EUROLINE Autoimmune Inflammatory Myopathies 16 Ag et cN-1A et HMGCR (IgG)
Test instruction

ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
DL 1530-1601-8 G DL 1530-5001-8 G DL 1530-6401-8 G	Mi-2α, Mi-2β, TIF1γ, MDA5, NXP2, SAE1, Ku, PM-Scl100, PM-Scl75, Jo-1, SRP, PL-7, PL-12, EJ, OJ, Ro-52, cN-1A, HMGCR	IgG	Ag-coated immunoblot strips	16 x 01 (16) 50 x 01 (50) 64 x 01 (64)

Indications: The EUROLINE test kit provides qualitative in vitro determination of human autoantibodies of the immunoglobulin class IgG to 18 different antigens **Mi-2α, Mi-2β, TIF1γ, MDA5, NXP2, SAE1, Ku, PM-Scl100, PM-Scl75, Jo-1, SRP, PL-7, PL-12, EJ, OJ, Ro-52, cN-1A and HMGCR** in serum or plasma to support the diagnosis of dermato- and polymyositis, idiopathic myositis, antisynthetase syndrome, inclusion body myositis (IBM), necrotising myositis and overlapping syndromes.

Application: The isolated presence of autoantibodies against individual myositis-specific antigens is characteristic for autoimmune myositides. Comprehensive studies in various centres in Europe have shown that the simultaneous investigation of myositis-specific antibodies in a large profile can significantly increase the serological detection rate. The EUROLINE Autoimmune Inflammatory Myopathies 16 Ag et cN-1A et HMGCR (IgG) enables serological diagnostics of inflammatory idiopathic myositides and further differentiation into dermato- and polymyositis, inclusion body myositis (IBM), and overlap diseases, and, for the first time, the automated analysis of 18 myositis-specific antibodies on one test strip.

Principles of the test: The test kit contains test strips coated with parallel lines of highly purified antigens. In the first reaction step, the immunoblot strips are incubated with diluted patient samples. In the case of positive samples, the specific IgG antibodies (also IgA and IgM) will bind to the corresponding antigenic site. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalysing a colour reaction.
The format DL 1530-5001-8 G belongs to the Immunoblot-PreQ system. The test strips are already placed into the incubation trays (EUROTray).

Contents of the test kit: (DL 1530-####-8 G)

Component	1601	6401	5001	Symbol
1. Test strips coated with the antigens Mi-2α, Mi-2β, TIF1γ, MDA5, NXP2, SAE1, Ku, PM-Scl100, PM-Scl75, Jo-1, SRP, PL-7, PL-12, EJ, OJ, Ro-52, cN-1A and HMGCR	16 strips	4 x 16 strips	5 x 10 strips	STRIPS
2. Positive control (IgG, human), 100x concentrate	1 x 0.02 ml	4 x 0.02 ml	5 x 100 µl	POS CONTROL 100x
3. Enzyme conjugate Alkaline phosphatase-labelled anti-human IgG (goat), 10x concentrate	1 x 3 ml	4 x 3 ml	---	CONJUGATE 10x
4. Enzyme conjugate Alkaline phosphatase-labelled anti-human IgG (goat), ready for use	---	---	4 x 30 ml	CONJUGATE
5. Sample buffer, ready for use	1 x 100 ml	3 x 100 ml	2 x 100 ml	SAMPLE BUFFER
6. Wash buffer, 10x concentrate	1 x 50 ml	1 x 100 ml	1 x 100 ml	WASH BUFFER 10x
7. Substrate solution Nitroblue tetrazolium chloride/5-Bromo-4-chloro-3-indolylphosphate (NBT/BCIP), ready for use	1 x 30 ml	4 x 30 ml	4 x 30 ml	SUBSTRATE
8. Incubation tray	2 x 8 channels	---	---	
9. Test instruction	1 booklet	1 booklet	1 booklet	

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

Updates with respect to the previous version are marked in grey.

The following components are not provided in the test kits but can be ordered at EUROIMMUN under the respective order numbers.
Performance of the test requires an **incubation tray**:
ZD 9895-0130 Incubation tray with 30 channels
ZD 9898-0144 Incubation tray with 44 channels (black, for the EUROBlotOne and EUROBlotCamera system)

If using Immunoblot-PreQ (DL 1530-5001-8 G), no additional incubation tray is needed.

For the creation of work protocols and the evaluation of incubated test strips using **EUROLineScan** green paper and adhesive foil are required:
ZD 9880-0101 Green paper (1 sheet)
ZD 9885-0116 Adhesive foil for approx. 16 test strips
ZD 9885-0130 Adhesive foil for approx. 30 test strips

If using Immunoblot-PreQ (DL 1530-5001-8 G), the strips should stay in the EUROTray during evaluation. For the evaluation we generally recommend using a EUROIMMUN camera system connected to EUROLineScan software. Strips need to be dry before starting the evaluation.

Preparation and stability of the reagents

Note: This test kit may only be used by trained personnel. Test strips and incubation trays are intended for single use. All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. Unopened, reagents are stable until the indicated expiry date when stored at +2°C to +8°C. After initial opening, reagents are stable for 12 months or until the expiry date, unless stated otherwise in the instructions. Opened reagents must also be stored at +2°C to +8°C and protected from contamination.

