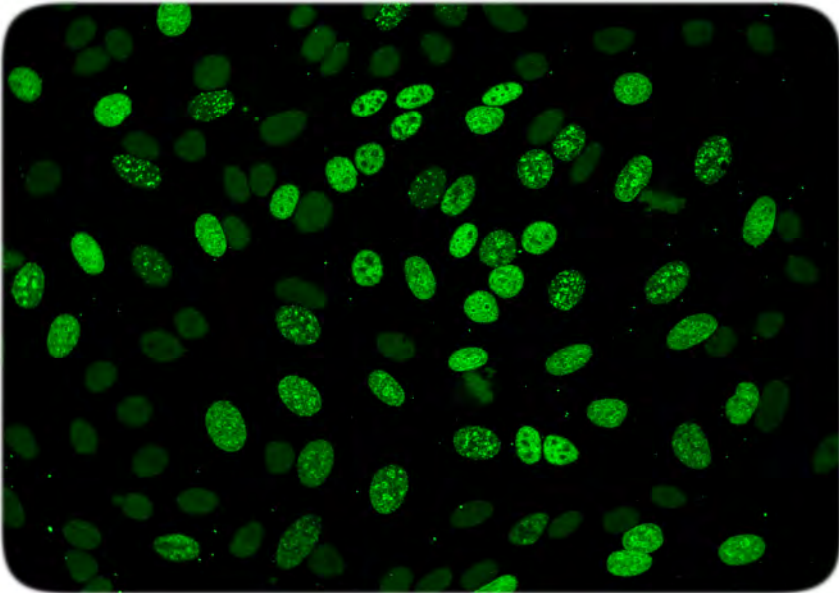


BARBARA FABIAN

ImmunoFluorescence Guide

FOR THE MODERN AUTOIMMUNITY LABORATORY



This guide clearly conveys the immense potential of fluorescence-coupled detection systems for the clarification of complex processes. 2nd edition



ImmunoFluorescence Guide

FOR THE MODERN AUTOIMMUNITY LABORATORY

PREFACE

Autoimmune findings are essential components of immunological diagnostics. Together with other serological and clinical data, determination of autoantibodies contributes significantly to the diagnosis of autoimmune diseases.

For many years, screening has been carried out by the detection of autoantibodies with indirect immunofluorescence tests on human epithelioma cells (HEp-2 cells). The advantages of this method are high sensitivity and a broad spectrum of detectable antibodies. In addition to antinuclear antibodies (ANA), staining of mitotic stages (metaphase) and cytoplasmic antibodies are also analysed.

Diagnostic tests on tissue sections (kidney, stomach and liver from rat or mouse) are a reasonable supplement to the analyses on HEp2 cells.

Indirect immunofluorescence assays allow for cost-effective and high-quality diagnostics. Subsequent application of other immunological tests such as ELISA or immunoblots in a stepwise diagnostic process further specifies positive findings.

In the modern autoimmune laboratory, experience and expertise of the personnel evaluating the test results remain the major criteria for high quality autoimmune diagnostics.

Barbara Fabian, September 2013

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LIST OF ABBREVIATIONS

ACR	American College of Rheumatology
AIH	autoimmune hepatitis
AB	antibody
CAVB	complete atrio-ventricular block
CLIFT	<i>Crithidia luciliae</i> immunofluorescence test
DM	dermatomyositis
dsDNA	double-stranded DNA
ELISA	enzyme-linked immunosorbent assay
GPA	gastric parietal cell antigen
HEp-2	human epithelioma cell line
hnRNP	heterogeneous nuclear ribonucleoprotein
IF	intrinsic factor
IIF	indirect immunofluorescence
KSL	kidney/stomach/liver tissues
LE	lupus erythematosus
LKM	liver/kidney microsomes
M2	inner mitochondrial membrane M2 protein
MCTD	mixed connective tissue disease
NOR	nucleolus organiser regions
NuMA	nuclear mitotic apparatus
PBC	primary biliary cirrhosis
PCNA	proliferating cell nuclear antigen
PM	polymyositis
prim. SS	primary Sjögren's syndrome
RA	rheumatoid arthritis
RNP	ribonucleoprotein
SLE	systemic lupus erythematosus
Sm AB	Smith antibody
SS	Sjögren's syndrome
SS-A	Sjögren's syndrome protein A
SS-B	Sjögren's syndrome protein B

HEp-2 CELLS – NUCLEAR ANTIBODIES

ANTIBODIES WITH HOMOGENEOUS PATTERN

- **dsDNA antibodies**
- **Histone antibodies**
- **Nucleosome antibodies**
- **Nuclear membrane antibodies – lamins**
- **Nuclear membrane antibodies – nuclear pores (gp210)**

DOUBLE-STRANDED DNA ANTIBODIES (dsDNA ANTIBODIES)

Autoantigen

Native double-stranded DNA (dsDNA)

Indirect immunofluorescence

HEp-2 → interphase nuclei: homogeneous
→ mitotic cells: positive

KSL → nuclei: homogeneous

Confirmation test

ELISA → ANA Detect, Anti-dsDNA

Immunoblot → Nucleo-9-Line

IIF → *Crithidia luciliae* IIF test (CLIFT):
fluorescence of the kinetoplasts

Clinical relevance

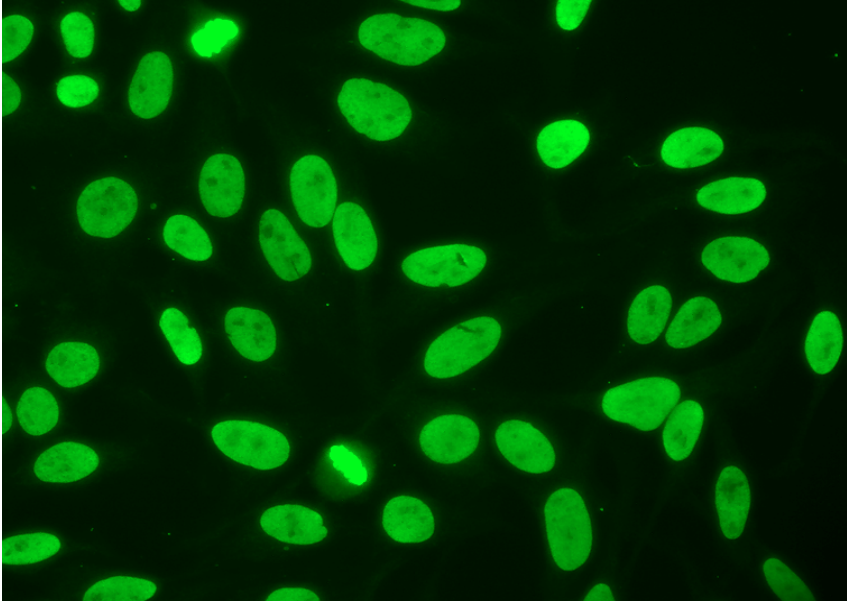
Diagnostic marker for SLE (ACR criteria!)

Prognostic marker

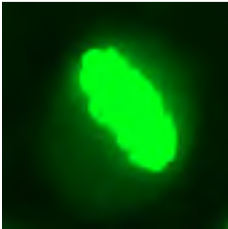
Frequency

SLE → 60-70% ⁽¹⁾

PBC → 8% ⁽¹⁰⁾



Hep-2 → interphase nuclei: homogeneous



Hep-2 → mitotic cells: positive

HISTONE ANTIBODIES

Autoantigen

Basic proteins H1, H2A, H2B, H3, H4

Indirect immunofluorescence

HEp-2 → interphase nuclei: homogeneous
→ mitotic cells: positive

Confirmation tests

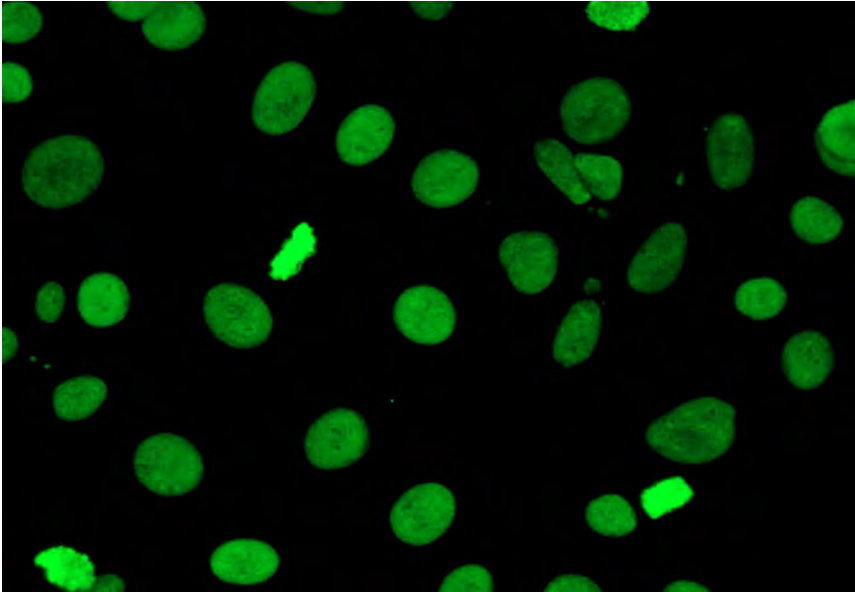
ELISA → ANA Detect, Anti-Histone

Clinical relevance

Indication of drug-induced lupus

Frequency

Drug-induced lupus → 90% ⁽⁴⁾
SLE → 50-70% ⁽²⁾



Hep-2 → interphase nuclei: homogeneous



Hep-2 → mitotic cells: positive

NUCLEOSOME ANTIBODIES

Autoantigen

Parts of the nucleosomes, structural components of chromatin

Indirect immunofluorescence

HEp-2 → interphase nuclei: homogeneous
→ mitotic cells: positive

Confirmation tests

ELISA → ANA Detect, Anti-Nucleosome

Immunoblot → Nucleo-9-Line

Clinical relevance

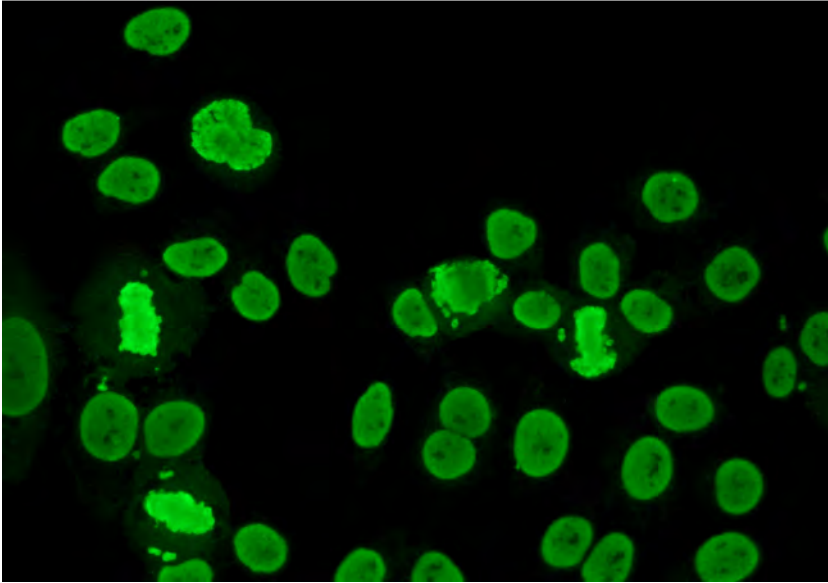
Diagnostic marker for SLE

even in anti-dsDNA negative SLE patients

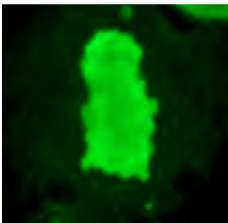
Early diagnostic marker for SLE

Frequency

SLE → 71%
Active SLE → 100%⁽⁵⁾
Systemic sclerosis → 46%⁽⁶⁾
MCTD → 45%⁽⁶⁾
SS → 4%



HEp-2 → interphase nuclei: homogeneous



HEp-2 → mitotic cells: positive

NUCLEAR MEMBRANE ANTIBODIES – LAMIN –

Autoantigen

Type V intermediate filaments in the nuclear membrane

Indirect immunofluorescence

HEp-2

- interphase nuclei: homogeneous ,
like “tissue paper”, with accentuated
continuous nuclear rim
- mitotic cells: negative

