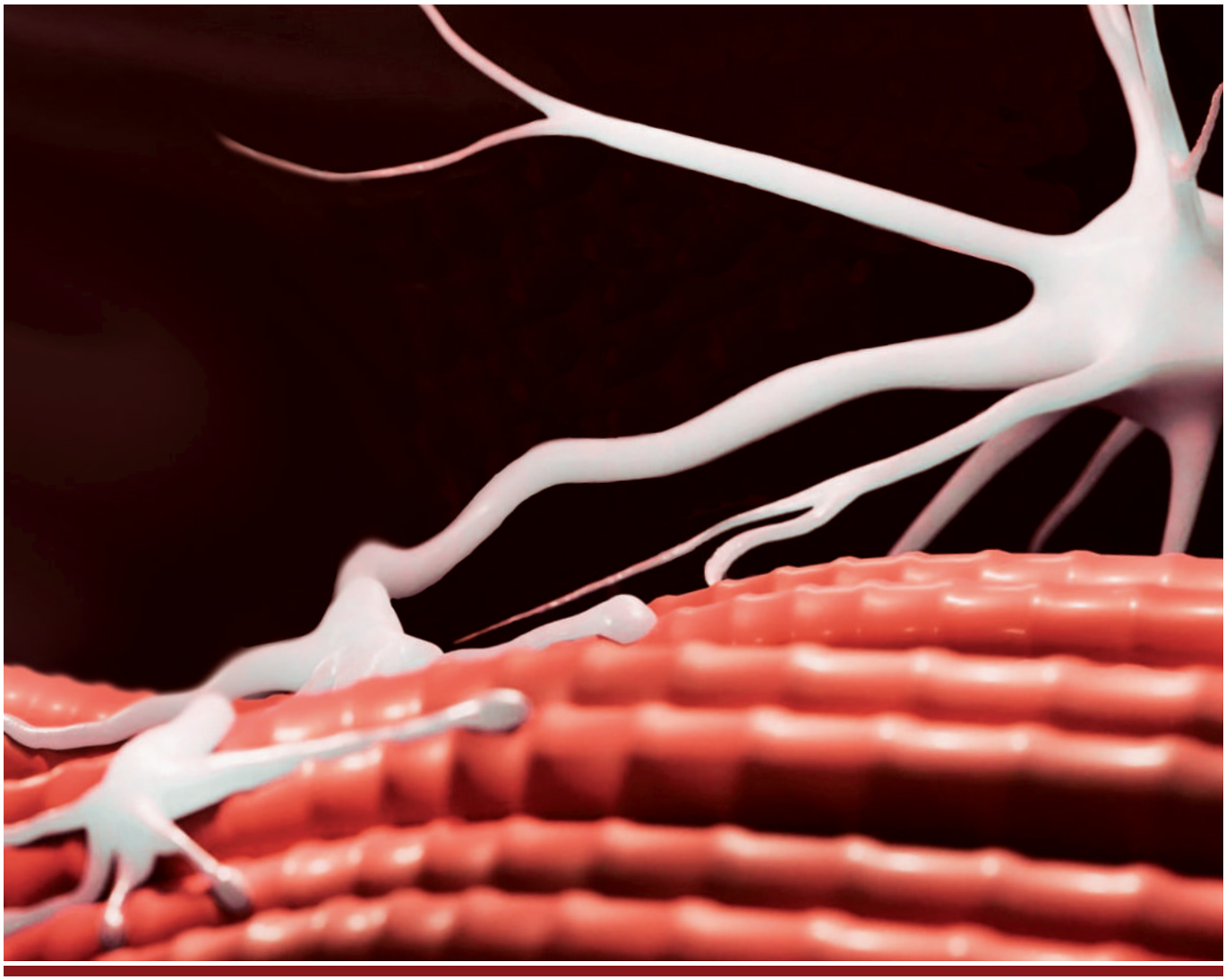
EUROIMMUN Medizinische Labordiagnostika AG

Myasthenia gravis

Innovative test systems for serological differential diagnostics

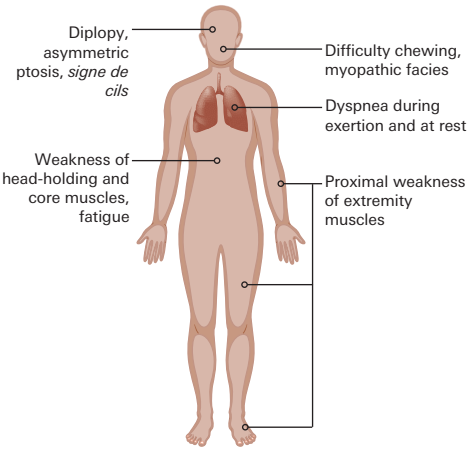


- **Exclusive:** Myasthenia Gravis Mosaic 2 IIFT — cell-based assay (CBA) with gold-standard potential for parallel analysis of autoantibodies against nicotinic acetylcholine receptors (AChR) and muscle-specific kinase (MuSK)
- Anti-Acetylcholine Receptor ELISA (IgG) — quantitative test for detection of autoantibodies against AChR
- Supplementary diagnostics: tests for detection of autoantibodies against skeletal muscle, titin and SOX1