- **Coated test strips:** Ready for use. Open the package with the test strips only when the strips have reached room temperature (+18°C to +25°C) to prevent condensation on the strips. After removal of the strips/Immunoblot-PreQ the package should be sealed tightly and stored at +2°C to +8°C.
- **Positive control:** The control is a 100x concentrate. For the preparation of the working-strength control the amount required should be removed from the bottle using a clean pipette tip and diluted 1:101 with sample buffer. Example: add 15 µl of control to 1.5 ml of sample buffer and mix thoroughly. The ready for use diluted control should be used at the same working day.
- **Enzyme conjugate:** The enzyme conjugate is supplied as a 10x concentrate. For the preparation of the working-strength enzyme conjugate the amount required should be removed from the bottle using a clean pipette tip and diluted 1:10 with sample buffer. For one test strip, dilute 0.15 ml enzyme conjugate with 1.35 ml sample buffer. The working-strength diluted enzyme conjugate should be used at the same working day.
- **Enzyme conjugate:** Ready for use
  Note: Only for DL 1530-5001-8 G!
- **Sample buffer:** Ready for use.
- **Wash buffer:** The wash buffer is supplied as a 10x concentrate. For the preparation of the working-strength wash buffer the amount required should be removed from the bottle using a clean pipette tip and diluted 1:10 with distilled water. For one test strip, dilute 1 ml in 9 ml of distilled water. The working-strength diluted wash buffer should be used at the same working day.
- **Substrate solution:** Ready for use. Close bottle immediately after use, as the contents are sensitive to light.

Storage and stability: The test kit must be stored at a temperature between +2°C and +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Waste disposal: Patient samples, controls and incubated test strips should be handled as infectious waste. Other reagents do not need to be collected separately, unless stated otherwise in official regulations.

Warning: The control of human origin has tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2. Nonetheless all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain sodium azide in a non-declarable concentration. Avoid skin contact.

Preparation and stability of the patient samples

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

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Sample dilution: The **patient samples** for analysis are diluted **1:101** with sample buffer using a clean pipette tip. For example, add 15 µl of sample to 1.5 ml sample buffer and mix well by vortexing. Sample pipettes are not suitable for mixing.

Incubation

If using Immunoblot-PreQ (DL 1530-5001-8 G), manual incubation is not possible. Please see below for options of automated incubation.

Pretreat: Remove the required amount of test strips from the package and place them each in an empty channel (Make sure that the surface of the test strips is not damaged!). The number on the test strip should be visible. Fill the channels of the incubation tray according to the number of serum samples to be tested with 1.5 ml sample buffer each.
Use of Immunoblot-PreQ: Set up the required antigen profiles according to the work protocol and insert into the incubation device.
Incubate for **5 minutes** at room temperature (+18°C to +25°C) on a rocking shaker. Afterwards aspirate off all the liquid.

Incubate: (1st step) Fill each channel with 1.5 ml of the diluted serum samples using a clean pipette tip.
Incubate for **30 minutes** at room temperature (+18°C to +25°C) on a rocking shaker.

Wash: Aspirate off the liquid from each channel and wash **3 x 5 minutes** each with 1.5 ml working-strength wash buffer on a rocking shaker.

Incubate: (2nd step) Pipette 1.5 ml diluted enzyme conjugate (alkaline phosphatase-labelled anti-human IgG) into each channel.
Incubate for **30 minutes** at room temperature (+18°C to +25°C) on a rocking shaker.

Wash: Aspirate off the liquid from each channel. Wash as described above.

Incubate: (3rd step) Pipette 1.5 ml substrate solution into the channels of the incubation tray.
Incubate for **10 minutes** at room temperature (+18°C to +25°C) on a rocking shaker.

Stop: Aspirate off the liquid from each channel and wash each strip **3 x 1 minute** with distilled water.

Evaluate: Place test strip on the evaluation protocol, air dry and evaluate.
Immunoblot-PreQ: The evaluation of the test strips is realised exclusively via the EUROIMMUN camera systems.

