



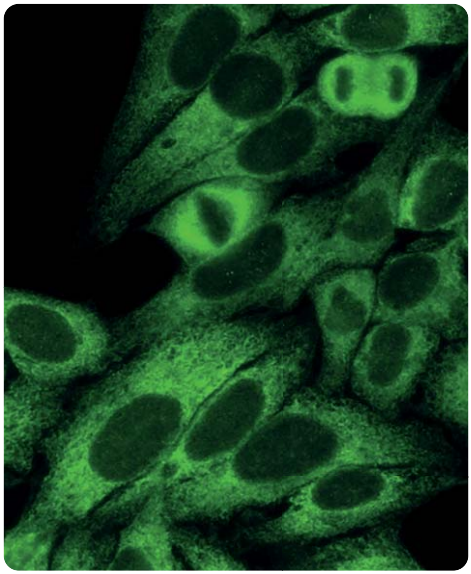
Primate liver

Autoantibodies against PL-7 and PL-12 show a fine speckled to homogenous cytoplasmic fluorescence with **HEp-2 cells**. The cell nuclei also show distinct clear dots in many cases. According to recent findings, the enzymes PL-7 and PL-12 are not solely localised in the cytoplasm, but are also found in the cell nucleus in some species.

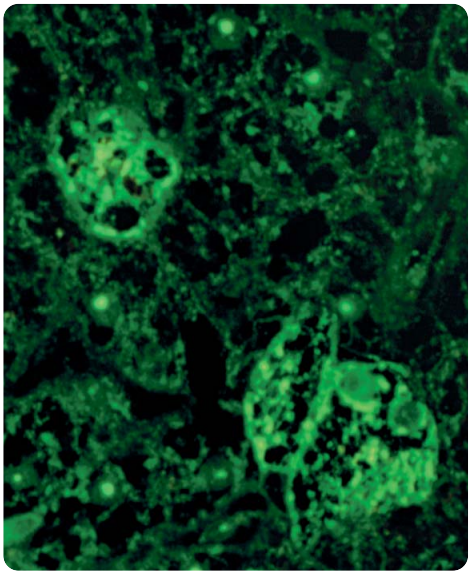
On frozen tissue sections of **primate liver** there is an unspecific fluorescence.

Clinical association: Antibodies against PL-7 and PL-12 occur in myositis with a prevalence of up to 4%.

Autoantibodies against ribosomal P proteins (AC-19)



HEp-2 cells



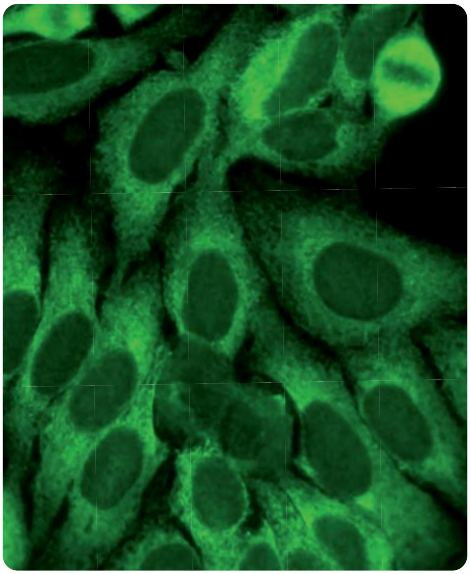
Primate liver

Autoantibodies against ribosomal P proteins cause a smooth to fine speckled staining of the cytoplasm when using **HEp-2 cells** as the substrate.

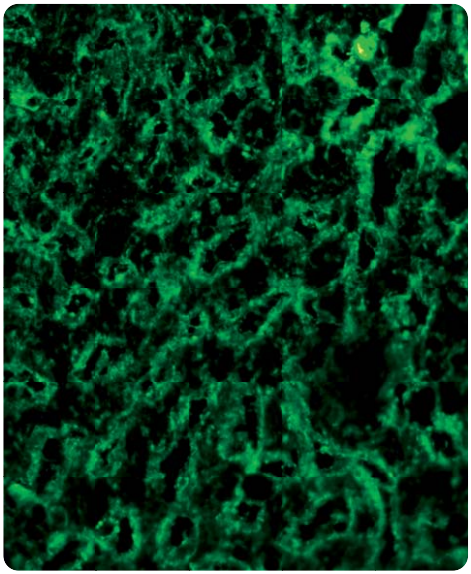
Hepatocytes of the **primate liver** show a cytoplasmic fluorescence of the entire surface with patchy accentuation. There is no reaction with low-titer samples.

Clinical association: Autoantibodies against ribosomal P proteins are a characteristic marker for SLE. The prevalence is around 10%.

Autoantibodies against SRP (AC-19)



HEp-2 cells



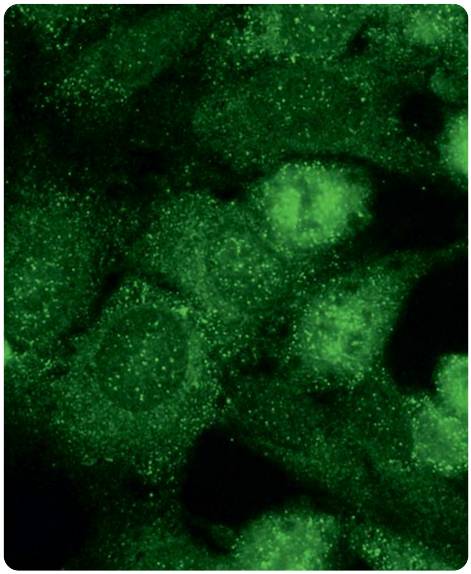
Primate liver

Autoantibodies against SRP produce a mainly cytoplasmic, smooth to fine speckled fluorescence on **HEp-2 cells**. In mitotic cells the fluorescence is perichromosomally intensified, the chromosomes are unaffected.

