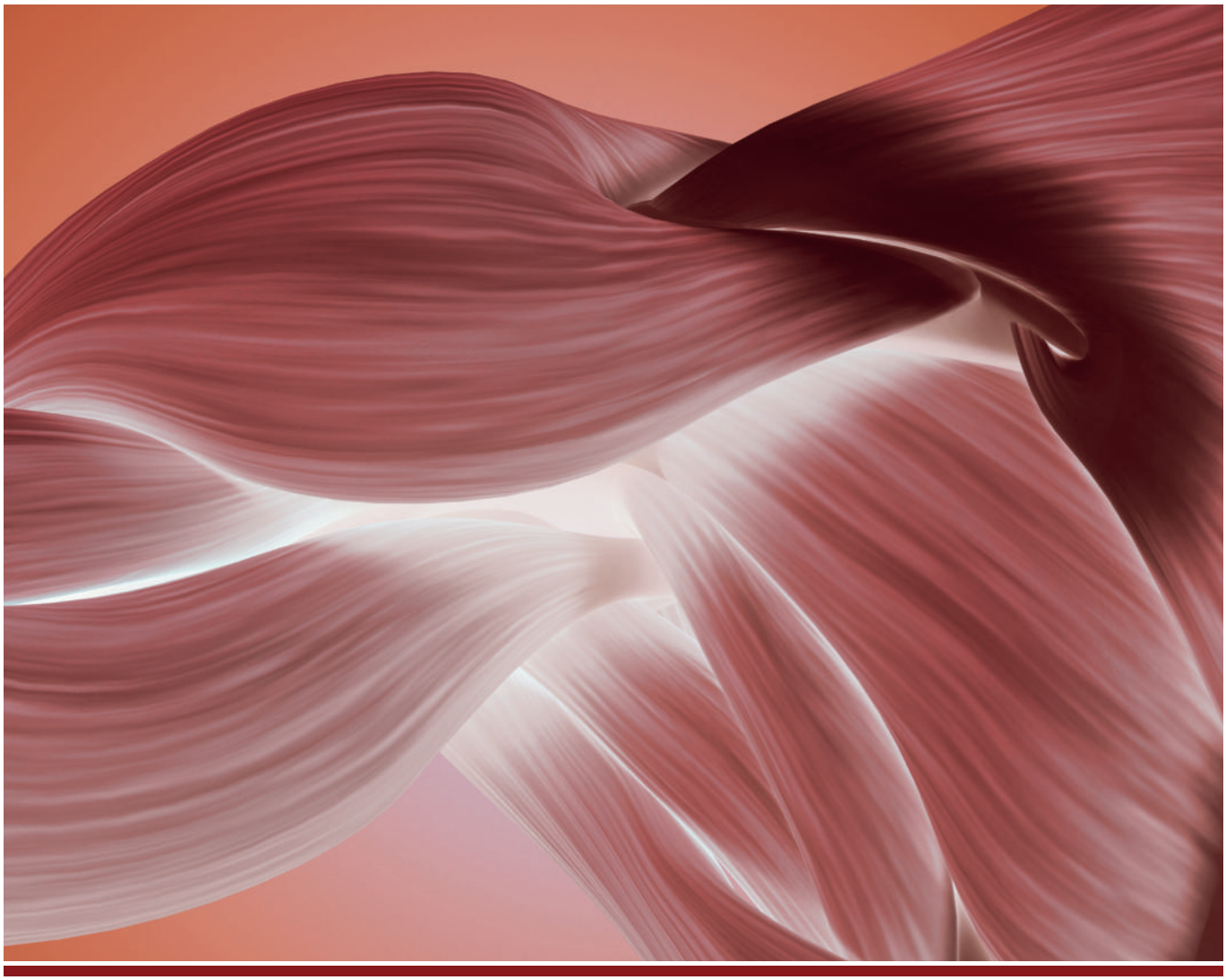
EUROIMMUN Medizinische Labordiagnostika AG

Comprehensive myositis diagnostics

Serological markers and exclusive test systems for laboratory analytics



- **IIFT mosaics – ANA gold standard for autoimmune diagnostics**
- **EUROLINE myositis profiles – monospecific detection of up to 20 myositis-relevant autoantibodies on one blot strip**
- **Exclusive* antigen for detection of anti-cN-1A autoantibodies – the only marker for serological diagnostics of inclusion body myositis (IBM)**