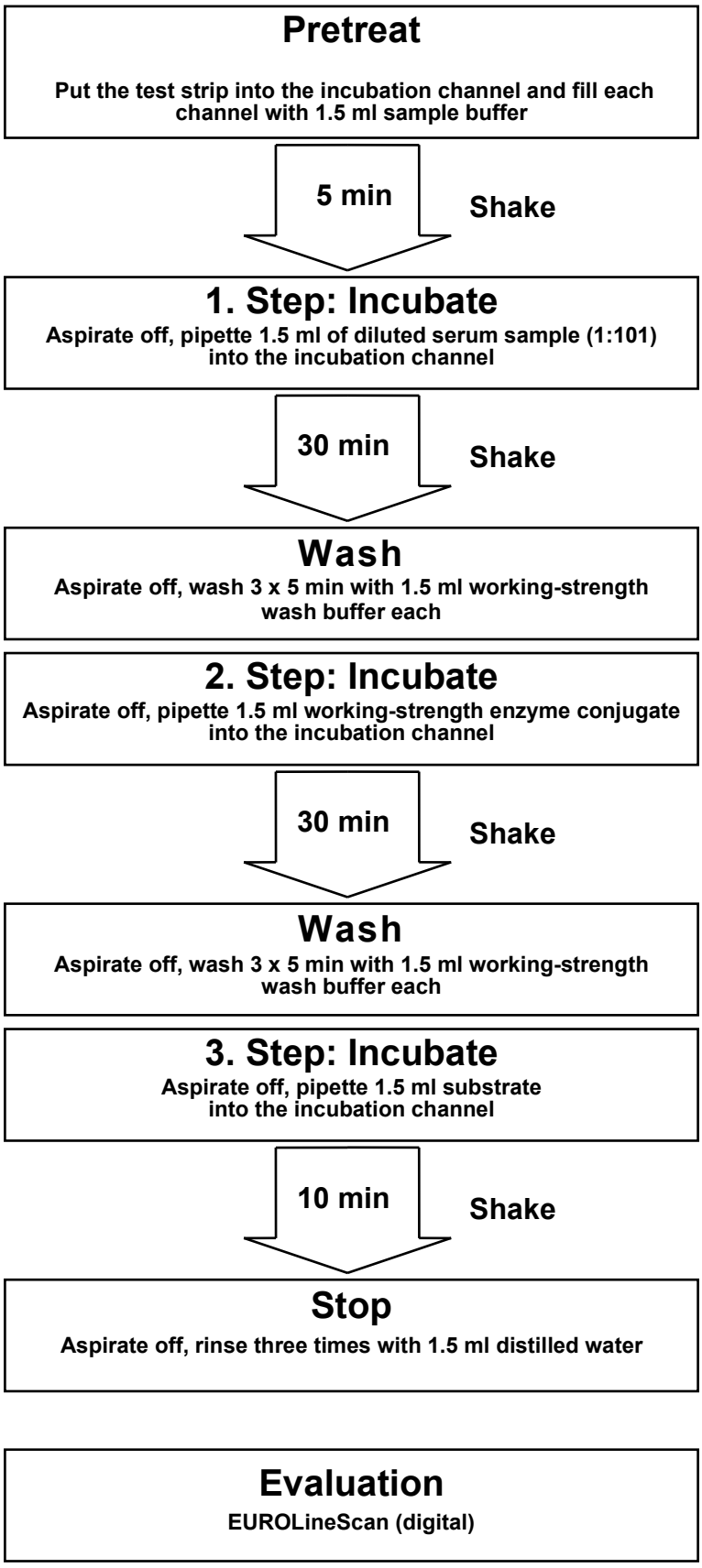
For automated incubation with the **EUROBlotMaster** select the program **Euro01 AAb EL30**.

For automated incubation with the **EUROBlotOne** select the program **Euro 01/02**.

For automated incubation of Immunoblot-PreQ with the **EUROBlotOne** see instruction manual EUROBlotOne (YG_0153_A_UK_CXX).

EUROLINE Autoimmune Inflammatory Myopathies 16 Ag et cN-1A et HMGCR (IgG)

Incubation protocol



Interpretation of results

Handling: For the evaluation of incubated test strips we generally recommend using the **EUROLineScan** software. After stopping the reaction using deionised or distilled water, place the incubated test strips onto the adhesive foil of the green work protocol using a pair of tweezers. The position of the test strips can be corrected while they are wet. As soon as all test strips have been placed onto the protocol, they should be pressed hard using filter paper and left to air-dry. After they have dried, the test strips will be stuck to the adhesive foil. The dry test strips are then scanned using a flatbed scanner (EUROIMMUN) and evaluated with **EUROLineScan**. Alternatively, imaging and evaluation are possible directly from the incubation trays (EUROBlotCamera and EUROBlotOne). For general information about the EUROLineScan program please refer to the EUROLineScan user manual (EUROIMMUN document no. YG_0006_A_UK_CXX). The code for entering the **test** into EUROLineScan is **Myositis-EL_8**.

If using Immunoblot-PreQ (DL 1530-5001-8 G), the strips should stay in the EUROTray during evaluation. For the evaluation we generally recommend using a EUROIMMUN camera system connected to EUROLineScan software. Strips need to be dry before starting the evaluation.

Note: Correct performance of the incubation is indicated by an intense staining of the control band.

Antigens and their arrangement on the strips: The EUROLINE test strips have been coated with the following antigens:

Mi-2α: Recombinant Mi-2α protein. The corresponding human cDNA was expressed with the baculovirus system in insect cells.

Mi-2β: Recombinant Mi-2β protein. The corresponding human cDNA was expressed with a baculovirus vector in insect cells.

TIF1γ: Recombinant TIF1γ protein. The corresponding human cDNA was expressed in E. coli.

MDA5: Purified MDA5 protein.

NXP2: Recombinant NXP2 protein. The corresponding human cDNA was expressed in E. coli.

SAE1: Recombinant SAE1 protein. The corresponding human cDNA was expressed in E. coli.

Ku: Recombinant Ku protein. The corresponding human cDNA was expressed with the baculovirus system in insect cells.

PM-Scl100: Recombinant PM-Scl protein (100 kDa). The corresponding human cDNA was expressed with the baculovirus system in insect cells.