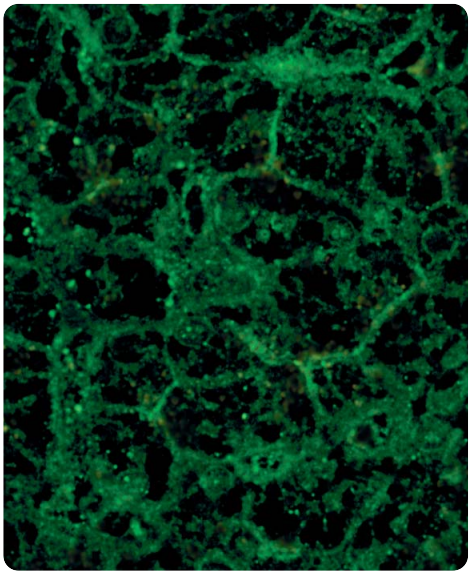
Hepatocytes of the **primate liver** generally show a fine speckled fluorescence distributed over the whole organ.

Clinical association: Antibodies against SRP can be found in polymyositis and dermatomyositis in approx. 5% of cases. They are also markers for necrotising myopathy, an autoimmune myopathy that differs from polymyositis, but can manifest with skin changes typical for dermatomyositis.

Autoantibodies against Jo-1 (AC-20)



HEp-2 cells



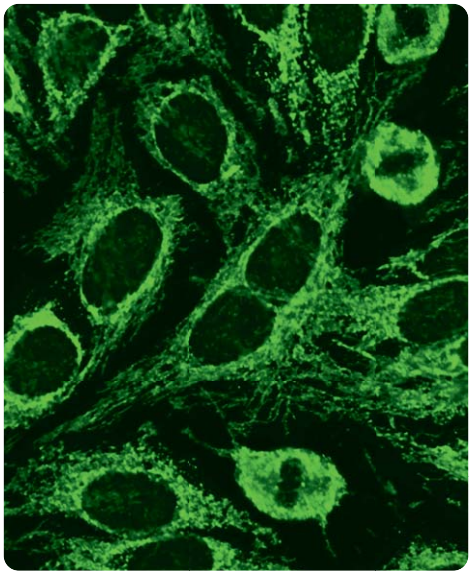
Primate liver

Antibodies against Jo-1 show a fine speckled to homogenous cytoplasmic fluorescence on **HEp-2 cells**. The cell nuclei also show distinct sharp dots in many cases. According to recent findings, Jo-1 target antigens are not solely localised in the cytoplasm, but are also found in the cell nucleus in some species.

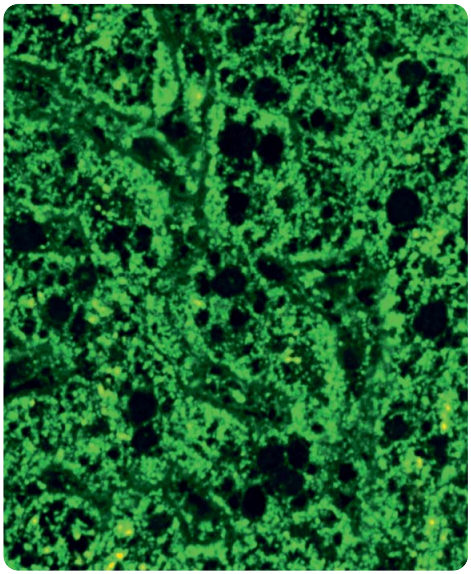
On frozen tissue sections of **primate liver** the cytoplasm is only slightly stained. The fluorescence cannot be used for diagnostics.

Clinical association: Antibodies against Jo-1 can be detected in polymyositis with a prevalence of 25–35%. They are often associated with other concurrent autoimmune diseases such as SLE, systemic sclerosis, interstitial lung fibrosis, Raynaud's syndrome and polysynovitis.

Autoantibodies against mitochondria (AC-21)



HEp-2 cells



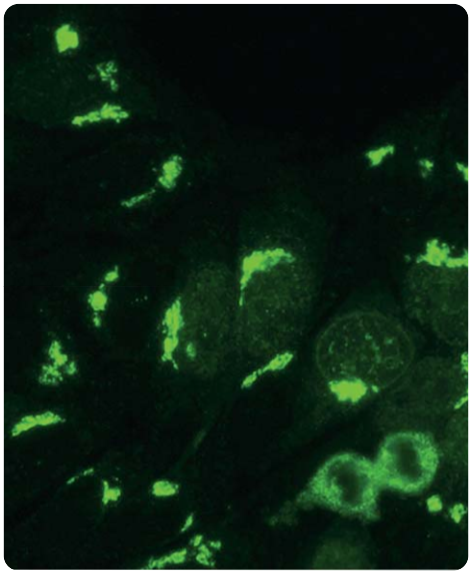
Primate liver

HEp-2-cells contain the antigens M2, M3, M5 and M9; here the antibodies produce a coarse speckled fluorescence of the cytoplasm which does not include the nucleus (previously, the likewise PBC-relevant nuclear dots also reacting were wrongly suspected of being stray mitochondria).