Myositis syndromes

Idiopathic inflammatory myopathies (IIM, also called myositides) are a group of heterogeneous autoimmune diseases of the skeletal musculature. The clinical picture is characterised by muscle weakness and pain which progressively develop into movement limitation. Other organs such as the skin, lung and heart are also often affected.[1] Around 13% of IIM cases are associated with a malignant tumour.[2] IIM are divided into six subgroups based on clinical, histological and immunopathological criteria: **polymyositis (PM)**, **dermatomyositis (DM)**, **inclusion body myositis (IBM)**, **necrotising myositis (NM)**, **anti-synthetase syndrome (ASS)** and **overlap myositis (OM)** according to the current German guidelines.[1]

PM shows the characteristics typical of most IIM. Symmetric and proximal pareses occur. Extramuscular manifestations can include myocarditis and life-threatening interstitial lung disease (ILD). PM is the rarest subgroup and makes up only approximately 5% of myositis cases. **DM** occurs much more frequently and accounts for around 31% of cases. It typically involves the skin, although polyarthritis or ILD can also occur. Different autoantibodies are associated with DM, some of which carry a high risk for neoplasias. **NM** can be distinguished from other IIM mainly morphologically, e.g. because of diffusely distributed muscle fibre necrosis. It accounts for approximately 19% of IIM cases. Nearly 70% of cases show typical autoantibodies. **IBM** is differentiated from other IIM by the presence of an asymmetric weakness of the proximal and distal muscles. Dysphagia is a typical symptom and also often the initial one. Mild extramuscular heart manifestations or neuropathies also rarely occur. IBM prognosis is often unfavourable. In most cases, it is therapy-refractory and takes a chronic course. **ASS** is characterised by autoantibodies against aminoacyl-t-RNA synthetases. It typically includes extramuscular manifestations such as ILD, arthritis and myocarditis, which can also occur without or at different times than muscular symptoms. Raynaud's syndrome, "mechanic's hands" and dysphagia may also occur. The ASS phenotype can vary depending on the autoantibodies. The heterogenous group of **OM** is the most common. It includes the clinical symptoms of both myositis (often ASS or DM) and another autoimmune disease, typically systemic sclerosis (SSc), systemic lupus erythematosus (SLE), Sjögren's syndrome (SjS) and rheumatoid arthritis (RA).[1]