PM-Scl75: Recombinant PM-Scl protein (75 kDa). The corresponding human cDNA was expressed with the baculovirus system in insect cells.

Jo-1: Jo-1 protein (histidyl-tRNA synthetase), purified by affinity chromatography from calf and rabbit thymus.

SRP: Recombinant SRP protein (54 kDa, signal recognition particle). The corresponding human cDNA was expressed with the baculovirus system in insect cells.

PL-7: Recombinant PL-7 protein (threonyl-tRNA synthetase). The corresponding human cDNA was expressed with the baculovirus system in insect cells.

PL-12: Recombinant PL-12 protein (alanyl-tRNA synthetase). The corresponding human cDNA was expressed with the baculovirus system in insect cells.

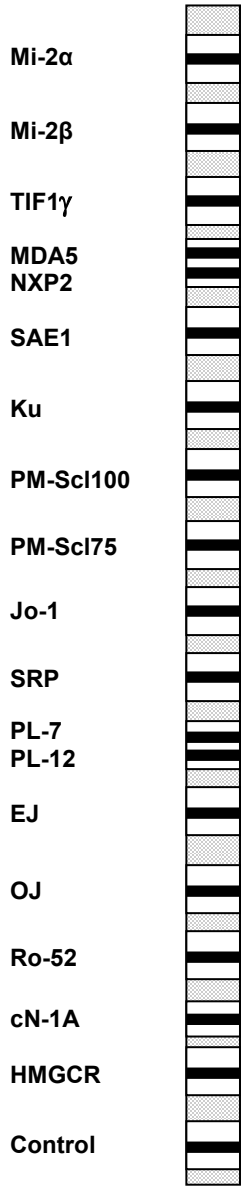
EJ: Recombinant EJ protein (glycyl-tRNA synthetase). The corresponding human cDNA was expressed in E. coli.

OJ: Recombinant OJ protein (isoleucyl-tRNA synthetase). The corresponding human cDNA was expressed in E. coli.

Ro-52: Recombinant Ro-52 (52 kDa). The corresponding human cDNA was expressed with the baculovirus system in insect cells.

cN-1A: Recombinant cN-1A protein. The corresponding human cDNA was expressed in E. coli.

HMGCR: Recombinant HMGCR protein. The corresponding human cDNA was expressed in E. coli



EUROIMMUN recommends interpreting results based on the signal intensity:

Signal **Visual evaluation**	**Signal intensity** **EUROLineScan** **Flatbed scanner**	**Result**	
No signal	0-5	o	Negative
Very weak band	6-10	(+)	Borderline
Medium to strong band	11-25 or 26-50	+, ++	Positive
Very strong band with an intensity comparable to the control band	>50	+++	Strong positive

Results in the **borderline range** (+) should be evaluated as increased but negative. The table above contains **values** for the evaluation using a flatbed scanner. The **values** for other instruments supported by EUROLineScan can be found in the EUROLineScan program. To do so mark the corresponding assay in the test list (main menu "Help"→"Test") and click on details and select **the corresponding instrument** in **"image source"**.

Parallel to the determination of autoantibodies against nucleic antigens with the EUROLINE, an indirect immunofluorescence test should always be performed – on the one hand to check the plausibility of e.g. false positive results, on the other hand to detect a wider spectrum of antibodies with the immunofluorescence.

For the antigens listed in the table below, please note:
Only those results for which there is a plausible correspondence between the screening test (indirect immunofluorescence with the substrate combination HEp-2 cells, liver/monkey, order no.: FA 1510) and the confirmatory test should be evaluated.
Antibodies against cytoplasmic antigens (Jo-1, SRP, PL-7, PL-12, EJ, OJ and Ro-52) are sometimes not clearly detectable with the IIFT. Parallel testing with screening and confirmatory test is recommended.

Results of the screening test indirect immunofluorescence with the substrate combination HEp-2 cells/liver	**Characteristics EUROLINE Autoimmune Inflammatory Myopathies 16 Ag et cN-1A et HMGCR**	**Result**
ANA positive: Fine granular fluorescence of cell nuclei, some nucleoli not visible.	Antigen band Mi-2α and/or Mi-2β	**Anti-Mi-2 positive**
ANA positive "Type Ku": Fine granular fluorescence in the cell nuclei, nucleoli partly positive. Primate liver: Fine granular, partly reticular fluorescence in the cell nuclei.	Antigen band Ku	**Anti-Ku positive**
ANA positive, nucleolar pattern: Homogeneous fluorescence of the nucleoli, at the same time a weaker, fine granular reaction in the nucleoplasm. Chromosomes of mitotic cells excluded, fine granular fluorescence outside of the chromosomes.	Antigen band PM-Scl100 and/or PM-Scl75	**Anti-PM-Scl positive**
Fine granular to homogeneous cytoplasmic fluorescence. The cell nuclei often show a distinct reaction (fine, defined dots).	Antigen band Jo-1	**Anti-Jo-1 positive**
Mainly granular fluorescence in the **cytoplasm**	Antigen band SRP	**Anti-SRP positive**
Frequently fine granular to homogeneous fluorescence in the **cytoplasm**.	Antigen band PL-7	**Anti-PL-7 positive**
	Antigen band PL-12	**Anti-PL-12 positive**
	Antigen band EJ	**Anti-EJ positive**
	Antigen band OJ	**Anti-OJ positive**
ANA positive or negative:	Antigen band Ro-52	**Anti-Ro-52 positive**

Isolated antibody reactions with Ro-52 should not be evaluated as anti-SS-A positive or specific for SLE or Sjögren's syndrome, since they can occur in many different autoimmune diseases.