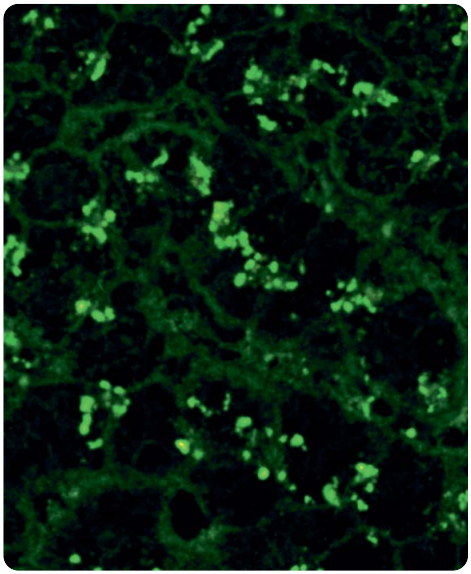
The **primate liver** shows a speckled fluorescence of the cytoplasm. The cell nuclei are dark. The reaction of the tissue is generally weaker than that of HEp-2 cells.

Clinical association: Autoantibodies against mitochondria can be detected in various diseases. They often occur together with other autoantibodies, e.g. with autoantibodies against cell nuclei. Antibodies to mitochondria are of particular significance for the diagnosis of primary biliary cholangitis (PBC). The prevalence is up to 95%.

Autoantibodies against Golgi apparatus (AC-22)



HEp-2 cells



Primate liver

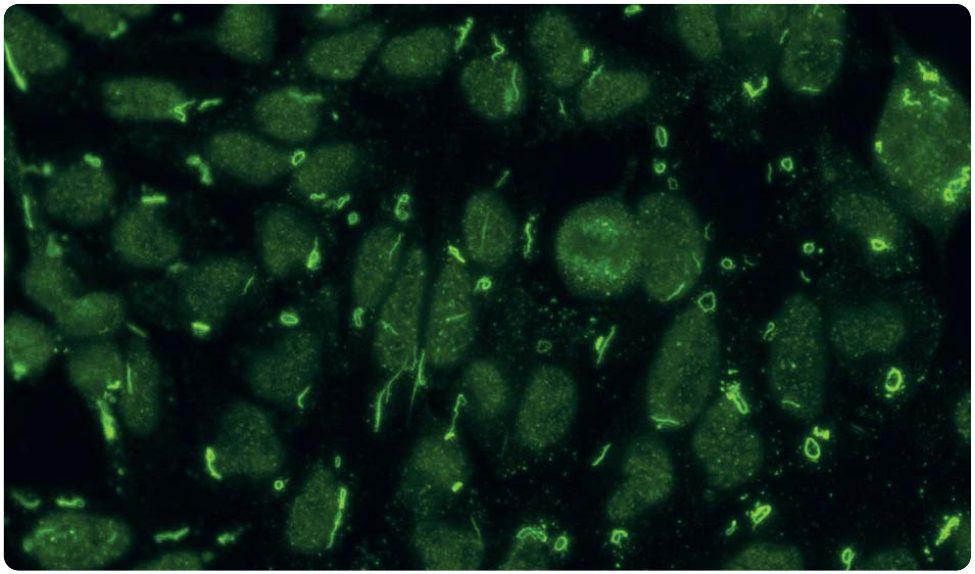
Autoantibodies against Golgi apparatus present in the indirect immunofluorescence on **HEp-2 cells** as reticular-granular structures which are in contact with the cell nucleus on one side. In cells which are in the mitosis, the Golgi apparatus is to a large extent dispersed. Here the antibodies show no reaction.

On **primate liver** the cytoplasm of hepatocytes is also stained.

Known target antigens: Giantin/macrogolgin, golgin-95/GM130, golgin-160, golgin-97 and golgin-245.

Clinical association: Autoantibodies against Golgi apparatus occur in different autoimmune diseases, particularly in SLE and Sjögren's syndrome and rheumatoid arthritis. Detection of these antibodies has little relevance due to their low disease specificity.

Cytoplasmic rods and rings (AC-23)



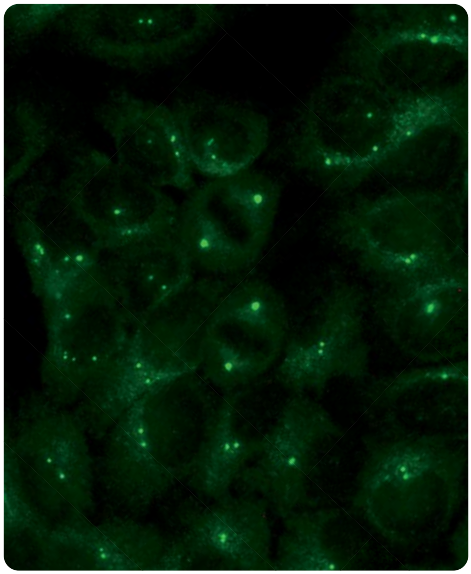
HEp-2 cells

Rods and rings are a cytoplasmic pattern on **HEp-2 cells** that has been described only recently. These filamentous structures, which are expressed in all stages of the cell cycle, present themselves as rings, rods or loops.