KSL (rat)

- nuclei: linear rim

Confirmation tests

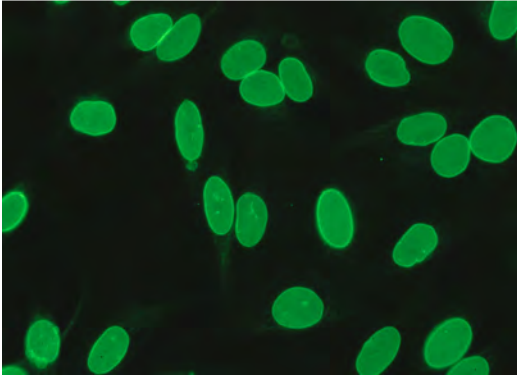
Currently not available in the routine laboratory

Clinical relevance

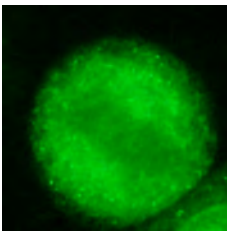
Indication of autoimmune liver diseases

Frequency

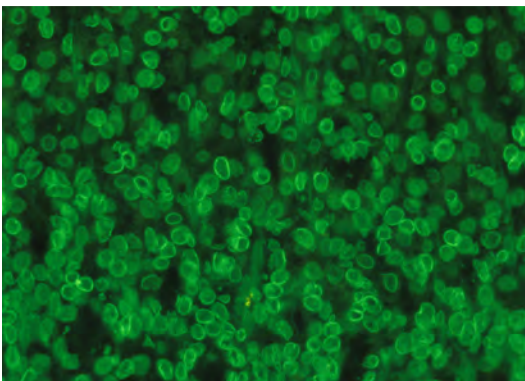
Rare in PBC, AIH, SLE



HEp-2 → interphase nuclei: homogenous, like “tissue paper”
with accentuated continuous nuclear rim



HEp-2 → mitotic cells: negative



KSL → stomach (rat): nuclei with linear rim

NUCLEAR MEMBRANE ANTIBODIES – NUCLEAR PORES (gp210) –

Autoantigen

Nuclear pore protein of 210 kDa

Indirect immunofluorescence

HEp-2 → interphase nuclei: pale with accentuated, punctate nuclear rim
→ mitotic cells: negative

Confirmation tests

ELISA → Anti-gp210

Immunoblot → Liver-9-Line

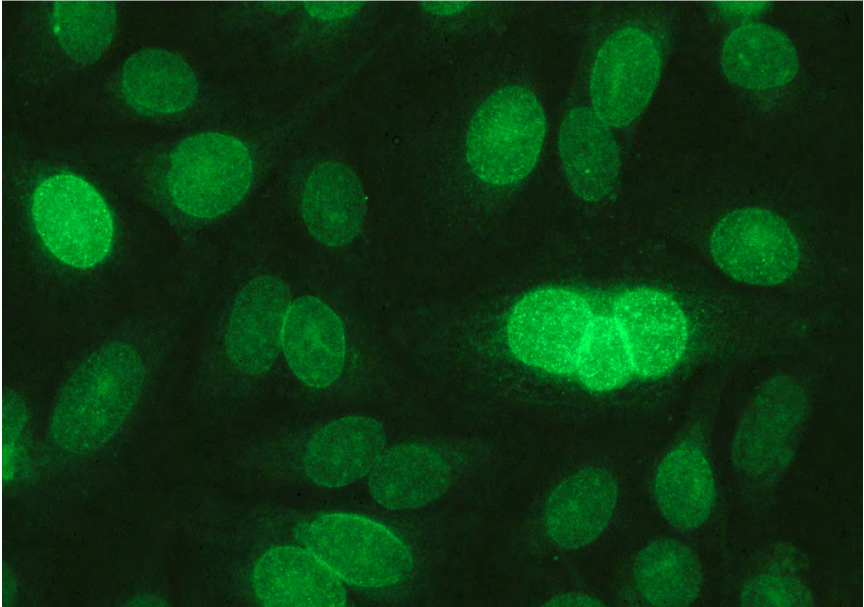
Clinical relevance

Prognostic marker for autoimmune liver diseases

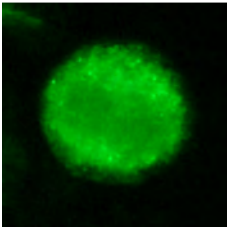
Frequency

PBC → 21%⁽¹⁰⁾

AIH → rare



HEp-2 → interphase nuclei: punctate nuclear rim



HEp-2 → mitotic cells: negative

HEp-2 CELLS – NUCLEAR ANTIBODIES

ANTIBODIES WITH GRANULAR PATTERN

- **SS-A/Ro antibodies**
- **SS-B/La antibodies**
- **NuMA antibodies**
- **U1-RNP antibodies**
- **Nuclear matrix protein antibodies**
- **Sm antibodies**
- **PCNA antibodies**

SS-A/R_o ANTIBODIES

Autoantigen

Two proteins of 52 kDa and 60 kDa from the ribonucleoprotein complex located in the cytoplasm and in the nucleus

Indirect immunofluorescence

HEp-2 → interphase nuclei: fine granular
→ mitotic cells: negative

Confirmation tests

ELISA → ANAcombi, ANA Detect, ANAScreen, Anti-SS-A, Anti-SS-A 52, Anti-SS-A 60, ENA-4-Profile, ENA-6-Profile, ENAcombi, ENAScreen

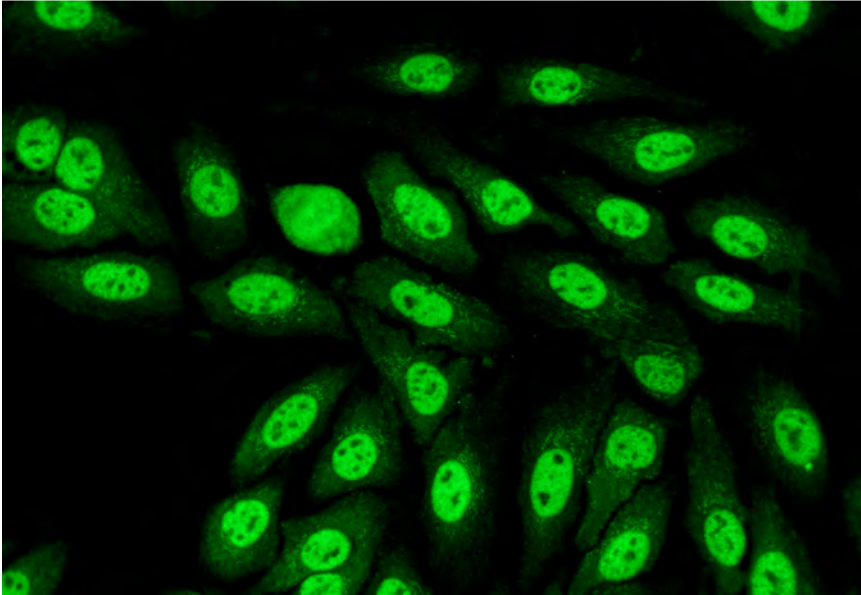
Immunoblot → ANA-9-Line, Nucleo-9-Line

Clinical relevance

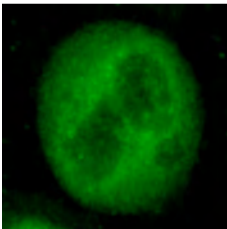
Diagnostic marker for Sjögren's syndrome (classification criteria!)
Early diagnostic marker for Sjögren's syndrome (even when patients are asymptomatic!)

Frequency

Prim. SS → 62% ⁽³⁾
SLE → 30-50% ⁽²⁾
CAVB in children of mothers with SS-A AB → 1-5% ⁽¹⁵⁾
PBC → 28% ⁽¹⁰⁾



Hep-2 → interphase nuclei: fine granular



Hep-2 → mitotic cells: negative

SS-B/La ANTIBODIES

Autoantigen

48 kDa protein of the ribonuclear protein complex located in the cytoplasm and in the nucleus

Indirect immunofluorescence

HEp-2 → interphase nuclei: fine granular
→ mitotic cells: negative

Confirmation tests

ELISA → ANA Detect, ANAcombi, ANAscreen,
Anti-SS-B, ENA-6-Profile, ENAcombi,
ENAscreen

Immunoblot → ANA-9-Line, Nucleo-9-Line

Clinical relevance

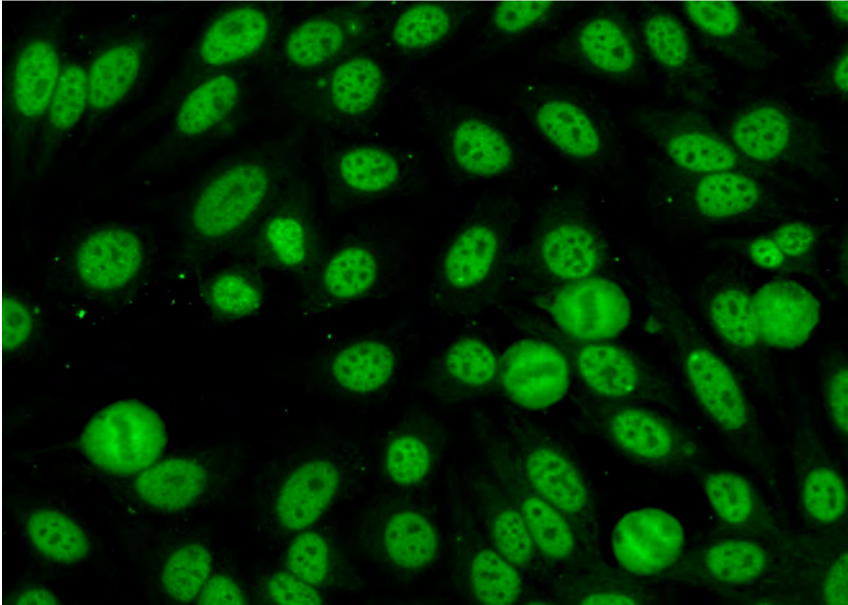
Early diagnostic marker for Sjögren's syndrome