For the medical diagnosis, the clinical symptoms of the patient and, if available, further findings should always be taken into account alongside the serological result. A negative serological result does not exclude the presence of a disease.

Test characteristics

Measurement range: The EUROLINE is a qualitative method. No measurement range is provided.

Cross-reactions: The high analytical specificity of the test system is guaranteed by the quality of the antigen substrates used (antigens and antigen sources). This EUROLINE specifically detects IgG class antibodies to Mi-2α, Mi-2β, TIF1γ, MDA5, NXP2, SAE1, Ku, PM-Scl100, PM-Scl75, Jo-1, SRP, PL-7, PL-12, EJ, OJ, Ro-52, cN-1A and HMGCR. No cross-reactions with other autoantibodies have been found.

Interference: Haemolytic, lipaemic and icteric sera up to concentrations of 5 mg/ml haemoglobin, 20 mg/ml triglycerides and 0.4 mg/ml bilirubin showed no effect on the analytical results of the present EUROLINE.

Inter- and intra-assay variation: The inter-assay variation was determined by multiple analyses of characteristic samples in several test runs over several days. The intra-assay variation was determined by multiple analyses of characteristic samples in one test run. In every case, the intensity of the bands was within the specified range. This EUROLINE displays excellent inter- and intra-assay reproducibility.

Clinical prevalences and specificity: In a study performed at the University of Uppsala, Sweden, 153 sera from patients with clinically characterised myositis (50 patients with dermatomyositis, 89 patients with polymyositis, 4 patients with juvenile dermatomyositis and 10 patients with inclusion body myositis) as well as 77 sera from control patients (26 patients with Sjögren's syndrome, 26 patients with SLE and 25 patients with systemic sclerosis) were tested for antibodies against Mi-2β, Ku, PM-Scl100, Jo-1, PL-7 and PL-12. The prevalence values ranged between 3% and 12%, with a specificity for myositis of 97% to 100%. The total detection rate for antibodies against Mi-2β, Ku, PM-Scl100, Jo-1, PL-7 and PL-12 in the myositis panel was 26%.

Anti-	Prevalence	Specificity
Mi-2β	3%	100%
Ku	3%	97%
PM-Scl100	7%	100%
Jo-1	12%	100%
PL-7	2%	100%
PL-12	0%	100%

A further study carried out at the University of Padua, Italy, showed similar results. In the investigation of 208 sera from patients with clinically characterised myositis and 214 sera from control patients (50 healthy persons, 13 patients with non-autoimmune myopathy, 23 sera from patients with CTD-associated myopathy, 65 patients with SLE, 34 patients with systemic sclerosis, 21 patients with primary Sjögren's syndrome, 8 patients with arthropathies) prevalence values of 4% to 21% were obtained, with a specificity for myositis of 95% to 100%. The total detection rate for antibodies against Mi-2β, Ku, PM-Scl100, Jo-1, PL-7 and PL-12 in the myositis panel was 37%.

Anti-	Prevalence	Specificity
Mi-2β	4%	98%
Ku	5%	95%
PM-Scl100	4%	100%
Jo-1	21%	100%
PL-7 or PL-12	4%	100%

In another study 194 patients with SLE, 131 patients with systemic sclerosis, 179 patients with polymyositis/dermatomyositis (PM/DM) and 50 patients with rheumatoid arthritis (RA) were examined for antibodies against SRP, EJ, OJ and PM-Scl75. The prevalence of antibodies against SRP was 4%, at a specificity for myositis of 99%. The prevalence values for antibodies against EJ, OJ and PM-Scl75 in the myositis and systemic sclerosis panel ranged between 1% and 6%, with a specificity for these diseases of 98% to 100%.

Anti-	Prevalence	Specificity
SRP	4%	99%
EJ	1%	100%
OJ	1%	100%
PM-Scl75	6%	98%

Sera from 264 patients with clinically characterised myositis and 120 healthy blood donors were tested for antibodies against Mi-2α, MDA5, NXP2, SAE1 and HMGCR. The prevalences ranged from 2 to 8%, with a specificity for myositis from 96 to 100%. The overall prevalence for antibodies against Mi-2α, MDA5, NXP2, SAE1 and HMGCR in the panel of myositis patients was 22% (57/264).

Anti-	Prevalence	Specificity
Mi-2α	7%	100%
MDA5	2%	100%
NXP2	2%	100%
SAE1	4%	100%
HMGCR	8%	96%

In a further study 10 anti-TIF1γ positive sera, which had been precharacterised using immunoprecipitation, and 120 sera from control persons were tested for antibodies against the TIF1γ antigen. The sensitivity was 100% (10/10) in comparison with the reference test. All samples from the 120 control persons were negative.