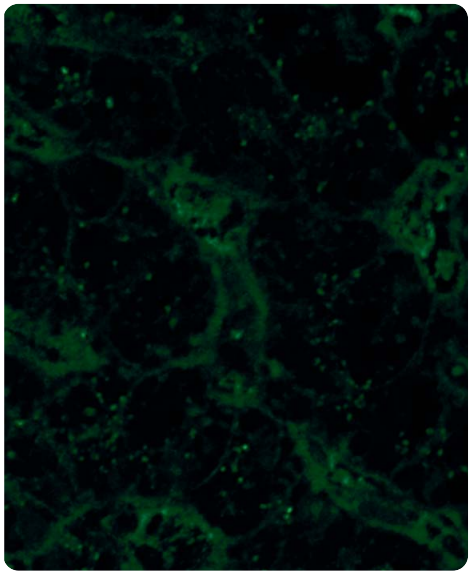
Known target antigens: It is assumed that the reaction is directed against the autoantigen inosine monophosphate dehydrogenase 2 (IMPDH2).

Clinical association: The depicted pattern was observed mainly in patients with hepatitis C infections, particularly after treatment with interferon-alpha or ribavirin (prevalence 35 %).

Autoantibodies against centrosomes (AC-24)



HEp-2 cells



Primate liver

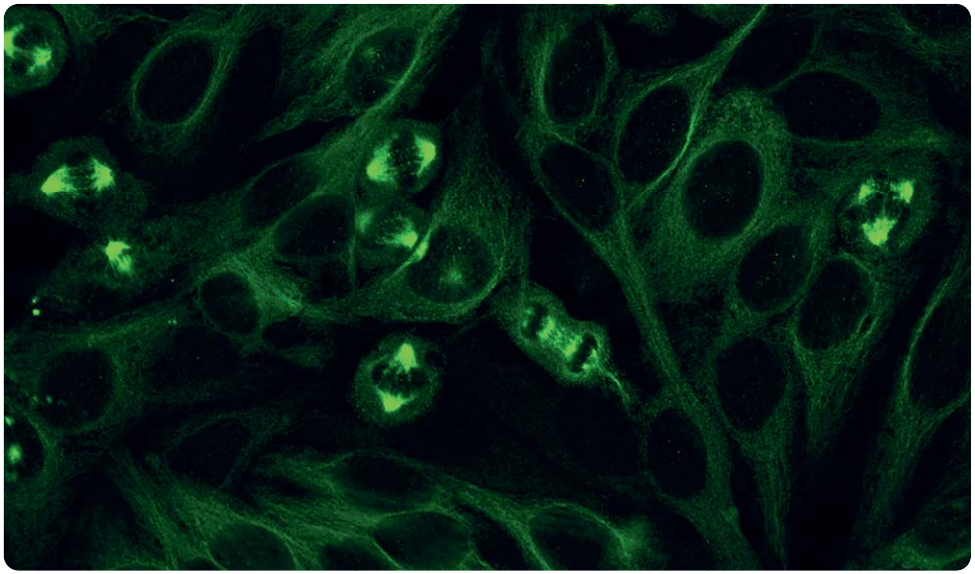
A typical positive result is characterised by fluorescing centrosomes in the cytoplasm of **HEp-2 cells**, namely one or two centrioles per cell. In mitotic cells the centrioles are located at two opposing poles.

On **primate liver**, high-titer samples produce small fluorescing dots in the cytoplasm of hepatocytes.

Known target antigens: Pericentrin, ninein, Cep250 and Cep110.

Clinical association: A high titer (>1:1,000) indicates progressive systemic sclerosis or Raynaud's syndrome, the prevalence however, only amounts to a few percent. The pattern was also observed in infections.

Autoantibodies against spindle fibres (AC-25)



HEp-2 cells

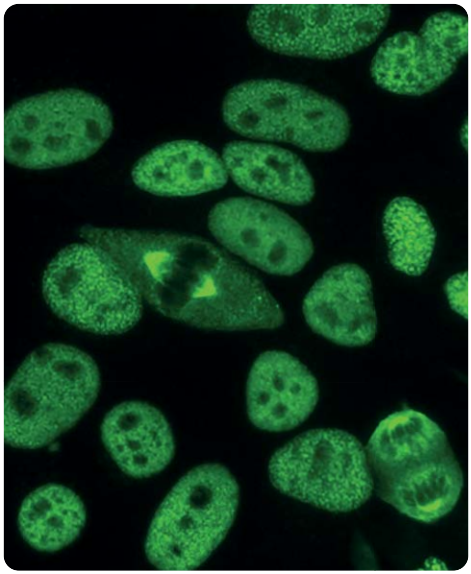
Using **HEp-2 cells**, antibodies against the spindle fibre antigen MSA-2 (HsEG5) can be detected. In the presence of these antibodies only the spindle fibres of the mitotic cells, but not the cell nuclei of the interphase cells are stained.

On tissue sections of **primate liver** a speckled fluorescence of the cell nuclei can be observed.