Higher diagnostic specificity when anti-SS-A and anti-SS-B are positive

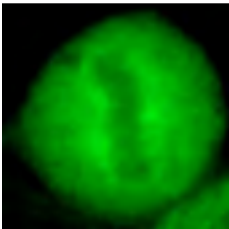
Frequency

Prim. SS → 43% ⁽³⁾

SLE → 10-15% ⁽²⁾



HEp-2 → interphase nuclei: fine granular



HEp-2 → mitotic cells: negative

NuMA ANTIBODIES (CENTROPHILIN ANTIBODIES)

Autoantigen

Nuclear mitotic spindle apparatus proteins

Indirect immunofluorescence

HEp-2 → interphase nuclei: fine granular
→ mitotic cells: positive at the spindle poles

Confirmation tests

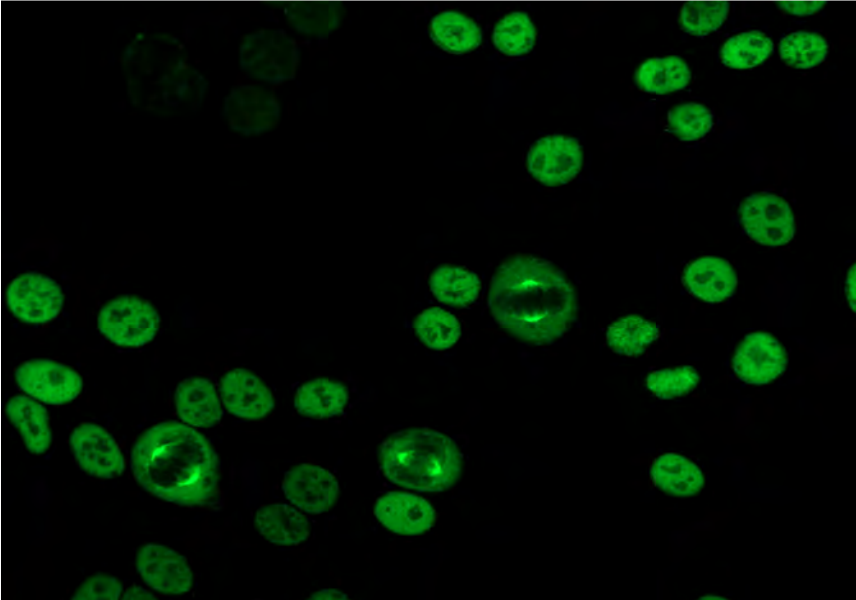
Currently not available in the routine laboratory

Clinical relevance

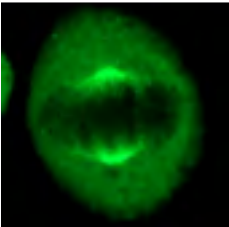
General indicator of autoimmune disease

Frequency

Rare for Sjögren's syndrome, SLE, systemic sclerosis, Sharp syndrome (MCTD), primary biliary cirrhosis



Hep-2 → interphase nuclei: fine granular



Hep-2 → mitotic cells: spindle poles positive

U1-RNP ANTIBODIES

Autoantigen

Ribonucleoproteins U1-70 kDa, U1-A, U1-C

Indirect immunofluorescence

HEp-2 → interphase nuclei: coarse granular ,
pattern with spared nucleoli
→ mitotic cells: negative

Confirmation tests

ELISA → ANAcombi, ANA Detect, ANAscreen,
Anti-RNP-70, Anti-RNP/Sm, ENA-4-Profile,
ENA-6-Profile, ENAcombi, ENAscreen

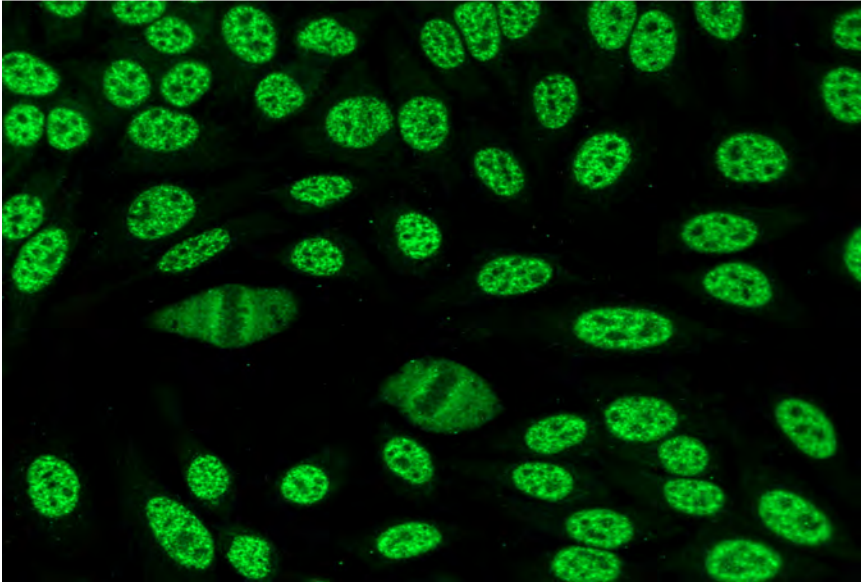
Immunoblot → ANA-9-Line, Nucleo-9-Line

Clinical relevance

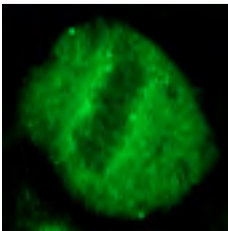
Diagnostic marker for Sharp syndrome (MCTD)

Frequency

MCTD → 100%
SLE → 30-40% ⁽²⁾
System. sclerosis → 2-5% ⁽²⁾



Hep-2 → interphase nuclei: coarse granular; nucleoli: negative



Hep-2 → mitotic cells: negative

NUCLEAR MATRIX PROTEIN ANTIBODIES (hnRNP ANTIBODIES)

Autoantigen

heterogenous nuclear ribonucleoprotein

Indirect immunofluorescence

HEp-2 → interphase nuclei: dots and lines arranged around negative nucleoli
→ mitotic cells: negative

Confirmation tests

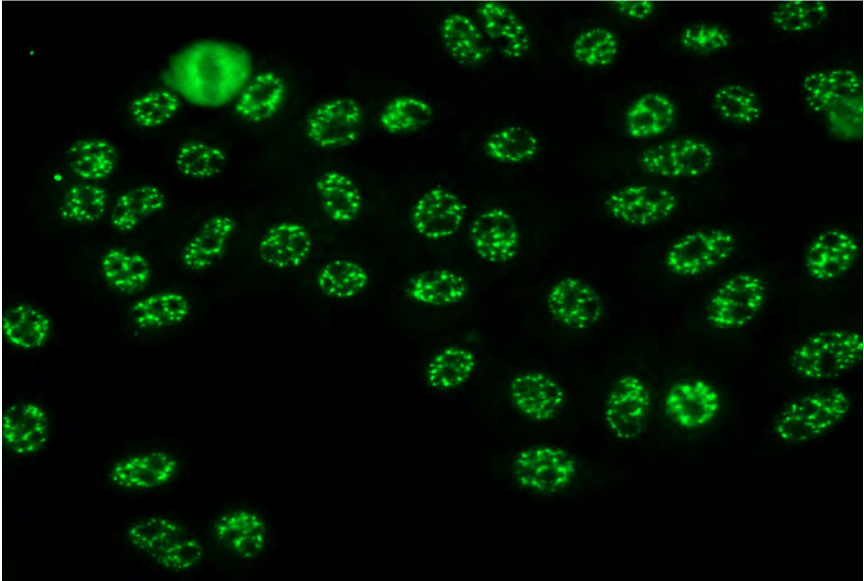
Currently not available in the routine laboratory

Clinical relevance

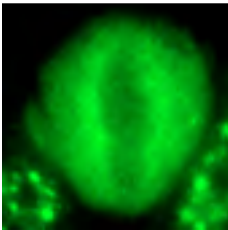
hnRNP – A2/RA 33 indicates early RA,
correlated with severe erosive arthritis in SLE patients

Frequency

hnRNP – A2/RA 33 for RA → 32%⁽⁸⁾



Hep-2 → interphase nuclei: dots and lines arranged around negative nucleoli



Hep-2 → mitotic cells: negative

Sm ANTIBODIES

Autoantigen

Sm-B, Sm-B´ and Sm-D ribonucleoproteins

Indirect immunofluorescence

HEp-2 → interphase nuclei: coarse granular
→ mitotic cells: negative

Confirmation tests

ELISA: → ANAcombi, ANA Detect, ANAScreen,
Anti-RNP/Sm, Anti-Sm, ENA-4-Profile,
ENA-6-Profile, ENAcombi, ENAScreen

Immunoblot → ANA-9-Line, Nucleo-9-Line

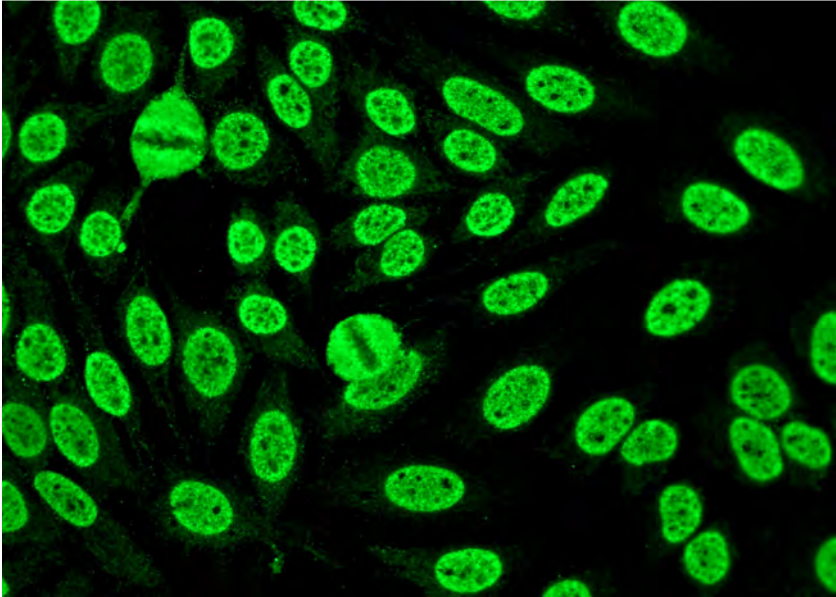
Clinical relevance

Diagnostic marker for SLE (ACR criteria!)