In the framework of a further study, sera of 197 patients with inclusion body myositis (IBM), 175 patients with different collagenoses (PM/DM, mixed collagenoses, undifferentiated collagenosis, systemic sclerosis, SLE, primary Sjögren's syndrome) and 161 healthy blood donors were investigated for antibodies against cN-1A. There were prevalences of 35% in IBM and 11% in the collagenoses panel.

Disease	Prevalence of antibodies against cN-1A	
	Serum samples	Anti-cN-1A positive (%)
Inclusion body myositis	197	35
PM/DM	52	8
Mixed collagenoses	3	0
Undifferentiated collagenosis	18	11
Systemic sclerosis	27	11
SLE/primary Sjögren's syndrome	75	13
Healthy blood donors	161	2

Antibodies against Ro-52: Sera from 591 patients with rheumatic autoimmune diseases, from 260 patients with autoimmune and infectious liver diseases and from 50 healthy blood donors were tested for antibodies against Ro-52 using EUROLINE. Antibodies against Ro-52 are not associated with a specific disease, but they can be found in both autoimmune and infectious diseases with a prevalence of 5% to 81%.

Disease	Prevalence of antibodies against Ro-52	
	Serum samples	Anti-Ro-52 positive (%)
Sjögren´s syndrome	88	81
Systemic sclerosis	81	28
Myositis	26	31
SLE	210	38
MCTD	21	19
Rheumatoid arthritis	165	5
Primary biliary cholangitis	100	27
Autoimmune hepatitis	60	35
Hepatitis B	50	10
Hepatitis C	50	22
Healthy blood donors	50	0

Clinical significance

Myositis is an inflammatory disease of skeletal muscles, which can be either hereditary or triggered by infections, toxins or immune system disorders.

The genetically caused form of myositis, fibrodysplasia ossificans progressiva (FOP), also known as fibrodysplasia ossificans multiplex progressiva, myositis ossificans progressiva or Münchmeyer's disease, is a progressive ossification of the connective and supporting tissues of the human body.

Infectious myositides can be divided into bacterial, viral, parasitic and fungal myositides. The bacterial diseases are divided into suppurative and non-suppurative forms. Suppurative myositides mainly occur acutely, are limited to single or a few neighbouring muscles, partly in the form of abscesses, and affect mostly previously healthy muscle. Histologically, the main feature is a pronounced inflammatory infiltrate. Viral myositides can proceed very blandly or, like HIV myositis, resemble polymyositis. They are caused by some types of enterovirus. Coxsackievirus type B causes inflammation of the chest and abdominal musculature, as well as a dry pleura inflammation. The disease is contagious; it can be transmitted from person to person (predominantly by smear infection). A high frequency of disease cases is observed in the summer months. Parasitic myositides mostly present in the form of toxoplasmosis, cysticercosis, echinococcosis or trichiniasis. They are rare in central Europe. They lead to focal inflammation, often with calcification.

The mechanisms of drug and toxic myopathies are very different. For example, zidovudine causes mitochondrial changes, interferon-alpha and D-penicillamine cause inflammatory myopathies, and cyclosporin causes vacuole formation and necrosis.

The autoimmunogenic myositides (idiopathic inflammatory myopathies) are systemic autoimmune diseases with inflammation of the skeletal musculature, symmetric and proximal accentuated pain and muscle weakness. They occur with an incidence of 0.1 to 1 per 100,000 per year, a prevalence of 1 to 6 per 100,000 and ratio of men to women of 1 to 2. They can be divided into polymyositis in adults (around 30%), dermatomyositis in adults (around 30%), paraneoplastic polymyositis of the lungs, ovaries, mammary glands, gastrointestinal tract and in myeloproliferative diseases (around 8%), infantile myositis/dermatomyositis with accompanying vasculitis (around 7%), as well as myositides in association with autoimmune diseases such as rheumatoid arthritis, lupus erythematosus, mixed connective tissue disease (MCTD) and rare forms such as granulomatosis, eosinophile, focal and inclusion body myositis (around 20%). It should be noted that dermato-/polymyositis is often of paraneoplastic origin, particularly in elderly patients. Dermatomyositis symptoms can occur before the tumour is even diagnostically detectable.

Histologically, myositides can be differentiated into purely interstitial myositides without fibre destruction, focal myositides with defined infiltrates and fibre lesions, and diffuse myositides. Clinically, the disease sometimes occurs acutely, affecting many muscles, with accentuated, inflammatory infiltration (especially in dermatomyositis). Other times it occurs as a particularly creeping disease, in which sometimes no infiltrates are identifiable and muscle fibre atrophy and muscle destruction are in the foreground of the histopathological picture. This is especially the case in inclusion body myositis and chronic pseudomyopathic granulomatosis myositis.

Polymyositis (PM) is a systemic inflammatory disease of the skeletal muscles of unknown etiology with perivascular lymphocytic infiltration. When the skin is involved, the disease is known as dermatomyositis (DM). Clinical symptoms of polymyositis are recurring bouts of fever, muscle weakness, arthralgia, possibly Raynaud's syndrome, trouble with swallowing and involvement of the inner organs. In dermatomyositis, skin symptoms appear as purple-coloured exanthema on the eye lids, nose bridge and cheeks, periorbital oedema, local erythema and scaly eczema dermatitis.