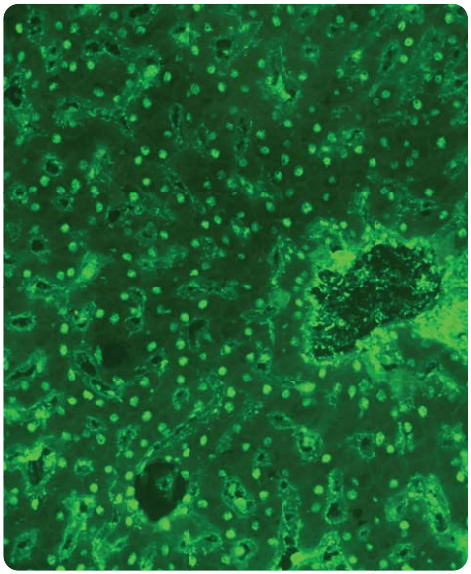
Known target antigen: HsEG5.

Clinical association: Autoantibodies against spindle fibres (MSA-2) occur rarely in Sjögren's syndrome, systemic lupus erythematosus (SLE) and other collagenoses.

Autoantibodies against NuMA (AC-26)



HEp-2 cells



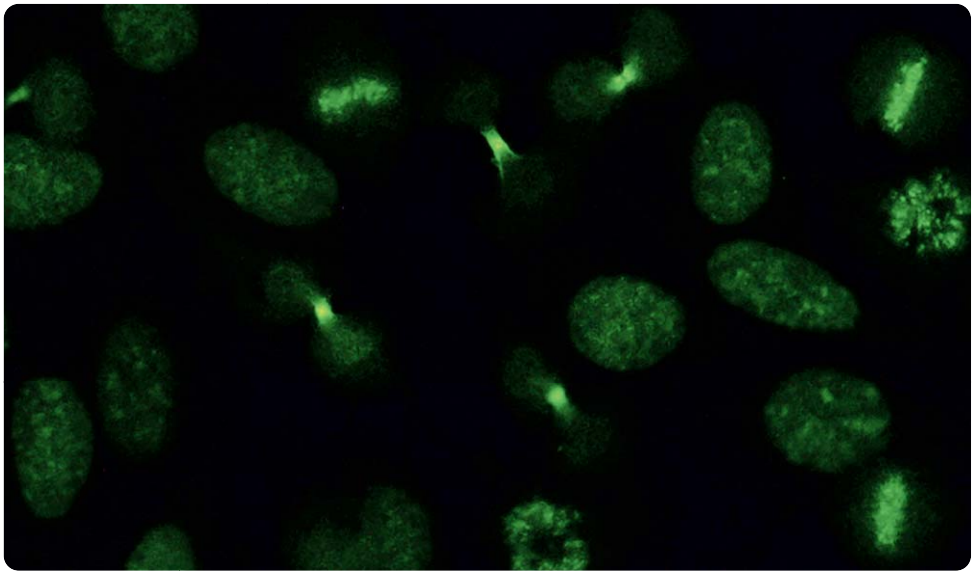
Primate liver

On **HEp-2 cells,** antibodies against NuMA (MSA-1) in the interphase show a fine speckled to reticular fluorescence of the nuclear matrix, with the exception of the nucleoli. In mitotic cells in the metaphase, the spindle fibres manifest as two opposing fans. The staining is most intense in the direction of the centrioles.

The **primate liver** shows a granular fluorescence.

Clinical association: Antibodies against NuMA (MSA-1) may occur, amongst other diseases, in Sjögren's syndrome and different forms of arthritis, sometimes also in anti-phospholipid syndrome (APS) and in systemic lupus erythematosus (SLE).

Autoantibodies against midbody (AC-27)

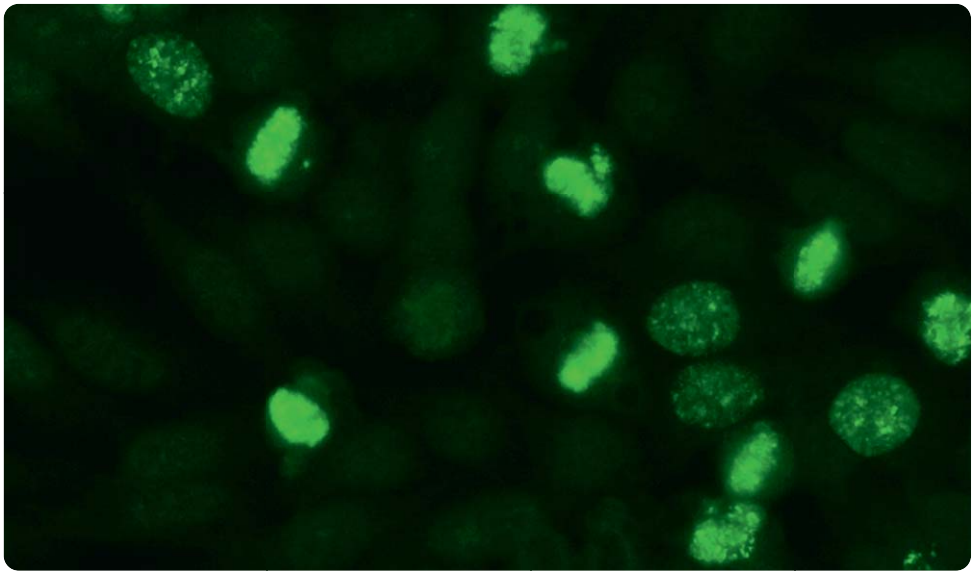


HEp-2 cells

In the indirect immunofluorescence test, **HEp-2 cells** in the metaphase of mitosis show a fine speckled fluorescence of the equatorial plane in the presence of midbody antibodies. In contrast to the pattern found with antibodies against centromeres, this fluorescing line remains in the middle until the end of mitosis. Their length corresponds to the whole cell width in the separation zone, and the line increasingly shortens until only a fluorescing dot is seen in the telophase, binding the daughter cells together ("goodbye kiss").