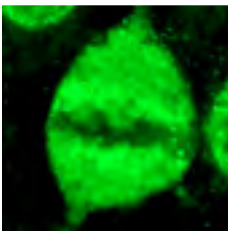
Prognostic marker

Frequency

SLE → 20-30% ⁽²⁾



Hep-2 → interphase nuclei: coarse granular



Hep-2 → mitotic cells: negative

PCNA ANTIBODIES (CYCLIN ANTIBODIES)

Autoantigen

Proliferating cell nuclear antigen

Indirect immunofluorescence

HEp-2

- interphase nuclei: pleomorphic,
dependent on cell cycle phase:
G1 and early S phase: negative to weak positive
middle and late S phase: fine to coarse granular
- mitotic cells: negative

Confirmation tests

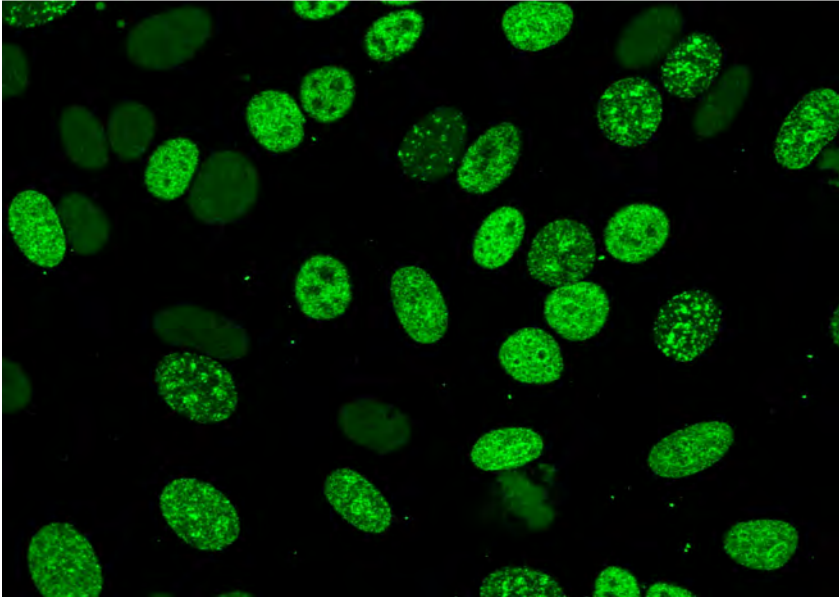
Currently not available in the routine laboratory

Clinical relevance

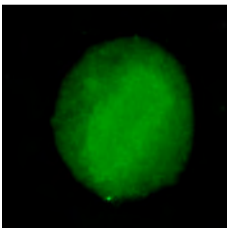
Diagnostic marker for SLE

Frequency

SLE → 3% ⁽²⁾



Hep-2 → interphase nuclei: pleomorphic, depending on cell cycle phase



Hep-2 → mitotic cells: negative

HEp-2 CELLS – NUCLEAR ANTIBODIES

ANTIBODIES WITH NUCLEOLAR PATTERN

- **Sci-70 antibodies**
- **PM-Sci-100 antibodies**
- **Fibrillarin antibodies**
- **NOR-90 antibodies**
- **RNA polymerase antibodies**

Sci-70 ANTIBODIES

Autoantigen

DNA topoisomerase I

Indirect immunofluorescence

HEp-2

- interphase nuclei: fine granular, nucleoli granular
- mitotic cells: positive

Confirmation tests

ELISA

- ANAcombi, ANA Detect, Anti-Sci-70, ANAscreen, ENA-6-Profile, ENAcombi, ENAscreen

Immunoblot

- ANA-9-Line, Nucleo-9-Line

Clinical relevance

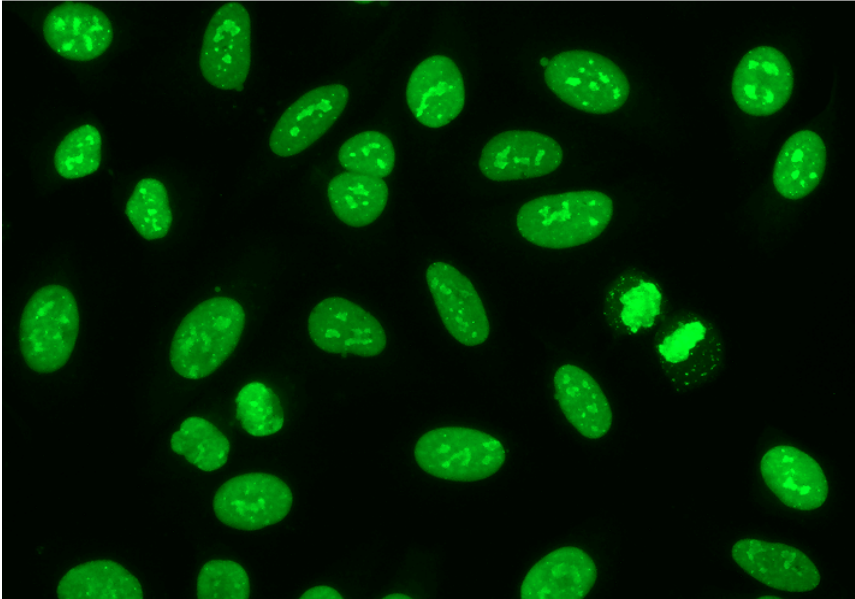
Diagnostic marker for sclerosis

Prognostic marker for sclerosis

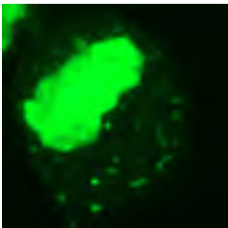
Early diagnostic marker for sclerosis

Frequency

- Systemic sclerosis** → 43% ⁽⁹⁾
- Diffuse forms of sclerosis** → 70% ⁽²⁾
- Lokalised forms of sclerosis** → 13% ⁽²⁾



Hep-2 → interphase nuclei: finely granular, nucleoli granular



Hep-2 → mitotic cells: positive

PM-Scl-100 ANTIBODIES

Autoantigen

20-110 kDa proteins of the nucleolar PM/Scl macromolecular complex (nucleolar exosome)

Indirect immunofluorescence

HEp-2 → interphase nuclei: weak fine granular
nucleoli homogeneous
→ mitotic cells: negative

Confirmation tests

ELISA → ANA Detect

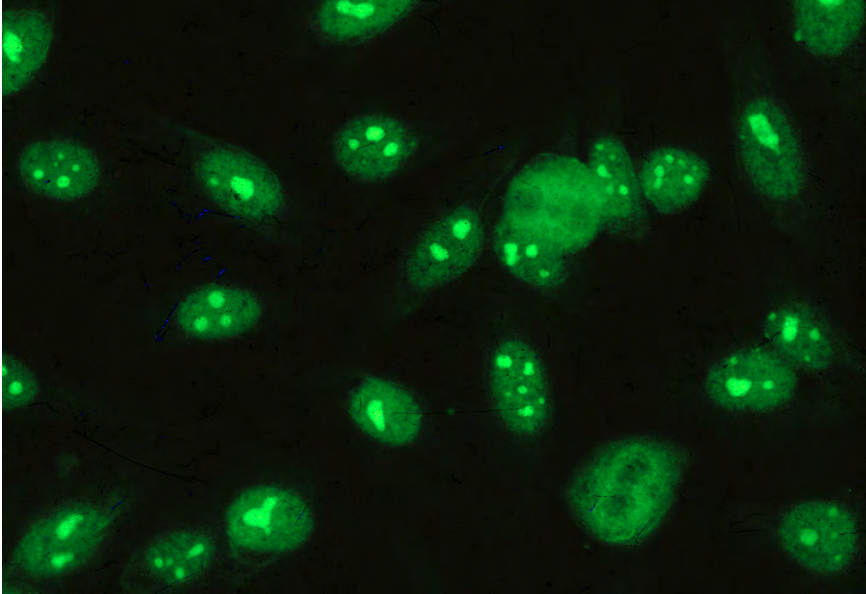
Immunoblot → Myositis plus

Clinical relevance

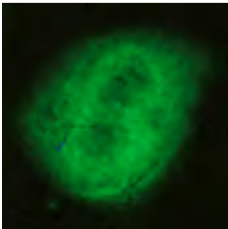
Diagnostic marker for polymyositis/sclerosis overlap syndromes
Prognostic marker

Frequency

Systemic sclerosis/myositis overlap → 50% ⁽⁹⁾



HEp-2 → interphase nuclei: fine granular, homogeneous nucleolar



HEp-2 → mitotic cells: negative

FIBRILLARIN ANTIBODIES (U3-RNP ANTIBODIES)

Autoantigen

34 kDa protein of the ribonucleoprotein complex

Indirect immunofluorescence

HEp-2

→ interphase nuclei: nucleoli clumpy

→ mitotic cells: "cloudy" positive

Confirmation tests

Currently not available in the routine laboratory

Clinical relevance

Diagnostic marker for sclerosis

Prognostic marker for sclerosis

Early diagnostic marker for sclerosis

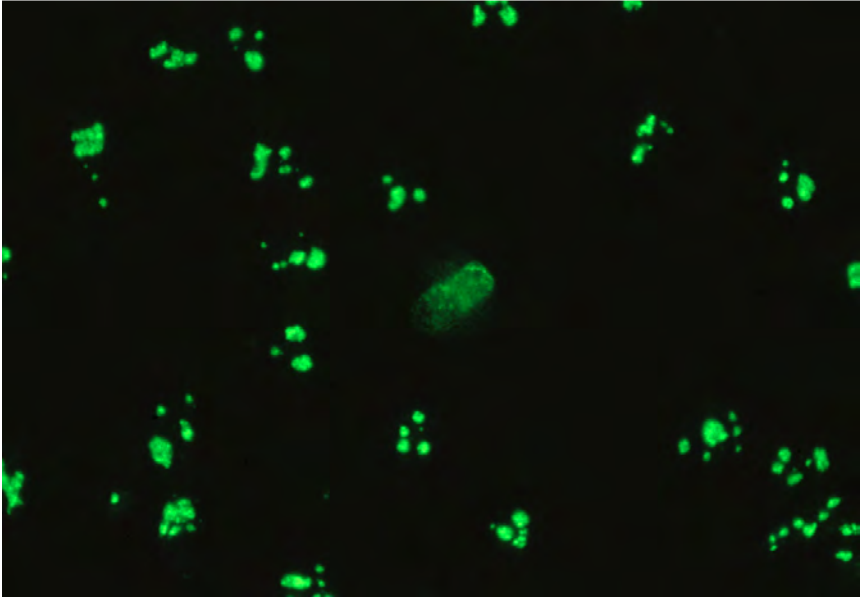
Frequency

Diffuse forms of sclerosis

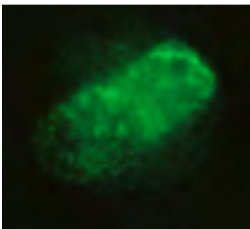
→ 5% ⁽²⁾

Limited forms of sclerosis

→ 10% ⁽²⁾



Hep-2 → interphase nuclei: nucleoli clumpy



Hep-2 → mitotic cells: "cloudy" positive

NOR-90 ANTIBODIES

Autoantigen

Nucleolus organizer region associated 90 kDa protein

Indirect immunofluorescence

Hep-2 → interphase nuclei: nucleoli granular
→ mitotic cells: isolated dots in the chromatin region