Findings in laboratory diagnostics include increased muscle enzyme values and unspecific signs of inflammation, such as increased CRP titer, fever, and acceleration of ESR. The detection of myositis-associated autoantibodies is of decisive importance for the diagnosis of dermatomyositis, as well as for assessment of the disease and treatment course. Although the mortality rate is increased by a factor of four (most frequent causes of death are heart and lung diseases), half of patients recover fully, although a slight weakness of the muscles may remain. In 30% of cases the disease can be stopped. 20% of patients experience deterioration despite therapeutic measures.

Autoantibodies of immunoglobulin class IgG against the 18 most important and most relevant of the currently known myositis-specific and myositis-associated DM/PM and overlap antigens can be detected in serum or plasma. These comprise Mi-2 (isoforms Mi-2α and Mi-2β), TIF1g, MDA5, NXP2, SAE1, Ku, proteins of the nucleolar PM-Scl macromolecular complex (PM-Scl100 and PM-Scl75), Jo-1, SRP, PL-7, PL-12, EJ, OJ, Ro-52, cN-1A and HMGCR.

Mi-2 (helicase protein part of NuRD complex)
Autoantibodies against Mi-2 (against nuclear helicase) with isoforms Mi-2α (CHD3) and Mi-2β (CHD4) have a high specificity of around 95% for myositides, especially for DM with hypertrophia of the nail folds. These antibodies can be found in 15% to 30% of patients with DM. Antibodies against Mi-2 can also be detected in 8% to 12% of patients with idiopathic myositis. Some patients with anti-Mi-2 antibodies have polymyositis and, in rare cases, also inclusion body myositis. Anti-Mi-2 antibodies can usually be serologically detected in the early stage of disease. In these cases the DM (including in adolescents) often has a favourable course. However, Mi-2-positive (predominantly Mi-2β-positive) DM may also be associated with neoplasia (e.g. colon or breast carcinoma).

Mi-2α (CHD3, chromodomain-helicase-DNA-binding protein 3)
Autoantibodies against Mi-2α, one of two isoforms of Mi-2, have largely the same serodiagnostic significance as autoantibodies against Mi-2, with a prevalence in DM of around 20%.

Mi-2β (CHD4, chromodomain-helicase-DNA-binding protein 4, ATP-dependent helicase CHD4)
Autoantibodies against Mi-2β (main isoform of Mi-2) are serologically detected more frequently in DM associated with neoplasia (e.g. colon or breast carcinoma).

TIF1g (transcriptional intermediary factor 1-gamma)
TIF1-gamma autoantibodies are detected in around 15% of DM patients and only in these persons. Hence, the detection of anti-TIF1-gamma antibodies is definitive for DM. In around 58% of anti-TIF1-gamma-positive patients, DM is associated with a malignant disease (e.g. pancreatic carcinoma).

MDA5 (melanoma differentiation-associated gene 5), synonym IFIH1 (interferon induced with helicase C domain 1)
Autoantibodies against MDA5 are detected in 13% to 26% of DM patients. They are highly specific for clinically amyopathic DM (95% of these patients are anti-MDA5-positive) or DM combined with interstitial lung disease.

NXP2 (MJ-p140-MU 140 kD protein, synonym MORC3, microrchidia family CW-type zinc-finger 3)
Autoantibodies against NXP2 are detected in 18% to 25% of cases of juvenile PM/DM (JDM) and in only around 1% of adult cases. This form of PM/DM is characterised by accompanying calcinosis and particularly severe and chronic disease courses. In adults the disease may be carcinoma-associated (breast, uterine or pancreatic carcinoma).

SAE (SAE1 / SAE2) - SUMO activating enzyme subunits 1 (40 kDa) and 2 (90 kDa)
Anti-SAE1 antibodies are highly specific markers for DM in 8% of cases and for adult DM associated with interstitial lung diseases (ILD) in 5% of cases.

Ku Autoantibodies against Ku (DNA-binding, non-histone protein) were originally described in poly-myositis-systemic sclerosis overlap syndrome. Since then, anti-Ku antibodies have been detected in other autoimmune diseases with varying frequencies (also depending on ethnic origin). They occur with a prevalence of up to 10% in systemic lupus erythematosus (SLE), a systemic autoimmune disease belonging to the group of collagenoses, which predominantly manifests with the so-called butterfly rash. 40% of patients with antibodies against Ku show symptoms of myositis or systemic sclerosis (SSc), a chronic autoimmune disease with fibrosis of the skin (scleroderma) on the joints and of inner organs such as the oesophagus, lungs, heart and kidneys. Autoantibodies against Ku can also occur in Sjögren's syndrome.