Clinical association: Raynaud syndrome, malignoma and progressive systemic sclerosis.

Autoantibodies against MCA (AC-28)



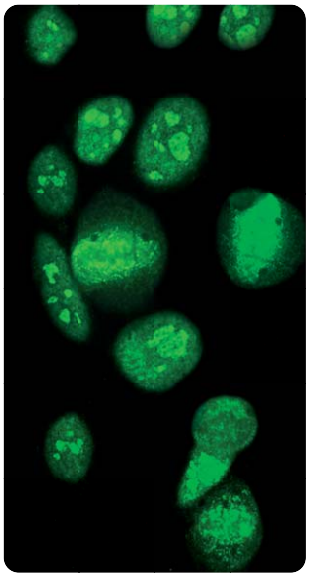
HEp-2 cells

On **Hep-2 cells**, the chromosomes in the pro- and methaphase show a dotted fluorescence. Interphase nuclei do not present any staining.

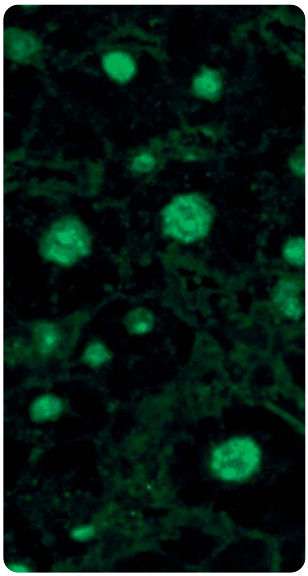
Known target antigens: Modified histon H3, MCA-1.

Clinical association: Polymyalgia rheumatica, discoid lupus erythematosus, Sjögren's syndrome and chronic lymphatic leukaemia.

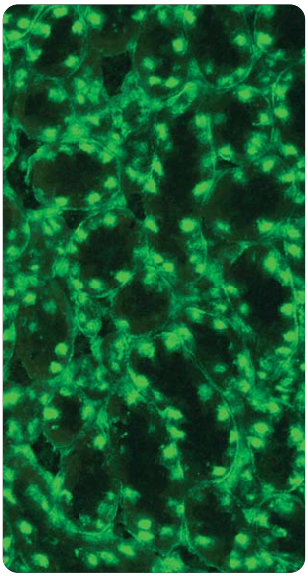
Autoantibodies against Topoisomerase I (Scl-70) (AC-29)



HEp-2 cells



Primate liver



Rat kidney

HEp-2 cells show a fine granular nuclear fluorescence in interphase cells. The nucleoli are positive, showing a homogeneous fluorescence. The cytoplasm is dark. In mitotic cells the border area of condensed chromosomes fluoresces, sometimes the entire chromosomal region is positive.

The **liver** exhibits a predominantly homogeneous fluorescence of the cell nuclei.

On **rat kidney** tissue sections, the cell nuclei of the tubular epithelium fluoresce.

Clinical association: Topoisomerase I antibodies are detected in 25–75% of patients with progessive systemic sclerosis (diffuse form), depending on the analysis method and the activity of the disease.

Dilution scheme for immunofluorescence

For all its immunofluorescence test systems EUROMMUN recommends choosing a **titration of serum samples** that provides the best basis for evaluation.

Previously, the accuracy was excessive due to use of quadratic dilution steps. On the other hand, titrating by a factor of 4 results in too rough a framework. Therefore, we recommend using a dilution scheme based on the square root of 10 to yield dilution steps of 1 : 10, 1 : 32, 1 : 100, 1 : 320, 1 : 1.000 and so on.

For every test parameter there is a **suitable starting dilution**. In order to simplify the test procedure and evaluation of results, **two antibody categories** are differentiated at EUROIMMUN: Antibodies of group I are already diagnostically relevant at at titer of 1:10, while those of group II first at 1:100.

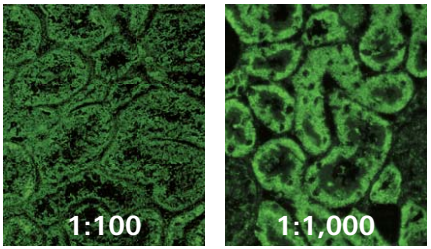
A **symbol from + to ++++** is attributed to a dilution step with a specific fluorescence. The differing clinical significance of antibody titers for the two groups is already incorporated into this scheme.