Confirmation tests

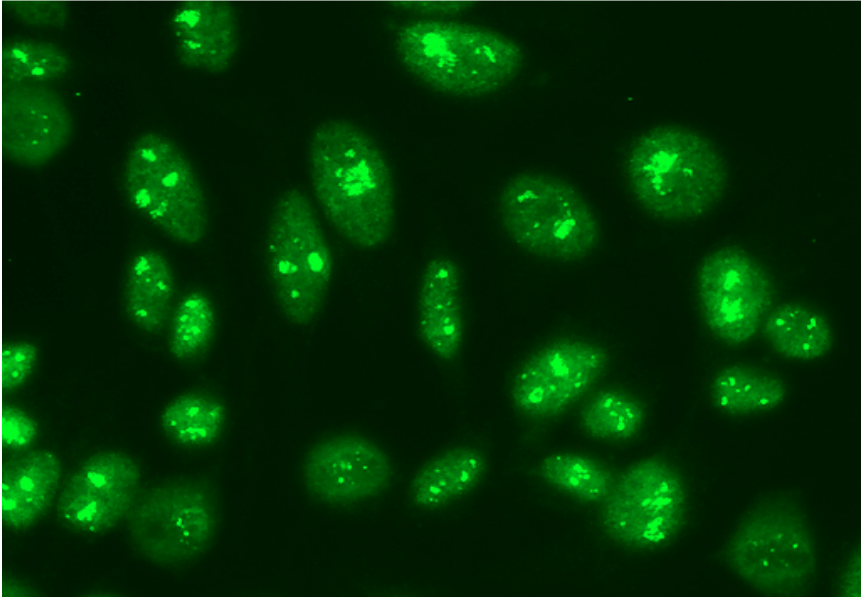
Currently not available in the routine laboratory

Clinical relevance

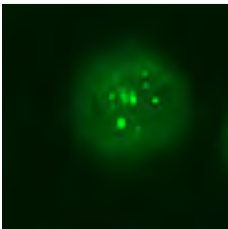
General indicator of autoimmune disease

Frequency

Rare for sclerosis, SLE



Hep-2 → interphase nuclei: nucleoli granular



Hep-2 → mitotic cells: isolated dots in the chromatin region

RNA POLYMERASE ANTIBODIES

Autoantigen

RNA polymerase complex, proteins of 10-220 kDa

Indirect immunofluorescence

HEp-2

- interphase nuclei: nucleoli granular
- mitotic cells: isolated dots in the chromatin region

Confirmation tests

Currently not available in the routine laboratory

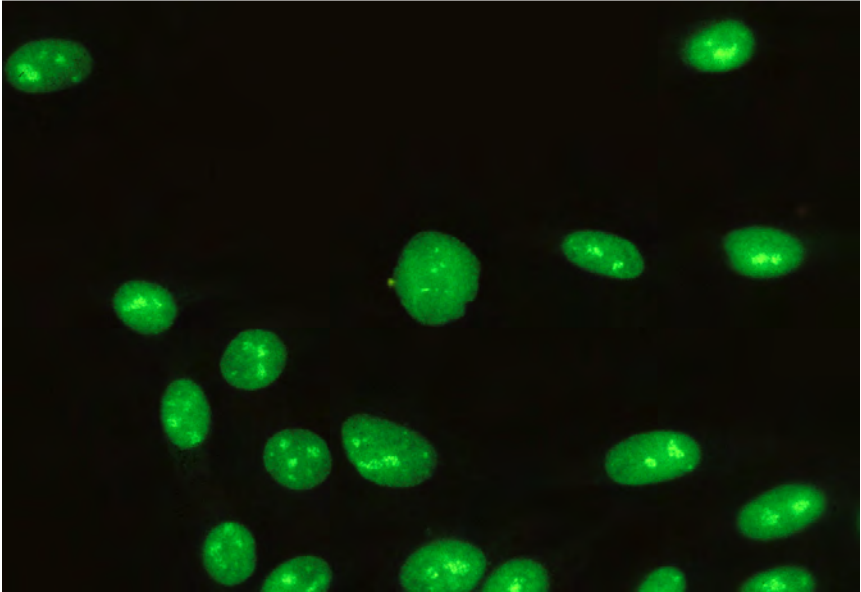
Clinical relevance

General indicator of autoimmune disease

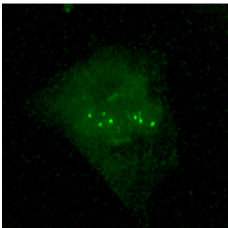
Frequency

Systemic sclerosis → 23% ⁽²⁾

Diffuse cutaneous sclerosis → 38% ⁽⁹⁾



Hep-2 → interphase nuclei: nucleoli granular



Hep-2 → mitotic cells: isolated dots in the chromatin region

HEp-2 CELLS – NUCLEAR ANTIBODIES

ANTIBODIES WITH DOTTED PATTERN

- **Nuclear dot antibodies**
- **Multiple dot antibodies**
- **Centromere antibodies**

NUCLEAR DOT ANTIBODIES (p80 COILIN ANTIBODIES)

Autoantigen

80 kDa protein localised in the Cajal bodies (coiled bodies) of the nucleus

Indirect immunofluorescence

HEp-2 → interphase nuclei: some dots (2 to 4)
→ mitotic cells: negative

Confirmation tests

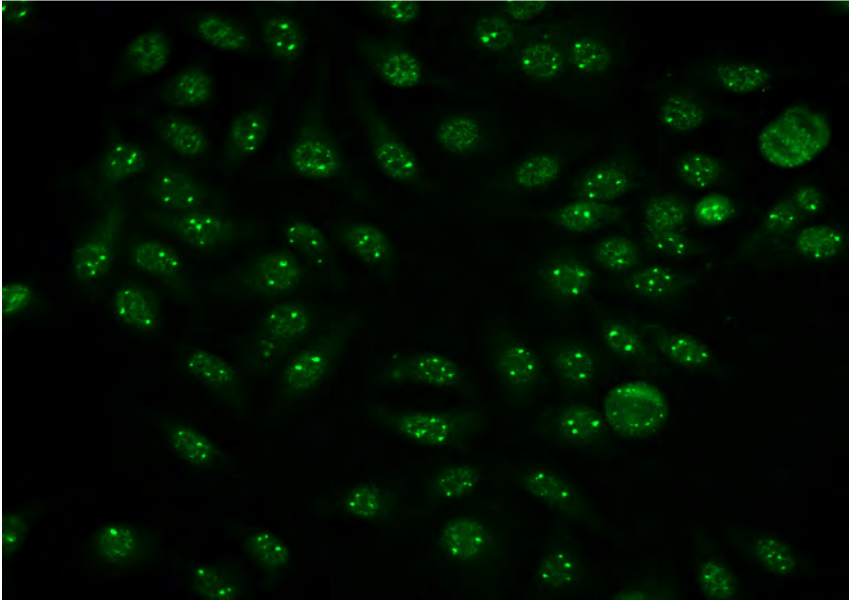
Currently not available in the routine laboratory

Clinical relevance

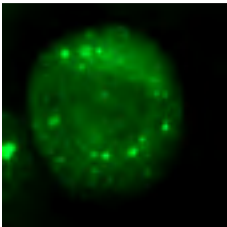
General indicator of autoimmune disease

Frequency

Rare for PBC, SS, sclerosis, chronic hepatitises



Hep-2 → interphase nuclei: some dots (2 to 4)



Hep-2 → mitotic cells: negative

MULTIPLE DOT ANTIBODIES (Sp100 ANTIBODIES)

Autoantigen

Soluble nuclear protein of 53 kDa (aberrant electrophoretic mobilities, with an apparent molecular weight of 100 kDa)

Indirect immunofluorescence

HEp-2 → interphase nuclei: dots (usually 3 to 10)
→ mitotic cells: negative

Confirmation tests

ELISA → Anti-Sp100

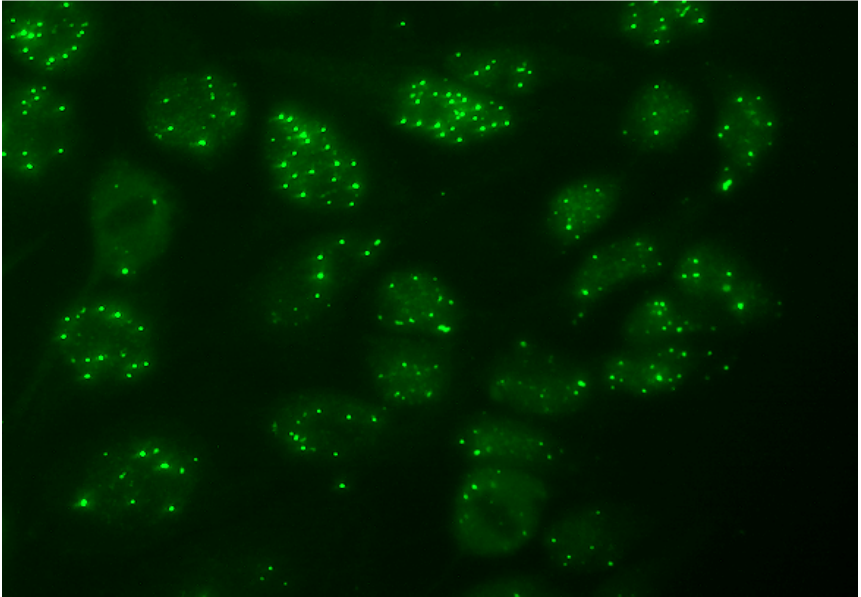
Immunoblot → Liver-9-Line

Clinical relevance

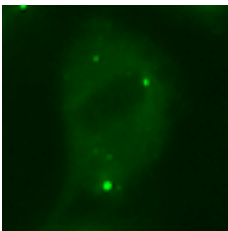
High specificity for PBC
Possible early diagnostic marker for PBC

Frequency

PBC → 33%⁽¹⁰⁾



Hep-2 → interphase nuclei: dots (3 to 10)



Hep-2 → mitotic cells: negative

CENTROMERE ANTIBODIES (CENP-B AB)