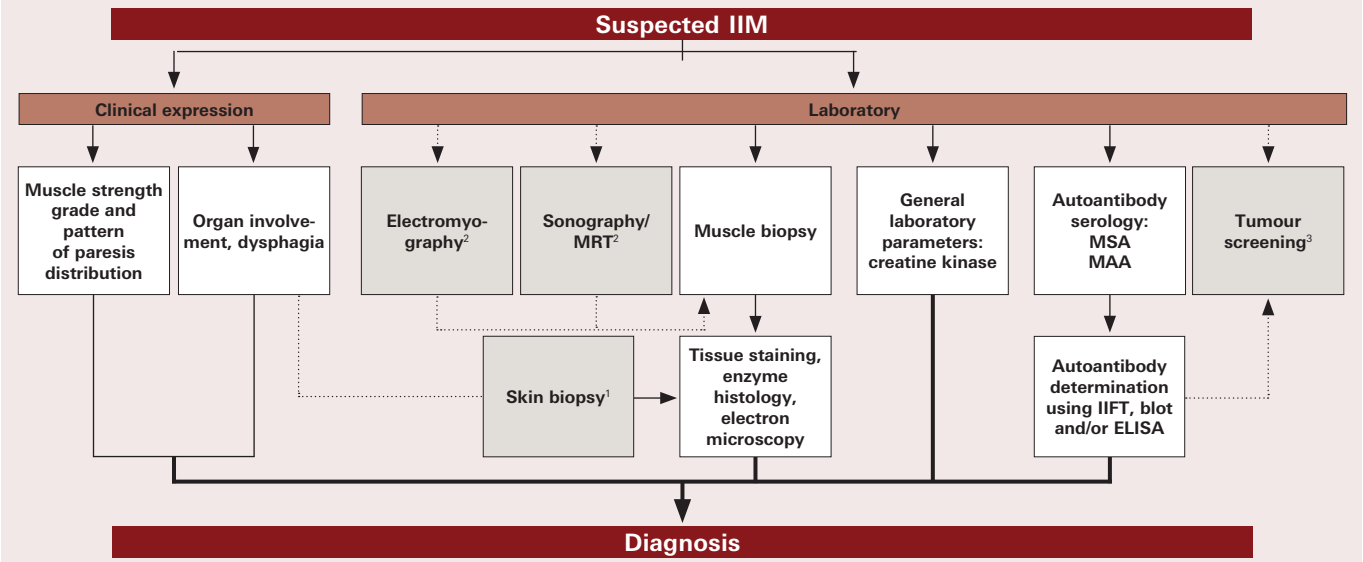
Myositis autoantibodies

Different autoantibodies have been identified in connection with IIM. They are differentiated into **myositis-specific antibodies (MSA)** and **myositis-associated antibodies (MAA)**.[1-3] MSA are rare and can only be detected in some IIM patients. They often appear in isolation and can be a strong indication for the presence of a certain subgroup of myositis. Some autoantibodies are associated with an increased risk for neoplasias or ILD. MAA also occur in other autoimmune diseases that may overlap with IIM and are detected in up to 50% of myositis patients.[4]

Myositis-specific antibodies				
Antibody	**Target antigen and/or mechanism**	**Associated IIM subgroups**	**Prevalence in myositis (%)[1]**	**Associated risks[1,3]**
Anti-Mi-2α	Nuclear transcription	DM	5–10	Only very rarely malignant tumours
Anti-Mi-2β	Nuclear transcription	DM		
Anti-SAE1	Post-translational modification	DM	5–10	Rarely malignant tumours
Anti-NXP2	Nuclear transcription and RNA metabolism	DM	< 5	Malignant tumours
Anti-MDA5	Melanoma differentiation antigen 5	DM	5	Rapidly progressive ILD
Anti-TIF1γ	Nuclear transcription and cellular differentiation	DM	20	Malignant tumours
Anti-SRP	Intracytoplasmic protein translocation	NM	5	-
Anti-HMGCR	3-hydroxy-3-methylglutaryl-CoA reductase	NM	6	-
Anti-Jo-1	Histidyl-tRNA synthetase	ASS	15–20	ILD
Anti-PL-7	Threonyl-tRNA synthetase	ASS	< 5	ILD
Anti-PL-12	Alanyl-tRNA synthetase	ASS	< 5	ILD
Anti-EJ	Glycyl-tRNA synthetase	ASS	5-10	ILD
Anti-OJ	Isoleucyt-tRNA synthetase	ASS	< 5	ILD
Anti-Ha	Tyrosyl-tRNA synthetase	ASS	< 1	ILD
Anti-Ks	Asparaginyl-tRNA synthetase	ASS	< 5	ILD
Anti-Zo	Phenylalanyl-tRNA synthetase	ASS	< 1	ILD
Anti-cN-1A	Cytosolic 5'-nucleotidase 1A	IBM	30 in IBM	-
Myositis-associated antibodies				
Antibody	**Target antigen and/or mechanism**	**Associated IIM subforms**	**Prevalence in myositis (%)[1]**	**Autoimmune disease[1-4]**
Anti-Ku	70–80 kDa catalytic subunit with DNA-PK	OM	20–30	SSc, SLE and other connective tissue diseases
Anti-PM-Scl75	Topoisomerase I, Exoribonuclease in the nuclear complex	OM, DM	8-10	SSc
Anti-PM-Scl100				
Anti-Ro-52	Ribosomal protein translation	OM	10–30	Connective tissue diseases

Differential diagnostics of myositis syndromes

The final diagnosis of IIM is complex and takes several years on average. The current IIM classification criteria published in 2017 by the European League Against Rheumatism (EULAR)/American College of Rheumatology (ACR) are based predominantly on clinical and histopathological features.[5] The criteria identify IIM with high sensitivity but may not reliably identify the subform. In recent years the advancement of knowledge and methods to detect myositis-relevant autoantibodies has increased the relevance of these markers in IIM diagnostics. In Germany, new guidelines published in 2022 by the German Society for Neurology (DGN) now recommend the determination of MSA and MAA in addition to an in-depth clinical examination including the determination of muscle strength grades and patterns of paresis distribution, a morphological analysis of a muscle biopsy and the measurement of general laboratory parameters.[1] For differential diagnostics, all examination results should be considered in parallel and given equal priority.