PM-Scl100/PM-Scl75 (antigens that are localised as exoribonucleases in the granular part of the nucleoli and in the nucleoplasm; proteins of the nucleolar PM-Scl macromolecular complex, PM-1)
Autoantibodies against the two main antigen-protein components PM-Scl100 and PM-Scl75 are classified by molecular masses. Anti-PM-Scl antibodies (antibodies against PM-Scl75 and PM-Scl100) are detected in 50% to 70% of patients with a so-called overlap syndrome. This combines the symptoms of polymyositis, dermatomyositis and systemic sclerosis (SSc). Patients with SSc exhibit mainly antibodies against PM-Scl75. Antibodies against PM-Scl75 can be detected in 3% of polymyositis cases, in 2% to 3% of patients with systemic sclerosis (SSc) and in 24% to 50% of patients with overlap syndrome. With tests that detect only anti-PM-Scl-100, the majority of SSc patients will be missed. There is no correlation between the antibody concentration and the disease activity. Due to the strong association between anti-PM-Scl antibodies and HLA class II alleles, these autoantibodies are detected exclusively in patients of Caucasian origin.

Jo-1 (histidyl-tRNA synthetase)
Autoantibodies against Jo-1 are found in polymyositis with a prevalence of 25% to 55% and a specificity of almost 100%.

SRP (signal recognition particle, ribonucleoprotein complex)
Autoantibodies against **SRP** can be detected in around 5% of cases of polymyositis (at a specificity of around 90%). Anti-SRP antibodies are a marker for necrotising myopathy (anti-SRP syndrome). The symptoms are acute, severe, proximal, symmetrical skeletal muscle weakness, and pain in muscles, including the heart muscle. Extramuscular signs of the disease can be interstitial lung diseases.

PL-7 (threonyl-tRNA synthetase)
Autoantibodies against PL-7 occur with a prevalence of around 3% to up to 6% in patients with myositis.

PL-12 (alanyl-tRNA synthetase)
Autoantibodies against PL-12 are detected with a prevalence of up to 3% in myositis patients.

EJ (glycyl-tRNA synthetase)
Autoantibodies against EJ are a diagnostic marker for polymyositis, occurring with a prevalence of 1% to 3%.

OJ (isoleucyl-tRNA synthetase)
Autoantibodies against OJ are associated with (poly)myositis (prevalence 3%).

Ro-52 Antibodies against Ro-52 are detected in myositis patients with a prevalence of 25%. Anti-Ro-52 also occurs in some rheumatic and non-rheumatic diseases. Anti-Ro-52 autoantibodies appear to play an important role in neonatal lupus and congenital heart block. In this case, certain epitopes are probably associated with the complications during pregnancy.

cN-1A (Mup44, cN1A, NT5C1A, NT5c1A, NT5C1a, sporadic inclusion body myositis autoantigen, 44-kDa-IBM autoantigen)
The detection of anti-cN-1A antibodies enables diagnosis of the rare inclusion body myositis (IBM), a degenerative autoimmune disease of the muscles. It is delimited from the sporadically occurring, hereditary, non-inflammatory form of inclusion body myositis. Anti-cN-1A antibodies destroy structures of the muscle cells/fibers and cause inflammatory reations with infiltration by cytotoxic T-cells. The complex pathogenesis encompasses also degenerative mechanisms. IBM is the most common chronic-inflammatory myopathy in older patients. It leads to muscle weakness and muscle atrophy of muscles near and distant of the trunk. Anti-cN-1A-positive IBM patients show especially severe courses of this autoimmune disease and increased motor impairment including the eye, facial and respiratory muscles. Prevalence of anti-cN-1A antibodies for IBM amount to 33% and up to 76%, for polymyositis 0% to 14%, for dermatomyositis 0% to 21%, for Sjögren syndrome 0% to 23% and for systemic lupus erythematosus 0% to 14% depending on the study. While autoantibodies against cN-1A for IBM are considered diagnostic markers, they are clinically irrelevant for other diseases. Also in 5% of healthy persons, anti-cN-1A antibodies are found.

HMGCR (3-hydroxy-3-methylglutaryl coenzyme A reductase, 100 kDa and 200 kDa dimers)
HMGCR catalyses the conversion of HMG-CoA into mevalonic acid, which is an important step of cholesterol biosynthesis. The expression of HMGCR is upregulated by statins.
A small number of patients who take statins develop necrotising myositis (NM) with anti-HMGCR antibodies. Anti-HMGCR antibodies occur in up to 75% of these patients. The titer correlates with the clinical activity of NM. NM is also associated with antibodies against SRP. NM with anti-HMGCR antibodies may also occur without the intake of statins.
Anti-HMGCR antibodies are detected in 6 to 7% of cases of idiopathic inflammatory myopathies. They are specific for myositis.
Patients with anti-HMGCR myopathy present with muscle weakness and high creatine kinase levels. Severe necrosis of the muscle fibres with only a few or no inflammatory infiltrates is also characteristic of the disease.
Anti-HMGCR antibodies, muscle weakness and high creatine kinase levels remain if the statin therapy is discontinued. Patients respond well to immunosuppressive drugs and relapses are frequent, if the therapy is stopped. Young adults with anti-HMGCR myopathy show a more severe course than older patients when taking statins and a poorer diagnosis in immunosuppressive therapy. Children with anti-HMGCR myopathy respond well to immunosuppressive medication. Independent of statin treatment, anti-HMGCR antibody-positive NM is associated with a slightly increased risk of cancer.

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