Autoantigen

Centromere B protein of 80 kDa, with centromeric DNA-associated proteins

Indirect immunofluorescence

HEp-2 → interphase nuclei: dots (46)
→ mitotic cells: punctate (like a zipper)

Confirmation tests

ELISA → ANAcombi, ANA Detect, ANAscreen,
Anti-Centromere-B

Immunoblot → ANA-9-Line, Nucleo-9-Line

Clinical relevance

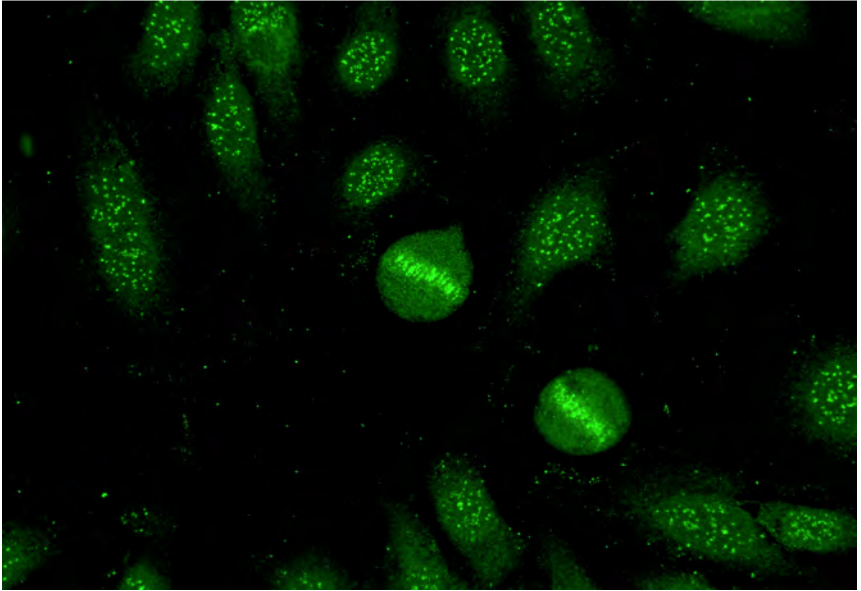
Diagnostic marker for systemic sclerosis

Prognostic marker for systemic sclerosis

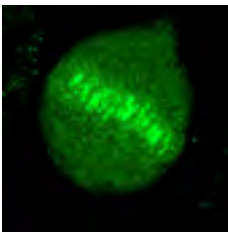
Early diagnostic marker for sclerosis

Frequency

Systemic sclerosis → 44% ⁽⁹⁾
Limited forms of sclerosis → 57-82% ⁽²⁾
Diffuse forms of sclerosis → 8% ⁽²⁾
PBC → 21% ⁽¹⁰⁾



HEp-2 → interphase nuclei: dots (46)



HEp-2 → mitotic cells: punctate

HEp-2 CELLS

CYTOPLASMIC ANTIBODIES

- **Jo-1 antibodies**
- **PL7, PL12 antibodies**
- **Ribosomal antibodies**
- **SRP antibodies**

Jo-1 ANTIBODIES

Autoantigen

Histidyl-tRNA synthetase

Indirect immunofluorescence

HEp-2

- interphase nuclei: negative
- mitotic cells: negative
- cytoplasm: diffuse granular fluorescence

Confirmation tests

ELISA

- ANAcombi, ANA Detect, ANAScreen, Anti-Jo-1, ENA-6-Profile, ENAcombi, ENAScreen

Immunoblot

- ANA-9-Line, Myositis plus, Nucleo-9-Line

Clinical relevance

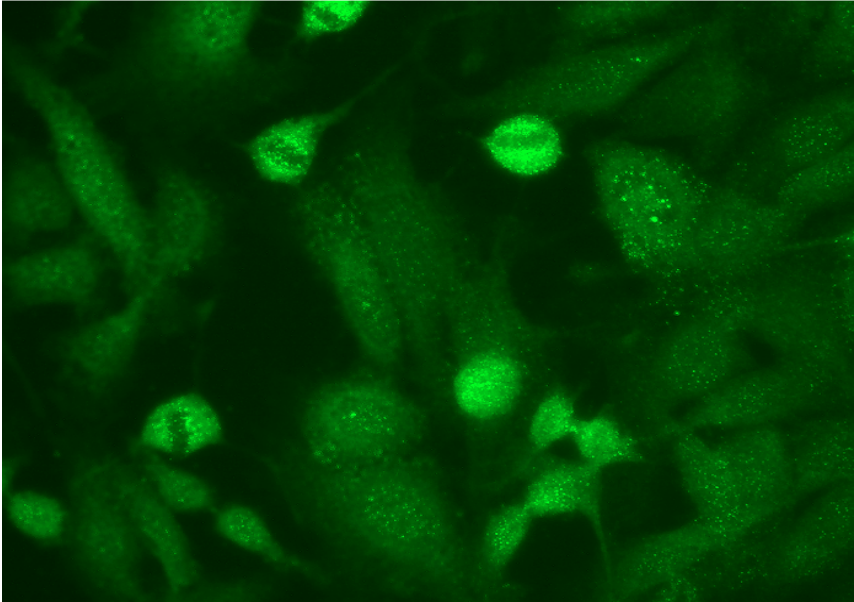
Diagnostic marker for idiopathic myositis

Prognostic marker

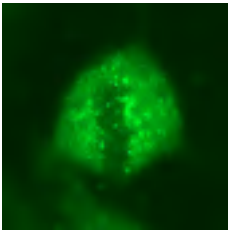
Frequency

PM/DM

- 20% ⁽²⁾



Hep-2 → cytoplasm: diffuse granular
→ interphase nuclei: negative



Hep-2 → mitotic cells: negative

PL7, PL12 ANTIBODIES

Autoantigen

PL7: threonyl-tRNA synthetase

PL12: alanyl-tRNA synthetase

Indirect immunofluorescence

Hep-2

→ interphase nuclei: negative

→ mitotic cells: negative

→ cytoplasm: diffuse, fine granular

Confirmation tests

Immunoblot

→ Myositis plus

Clinical relevance

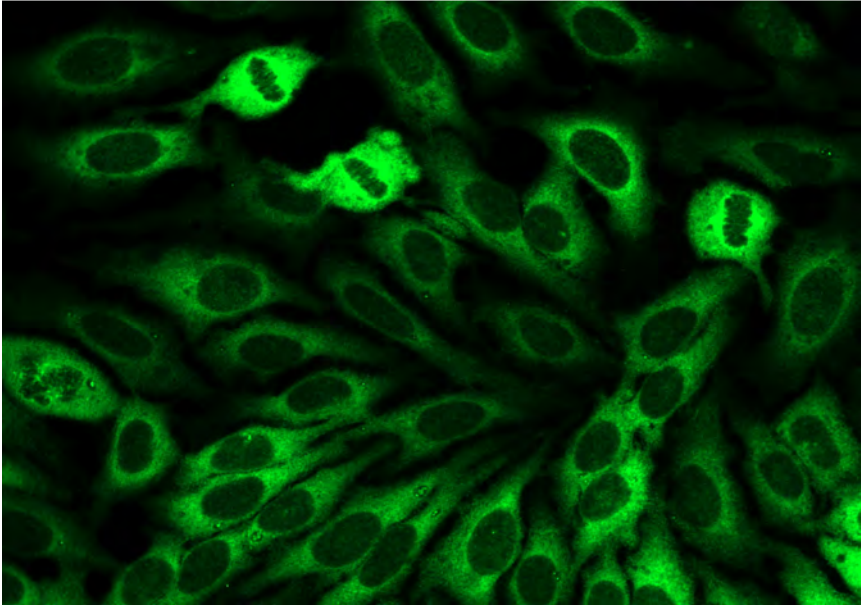
Diagnostic marker for idiopathic myositis

Frequency:

Myositis:

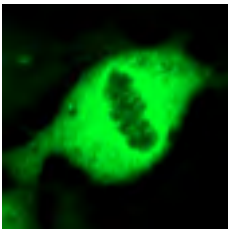
anti-PL7 → 2%⁽¹²⁾

anti-PL12 → 1%⁽¹²⁾



Hep-2 (anti-PL12)

- cytoplasm: diffuse, fine granular
- interphase nuclei: negative



Hep-2 → mitotic cells: negative

RIBOSOMAL ANTIBODIES (Rib-P ANTIBODIES)

Autoantigen

proteins of the ribosomal complex (ribosomal P proteins)

Indirect immunofluorescence

HEp-2 → interphase nuclei: nucleoli positive
→ mitotic cells: negative
→ cytoplasm: fine granular

Confirmation tests

ELISA → Anti-Rib-P

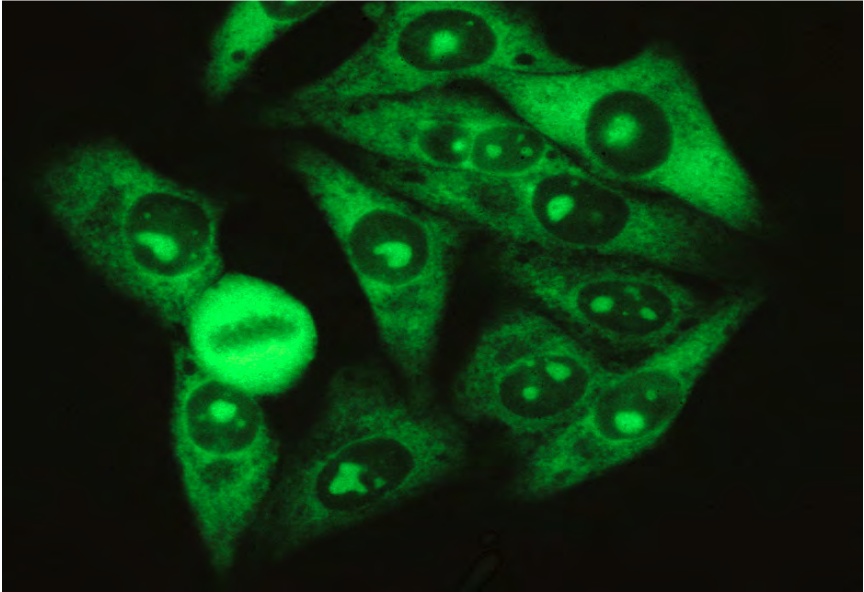
Immunoblot → ANA-9-Line, Myositis plus

Clinical relevance

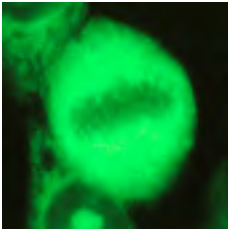
Diagnostic marker for SLE

Frequency:

SLE → 15% ⁽¹⁾



Hep-2 → cytoplasm: fine granular
→ interphase nuclei: nucleoli positive



Hep-2 → mitotic cells: negative

SRP ANTIBODIES

Autoantigen

Signal recognition particle

Indirect immunofluorescence

HEp-2

- interphase nuclei: negative
- mitotic cells: negative
- cytoplasm: diffuse finely granular