Serum dilution	1:10	1:32	1:100	1:320	1:1,000	1:3,200	1:10,000
Evaluation, group I	+	++	++	+++	+++	++++	++++
Evaluation, group II			+	++	+++	++++	++++

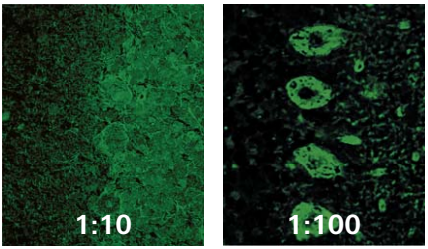
= suitable starting dilutions
+ = weak positive, ++ = positive, +++ = strong positive, ++++ = very strong positive
Group I: most organ-specific autoantibodies, ANCA, autoantibodies against dsDNA
Group II: ANA, AMA, ASMA, autoantibodies against skeletal muscle

In order to make optimal use of the great potential of indirect immunofluorescence, experts in this area **always use two parallel dilutions to test for most autoantibodies**, for the following reasons:

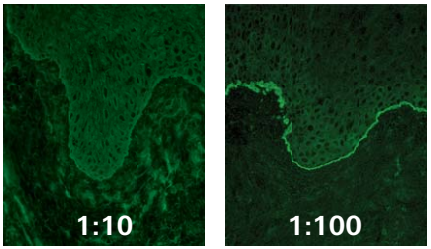
Blocking effect: In two out of every 100 high-titer sera an untypical result is seen with the starting dilution. Some strongly positive sera even react as false negative if they are not sufficiently diluted.



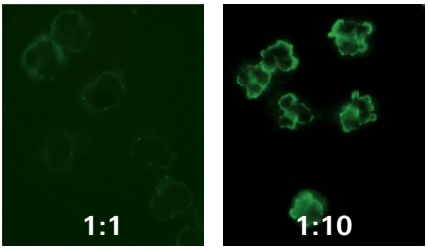
AMA (kidney)



Anti-Yo (cerebellum)

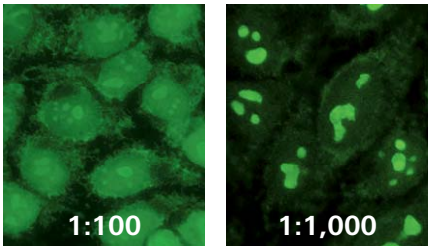


Anti-epid. basal membrane (tongue)

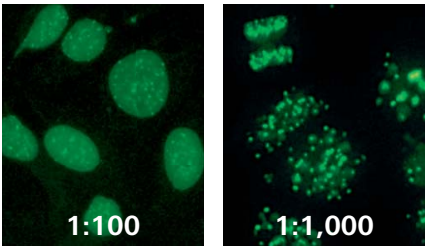


pANCA (granulocytes)

Autoantibody masking: If unspecific antibodies or additional, visually dominant autoantibodies are present in too high a concentration, they can mask a relevant antibody.



Anti-ribosomes and anti-nucleoli

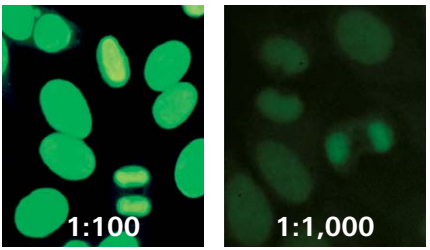


ANA, homog. and anti-centromeres

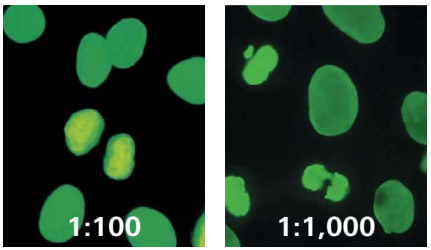
Titer estimation: When two dilutions made at an interval of factor 10 are used, a titer can be determined for most positive results without further incubations (see table). Results are obtained one day earlier than with stepwise titrations.

Fluorescence at 1:100	1:1,000	AAb titer
weak	negative	1:100
strong	negative	1:320
strong	weak	1:1,000
strong	moderate	1:3,200
strong	strong	1:10,000

In contrast, it is not possible to quantify a positive result from a single dilution: AAb show very different abating behaviour depending on the avidity. This is detected by the parallel analysis. Photometric systems based on cytochemical ELISA or fluorescence are hence obsolete.



ANA, homogeneous (titer 1:1,000)



ANA, homogeneous (titer 1:10,000)

EUROPattern: Automated evaluation of IIFT

EUROPattern Microscope

In order to provide diagnostic laboratories with maximum support in ANA diagnostics, EUROIMMUN has developed EUROPattern, a system for automated recording of immunofluorescence images and computer-aided evaluation with a continuously increasing range of substrates.

The EUROPattern Microscope can automatically process up to 500 incubation fields in less than 2 hours. For this, the mechanical stage moves into the magazine and picks up one carrier plate with slides, which are securely identified by means of a data matrix code. All fields on the slides are brought into view one by one in a precise manner. The substrates are focussed without causing fading of the fluorescence and high-quality fluorescence images are taken. Besides cell substrates, EUROIMMUN also provides tissues and purified antigens (EUROPLUS) for automated image recording. The fluorescence images are automatically archived and are available for interpretation of the recent and any subsequent analysis.