[1] recommended in case of skin involvement
[2] to support determination of a suitable biopsy site
[3] particularly recommended in the case of autoantibodies associated with malignant tumours

EUROIMMUN test systems for autoantibody determination

Indirect immunofluorescence test (IIFT)

The IIFT with human epithelial cells and primate liver is the gold standard for screening of antibodies against nuclear antigens (ANA) thanks to its high sensitivity and specificity. Many myositis autoantibodies show a characteristic fluorescence pattern. Some examples: Autoantibodies against PM-Scl show a homogeneous nucleolar fluorescence of the epithelial cells (Fig. 1A). Autoantibodies against Ku present as a nuclear, finely spotted fluorescence with partially spared nucleoli (Fig. 1B). Anti-Jo-1 autoantibodies show a pattern of cytoplasmic, tightly spotted fluorescence and often a distinct reaction of cell nuclei (Fig. 1C). A dense, finely spotted fluorescence of the cytoplasm is produced by, e.g., autoantibodies against PL-7 (Fig. 1D). Not all autoantibodies can be detected using IIFT. It is therefore useful to simultaneously perform a monospecific determination using line blot or ELISA.[6]

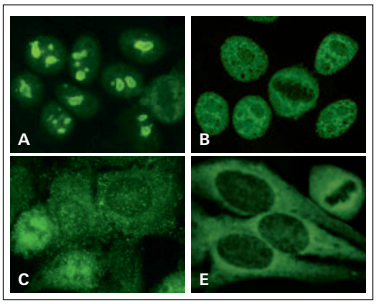


Fig. 1 IIFT: HEp-2 test results

EUROLINE profiles

EUROIMMUN offers line blots with antigen combinations for different clinical circumstances. These allow a more efficient and comprehensive analysis than sequential testing. The multiparameter tests cover both MSA and MAA and have particularly high diagnostic value. The inclusion of antigens for the determination of autoantibodies with low prevalences increases the serological detection rate.[7-9] The **EUROLINE Profile Autoimmune Inflammatory Myopathies 20 Ag (IgG)** (Fig. 2), for example, combines 20 target antigens in a single test strip including – the exclusive* – cN-1A. Autoantibodies against cN-1A are the first and only known serological marker for IBM.[6] The recent addition of Ha, Ks and Zo expands the tRNA synthetase antigen spectrum for ASS differential diagnostics.

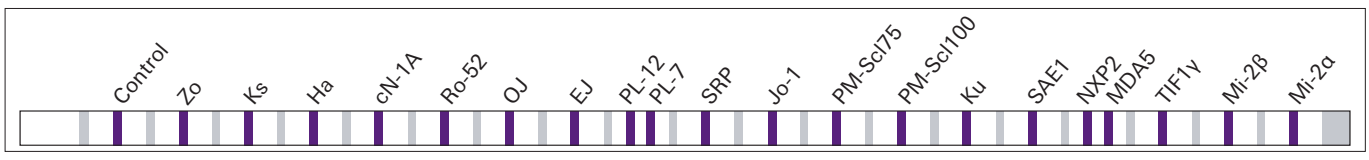


Fig. 2: EUROLINE Profile Autoimmune Inflammatory Myopathies 20 Ag (IgG)

ELISA

The Anti-Jo-1 ELISA (IgG) allows for semiquantitative or quantitative determination of anti-Jo-1, the most characterised and frequent MSA. Several studies also showed a correlation between the anti-Jo-1 autoantibody titer and ASS disease activity.[10,11] Anti-Jo-1 autoantibodies often occur together with anti-Ro-52 autoantibodies (58% of cases positive for anti-Ro-52). The combination is associated with severe disease courses.[1]

Autoantibodies against cN-1A are the only known biomarker for IBM.[6] This subform in particular has a high misdiagnosis rate and a diagnostic delay of 5–8 years.[12] The semiquantitative Anti-cN-1A ELISA (IgG) has a specificity of over 96% and a sensitivity of up to 39%.[13] High autoantibody titers can indicate a severe course of disease.[1]