Confirmation tests

Immunoblot

- Myositis plus

Clinical relevance

Diagnostic marker for polymyositis

Prognostic marker

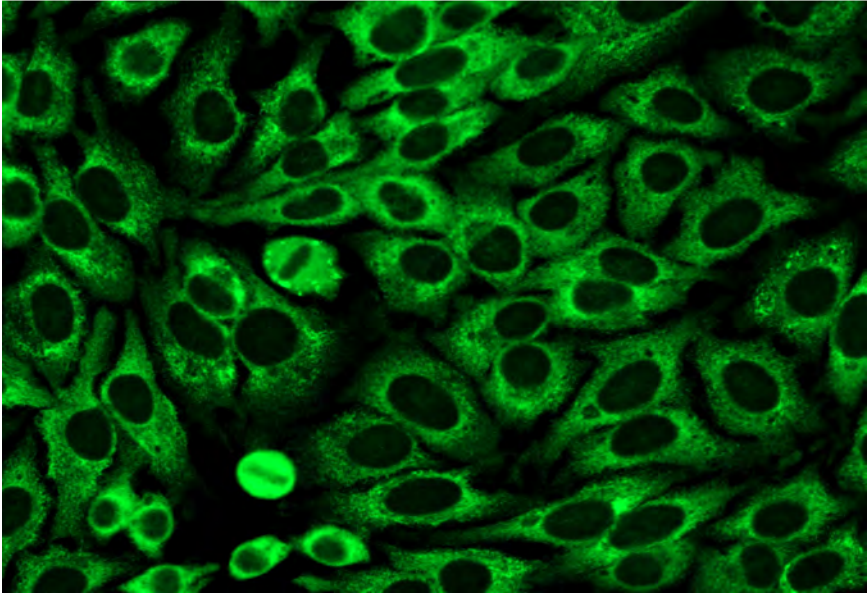
Frequency

Polymyositis

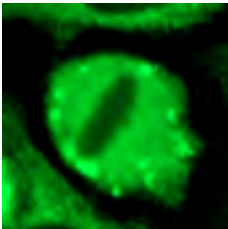
- 5% ⁽²⁾

Dermatomyositis

- 0% ⁽²⁾



Hep-2 → cytoplasm: diffuse fine granular
→ interphase nuclei: negative



Hep-2 → mitotic cells: negative

KSL TISSUES

ANTIBODIES ON KIDNEY/STOMACH/LIVER TISSUES

- **Antimitochondrial antibodies (AMA-M2)**
- **F-actin antibodies**
- **LKM-1 antibodies**
- **Parietal cell antibodies**

ANTIMITOCHONDRIAL ANTIBODIES (AMA-M2)

Autoantigen

alpha-ketoacid dehydrogenase complex of the inner mitochondrial membrane (PDC-E2)

Indirect immunofluorescence

HEp-2

- interphase nuclei: negative
- mitotic cells: negative
- cytoplasm: coarse granular

KSL (rat)

- kidney: distal und proximal tubular cells granular positive
- stomach: parietal cells granular
- liver: hepatocytes granular

Confirmation tests

ELISA

- AMA-M2

Immunoblot

- Liver-9-Line, Myositis plus

Clinical relevance

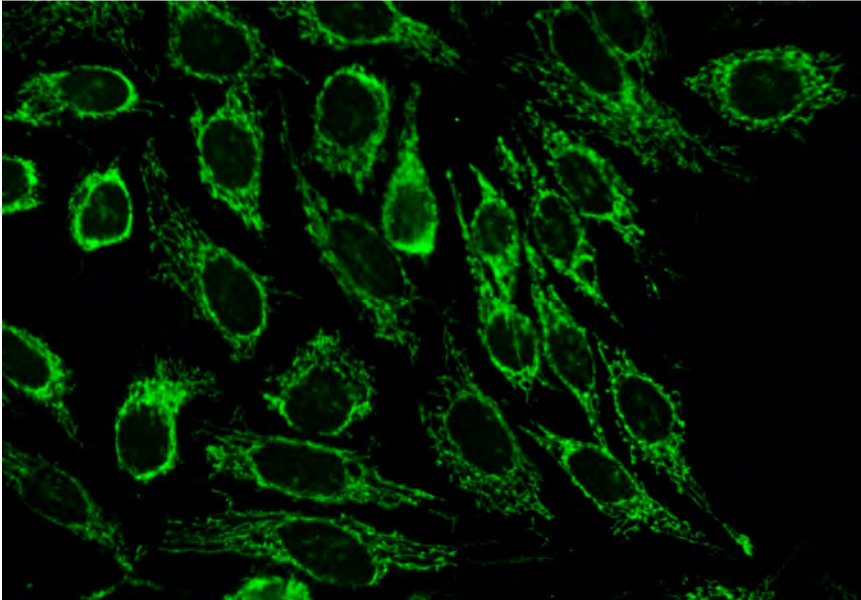
Diagnostic marker for PBC

Early diagnostic marker for PBC

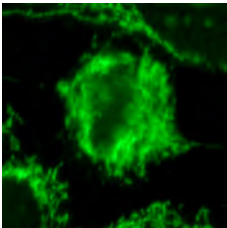
Frequency

PBC

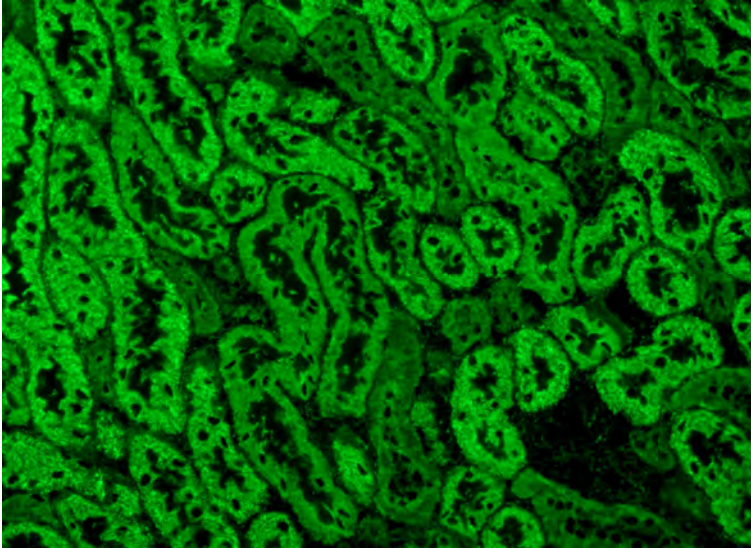
- 90-95% ⁽¹⁰⁾



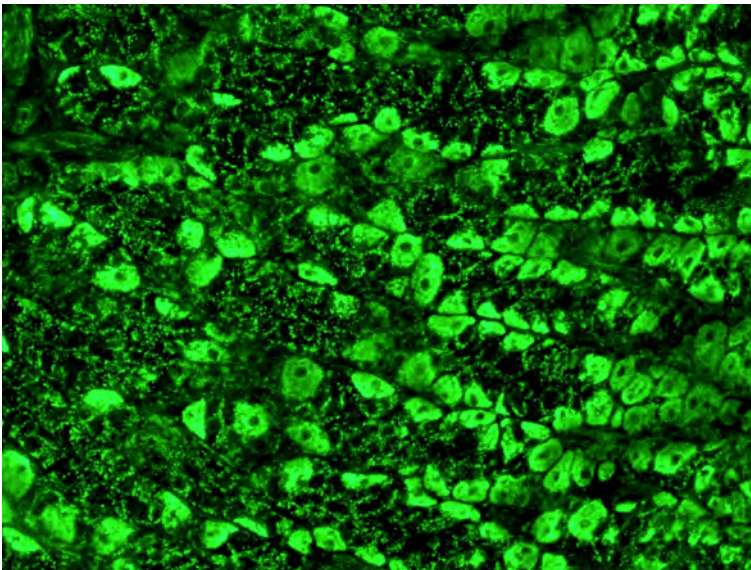
Hep-2 → cytoplasm: coarse granular
→ interphase nuclei: negative



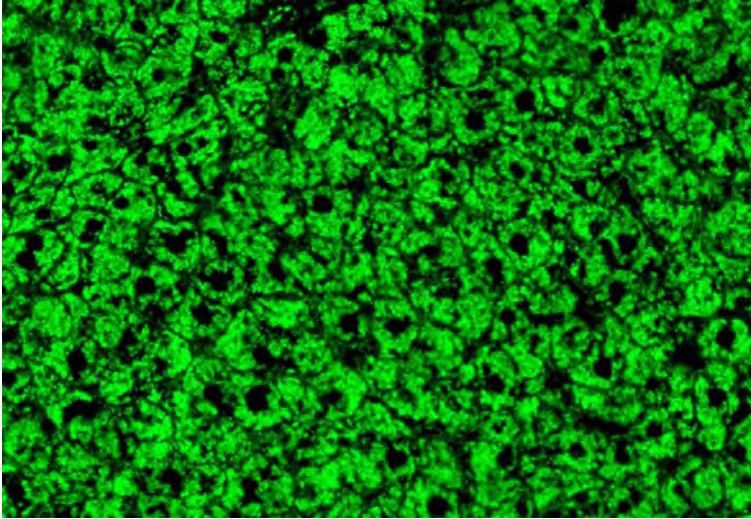
Hep-2 → mitotic cells: negative



KSL → kidney (rat): distal and proximal tubular cells granular positive



KSL → stomach (rat): parietal cells granular



KSL → liver (rat): hepatocytes granular

ACTIN ANTIBODIES

Autoantigen

F-actin 41 kDa

Indirect immunofluorescence

HEp-2

- interphase nuclei: negative
- mitotic cells: negative
- cytoplasm: fine granular to fibrous

KSL (rat)

- kidney: glomeruli and vessels positive; tubuli: needle-like, peritubular fluorescence
- stomach: muscle and interparietal septa positive