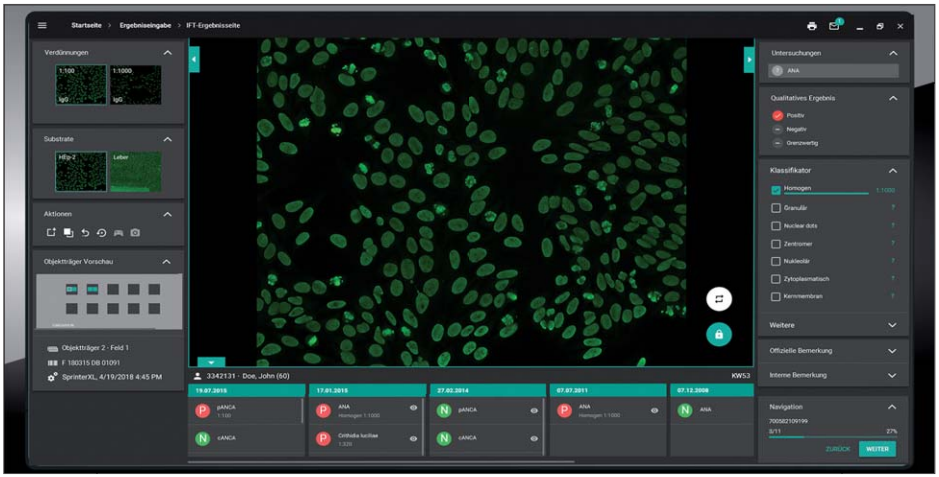
Based on the recorded immunofluorescence images, EUROPattern fully automatically generates a diagnosis suggestion with all kinds of substrates. At present, this includes HEp-2/HEp-20-10 cells, granulocytes (various fixing methods),

Crithidia luciliae, EUROPLUS and recombinant cells (e.g. aquaporin-4, PLA2R, DPPX). EUROPattern classifies the fluorescence images into positive, negative or borderline using modern mathematical procedures and identifies the patterns.

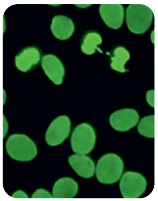
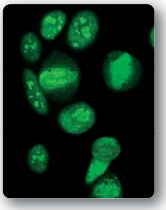
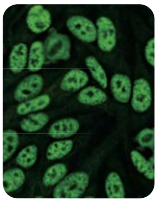
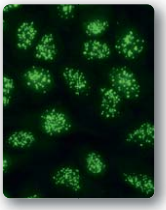
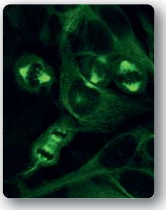
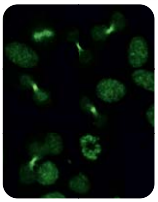
For each pattern a titer is automatically calculated from the fluorescence intensities of the incubated dilutions, which ensures reproducible quantification.

The automatically generated diagnosis suggestion for each patient, including titers and confidence value, is displayed on the screen together with the fluorescence images. The diagnostician can verify the final result with one mouse click, taking into account the detailed patient history. Furthermore, batch processing of negative samples is supported. Thus, EUROPattern ensures a quick and secure processing of IIFT in laboratory diagnostics.

EUROPattern is an extension module for the laboratory management software EUROLabOffice. It can be easily integrated into existing work processes and automation solutions.



Presentation of results in EUROPattern

SYSTEMIC AUTOANTIBODIES AND ASSOCIATED AUTOIMMUNE DISEASES

Domains of immunofluorescence

Diagnostically
■ essential
□ relevant

Dated July 2017

Autoantibodies against	Anti-phospholipid syndrome	Habitual abortions	Drug-induced lupus erythematosus	Neonatal lupus erythematosus	Polymyositis/Dermatomyositis	Inclusion body myosits	Progressive systemic sclerosis	Rheumatoid arthritis	Sharp syndrome (MCTD)	Sjögren's syndrome	Systemic lupus erythematosus	Autoimmune hepatitis	Primary biliary cirrhosis	Paraneoplastic autoimmunity
Cell nuclei (ANA)	□	■	■	■	■		■	□	■	■	■	■	■	□
CENP-F (cyclin II – mitosin)														□
dsDNA											■			
Nucleosomes											■			
Sm											■			
U1-RNP					□				■		□			
SS-A (Ro, 60 kDa)				■						■	■		■	
SS-B (La)				□						■	□			
Histones			■								□			
PCNA (cyclin I)											■			
RNA helicase A											■			
MSA-1 (NuMA)										□	■			
MSA-2 (HsEg5)					□			□		□	□			
Topoisomerase I (Scl-70)							■							
Fibrillarin (U3-RNP)					□		■							
RNA polymerases I, II, III							■							
NOR-90, PDGF-R							■							
Centromeres (CENP-A, CENP-B)							■						■	
PM-Scl (1, 75, 100)					□		■							
Ku					■									
Mi-2					■									
SRP					■									
cN-1A (Mup44)						■								
Jo-1					■									
TIF1-gamma					■									
MDA5, NXP2, SAE1					■									
PL-7, PL-12, OJ, EJ, SC, KS					■									
Nuclear dots													■	
Sp100, Sp140, SUMO, PML													■	
Nuclear membrane, lamin B recept.													■	
GP210, NUP62													■	
Cardiolipin	■	■									□			
Beta-2 glycoprotein	■	■									□			
Phosphatidylserine	□	□												
Coagulation factor (lupus anticoag.)	■	■									□			
Prothrombin, annexin A5	□	□												
C1q											□			
Mitochondria (AMA)												□	■	
Mitochondria (AMA M2-3E/BPO)													■	
Ribosomal P proteins											■			
ASMA												■		
F-actin												■		
IgG (rheumatoid factors)								■						
Citrullinated peptides (CCP)								■						
Sa								□						
Filaggrin, RA keratin								□						
Citrullinated enolase peptide (CEP-1)								□