Product selection

Test system	Test name	Antibodies against	Substrate	Order number
IIFT	IIFT: HEp-2	Cell nuclei (ANA)	HEp-2 cells (human)	FA 1520-####
	IIFT: HEp-20-10	Cell nuclei (ANA) + mitotic phases	HEp-20-10 cells (human)	FA 1522-####
	IIFT Mosaic: HEp-2/Liver (Monkey)	Cell nuclei (ANA global test)	HEp-2 cells (human) Liver (monkey)	FA 1510-####-1
	HEp-20-10/Liver (Monkey)	Cell nuclei (ANA global test) + mitotic phases	HEp-20-10 (human) Liver (monkey)	FA 1512-####-1
EUROLINE	EUROLINE Myositis Antigen Profile (IgG)	Mi-2β, Ku, PM-Scl, Jo-1, PL-7, PL-12, Ro-52	Antigen-coated test strips	DL 1530-#### G
	EUROLINE Myositis Antigen Profile 3 (IgG)	Mi-2β, Ku, PM-Scl100, PM-Scl75, Jo-1, SRP, PL-7, PL-12, EJ, OJ, Ro-52		DL 1530-####-3 G
	Autoimmune Inflammatory Myopathies 16 Ag (IgG)*	Mi-2α, Mi-2β, TIF1γ, MDA5, NXP2, SAE1, Ku, PM-Scl100, PM-Scl75, Jo-1, SRP, PL-7, PL-12, EJ, OJ, Ro-52		DL 1530-####-4 G
	Autoimmune Inflammatory Myopathies 16 Ag et cN-1A (IgG)*	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		DL 1530-####-7 G
	Autoimmune Inflammatory Myopathies 20 Ag (IgG)	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, Ha, Ks, Zo		DL 1530-####-9 G
ELISA	Anti-Jo-1 ELISA (IgG)	Jo-1	Antigen-coated microplate wells	EA 1661-9601 G
	Anti-cN-1A ELISA (IgG)*	cN-1A		EA 1675-4801 G

* Protected by patent applications EP2729810 and CN103688173

Regulatory status of the products must be verified for the user's individual jurisdiction.
Please contact your country representative for product availability and information.

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[1] Wiendl H, et al. **Myositissyndrome, S2k-Leitlinie.** Deutsche Gesellschaft für Neurologie, Leitlinien für Diagnostik und Therapie in der Neurologie (2022). [in German] [2] Lilleker JB, et al. **The EuroMyositis registry: an international collaborative tool to facilitate myositis research.** Ann Rheum Dis 77(1):30-39 (2018). [3] McHugh NJ, et al. **Autoantibodies in myositis.** Nat Rev Rheumatol 14(5):290-302 (2018). [4] Senécal JL, et al. **Editorial: A new classification of adult autoimmune myositis.** Arthritis Rheumatol 69(5):878-884 (2017). [5] Lundberg IE, et al. **European League Against Rheumatism/American College of Rheumatology classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups.** Ann Rheum Dis 76(12):1955-64 (2017). [6] Mende M, et al. **Autoantibodies in myositis. How to achieve a comprehensive strategy for serological testing.** Mediterr J Rheumatol 30(3):155-161 (2019). [7] Rönnelid J, et al. **Use of a commercial line blot assay as a screening test for autoantibodies in inflammatory myopathies.** Autoimmun Rev. 9(1):58-61 (2009). [8] Gunawardena H, et al. **Newly identified autoantibodies: relationship to idiopathic inflammatory myopathy subsets and pathogenesis.** Curr Opin Rheumatol 20(6):675-80 (2008). [9] Targoff IN. **Myositis specific autoantibodies.** Curr Rheumatol Rep 8(3):196-203 (2006). [10] Stone KB, et al. **Anti-Jo-1 antibody levels correlate with disease activity in idiopathic inflammatory myopathy.** Arthritis Rheum 56(9):3125-31 (2007). [11] Gomard-Mennesson E, et al. **Clinical significance of anti-histidyl-tRNA synthetase (Jo1) autoantibodies.** Ann N Y Acad Sci 1109:414-20 (2007). [12] Molberg Ø, et al. **Epidemiology of sporadic inclusion body myositis.** Curr Opin Rheumatol 28(6):657-60 (2016). [13] Kramp SL, et al. **Development and evaluation of a standardized ELISA for the determination of autoantibodies against cN-1A (Mup44, NT5C1A) in sporadic inclusion body myositis.** Auto Immun Highlights 7(1):16 (2016).

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