Confirmation tests

Immunoblot

- Liver-9-Line

Clinical relevance

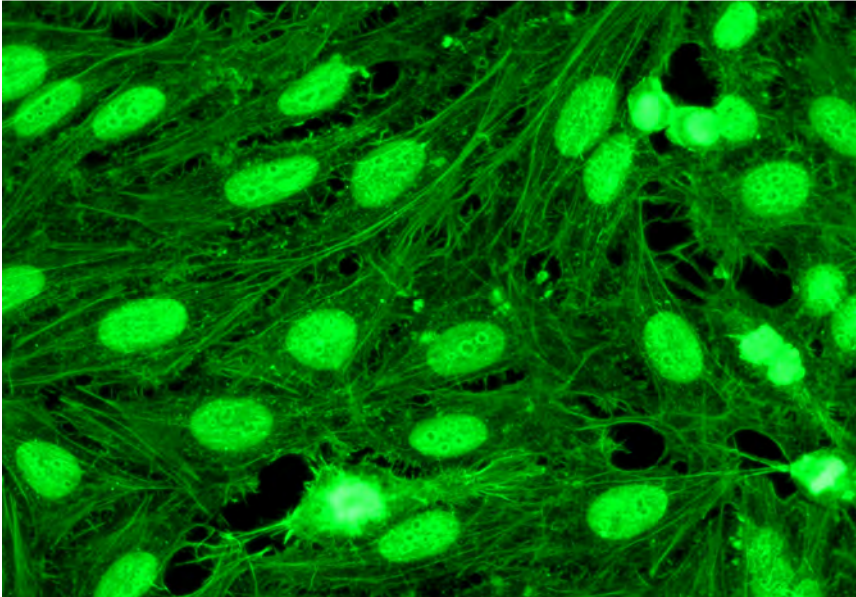
Diagnostic marker for AIH type 1

Prognostic marker

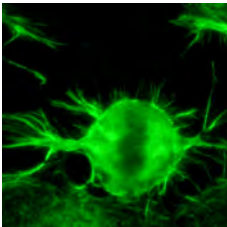
Frequency

AIH type 1 (high titre) → 58% ⁽¹⁴⁾

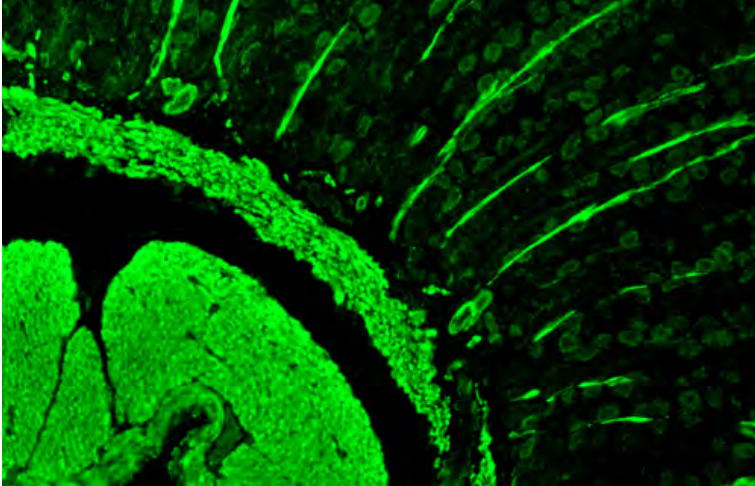
PBC (low titre) → 8% ⁽¹⁴⁾



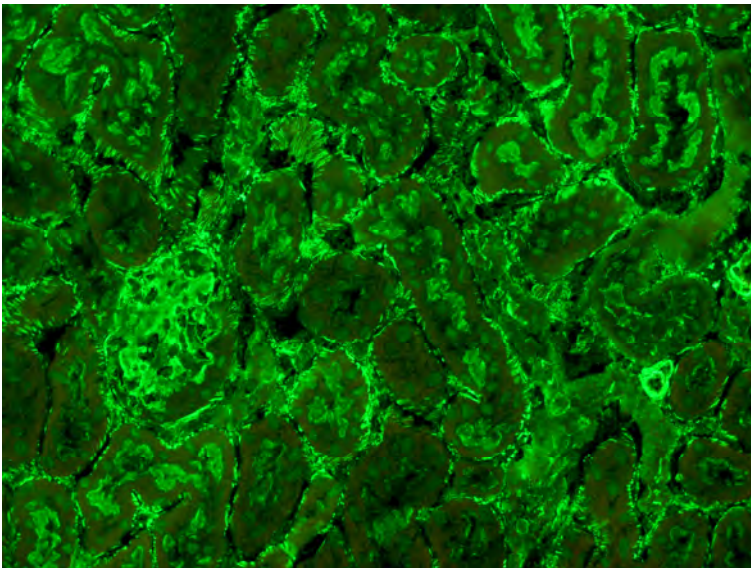
Hep-2 → cytoplasm: finely granular to fibrous
→ interphase nuclei: ANA positive possible for AIH type 1



Hep-2 → mitotic cells: negative



KSL → stomach (rat): muscle and interparietal septa positive



KSL → kidney (rat): glomeruli und vessels positive;
tubuli: needle-like, peritubular fluorescence

LKM-1 ANTIBODIES (LIVER/KIDNEY MICROSOMAL ANTIBODIES TYPE 1)

Autoantigen

Cytochrome P450 2D6

Indirect immunofluorescence

HEp-2 → interphase nuclei: negative
→ mitotic cells: negative
→ cytoplasm: negative

KSL (rat) → kidney: proximal tubuli positive, distal tubuli negative
→ stomach: negative
→ liver: hepatocytes positive

Confirmation tests

ELISA → Anti-LKM-1

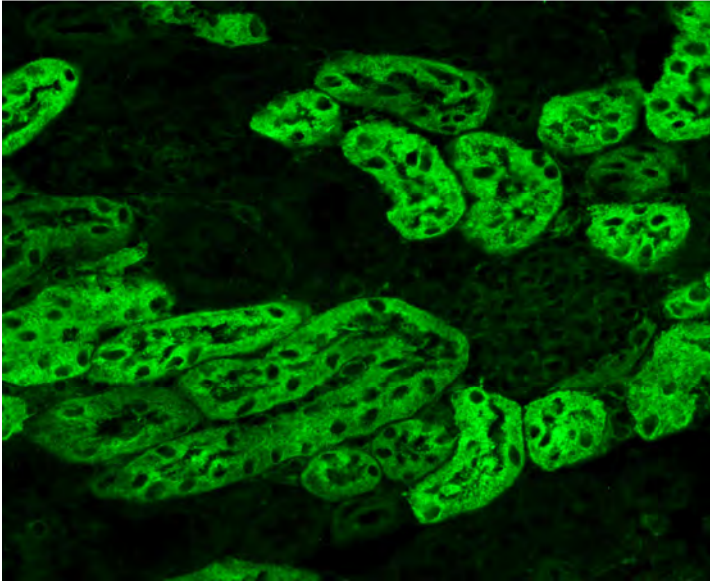
Immunoblot → Liver-9-Line

Clinical relevance

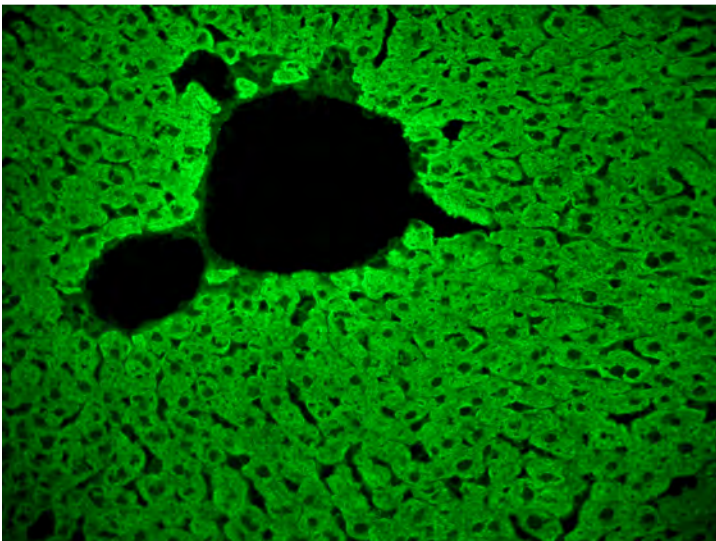
Diagnostic marker for AIH type 2

Frequency

AIH → 3-4% ⁽¹³⁾



KSL → kidney (rat): proximal tubuli positive



KSL → liver (rat): hepatocytes positive

GASTRIC PARIETAL CELL ANTIBODIES (GPA ANTIBODIES)

Autoantigen

Gastric parietal cell antigen (alpha and beta subunits of gastric parietal cell H⁺/K⁺ ATPase)

Indirect immunofluorescence

HEp-2 → interphase nuclei: negative
→ mitotic cells: negative

KSL (rat) → stomach: parietal cells clumpy positive
→ kidney: negative
→ liver: negative

Confirmation tests

ELISA → Anti-Intrinsic Factor, Anti-Parietal Cell

Immunoblot → Gastro-5-Line

Clinical relevance

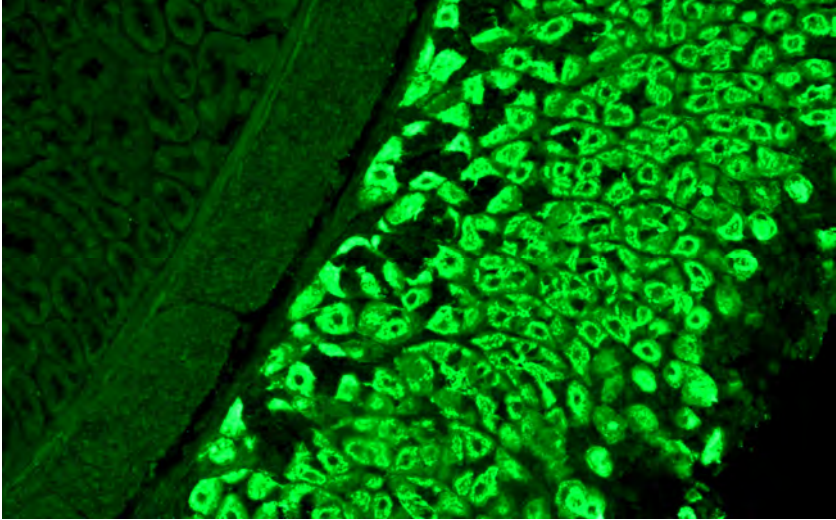
Diagnostic marker of pernicious anaemia (together with antibodies against intrinsic factor)

Early diagnostic marker for pernicious anaemia

Frequency

Pernicious anaemia:

GPA antibodies	→ sensitivity 82%	→ specificity 90% ⁽¹¹⁾
IF antibodies	→ sensitivity 37%	→ specificity 100% ⁽¹¹⁾
GPA and IF antibodies	→ sensitivity 73%	→ specificity 100% ⁽¹¹⁾



KSL → stomach (rat): parietal cells clumpy

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The community GRÜNER CLUB AUTOIMMUN was founded in January 2011 by the German company ORGENTEC Diagnostika.

The intention of this club is to improve communication and exchange of experiences between individuals working in the field of autoimmunity. Good collaboration between laboratories, clinicians, and companies leads to an improvement in autoimmunity results. This should also increase the influence of autoimmunity results in clinical diagnostics.

The platform for this exchange of information is a protected website. The site will provide information about autoantibodies, diseases, and diagnostics. In addition, there is a forum for technical questions and answers. Immunofluorescence images from interesting and unusual cases will also be presented.

The club offers workshops for continuing education. Interesting topics are presented so as to cover both theory and practice to serve the exchange of knowledge between co-workers.

The goal is to improve the quality of products used and the results obtained.

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