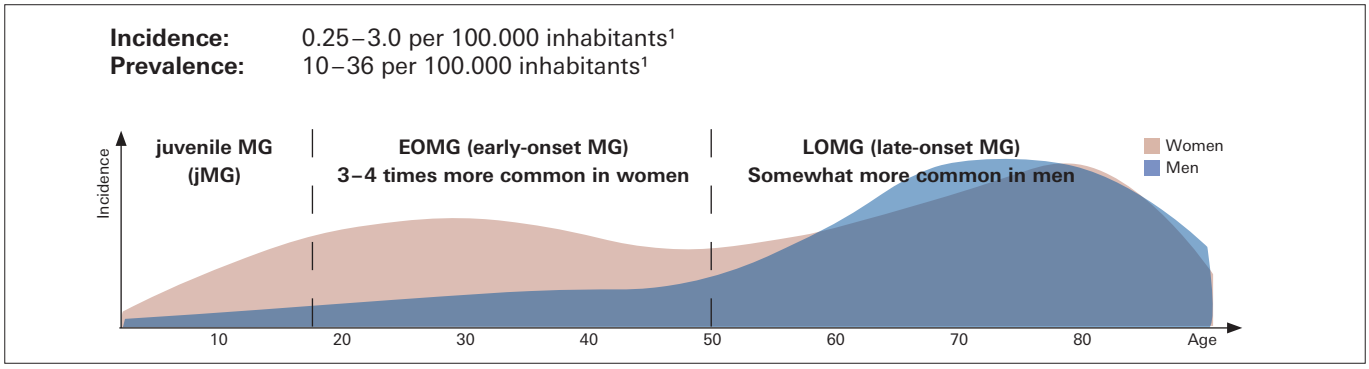
Myasthenia gravis

Myasthenia gravis (MG), along with Lambert-Eaton myasthenic syndrome, (LEMS), is an autoimmune-mediated myasthenic disorder. The main symptom of the disease is exertion-dependent muscle weakness which improves with rest. This is caused by disruption of neuromuscular transmission.

MG often begins with ocular symptoms such as diplopy and paralysis of the outer eye muscles and eyelid lifter muscles, leading to the typical asymmetrical ptosis. In 70–80% of patients, the disease generalises in the following two to three years. The muscle weakness is then rarely restricted to one muscle group, but can affect the entire musculoskeletal system as well as the breathing and chewing muscles (generalised MG). Pure ocular disease courses occur in only 10–15% of patients (ocular MG).[1]



Typical symptoms of MG

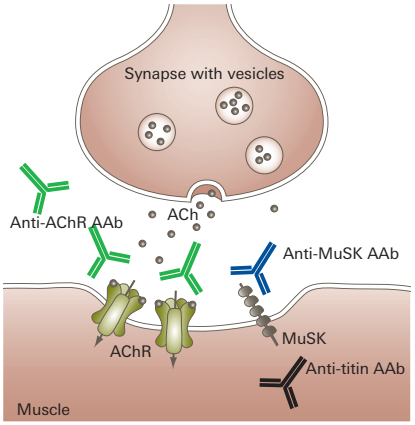


Schematic representation of age- and sex-specific incidence rates, modified from Carr et al., 2010[2]

MG-specific autoantibodies

The pathomechanism of MG is based on the loss of function of nicotinic acetycholine receptors (AChR) at the motor endplates due to attacks by different autoantibodies.[1,3] Autoantibodies against AChR are detected in around 80% of MG patients and autoantibodies against muscle-specific kinase (MuSK) in around 3% of MG patients. 15% of MG cases are seronegative and are assumed to be associated with pathognomonic autoantibodies that have not yet been identified.[1,3]

Around 15% of MG patients have a paraneoplastic syndrome due to thymoma, mostly together with anti-AChR autoantibodies. Half of these patients also exhibit autoantibodies against titin, a structural protein of skeletal muscle. 70% of anti-AChR-positive EOMG patients have thymitis, which is assumed to be the cause of the autoimmune reaction. LOMG patients on the other hand do not generally show inflammatory changes in the thymus, but in 50% of cases they exhibit anti-titin autoantibodies.[1]



MG-associated autoantibodies (AAb) and their target structures, modified from Huang et al., 2019[3]

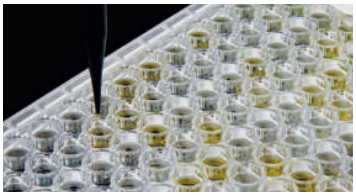
MG diagnostics

Diagnosis of MG is based on anamnesis and investigation of exertion-dependent muscle weakness, which is then confirmed by positive antibody detection or a positive result from electrophysiology or pharmacological testing.[1]

Autoantibody analysis plays an important role in therapy decision-making. Anti-AChR autoantibodies primarily belong to the IgG1 subclass and exert their pathological effect by activating the complement cascade. Anti-MuSK autoantibodies on the other hand belong to the non-complement-binding IgG4 subclass, so that therapeutic use of complement inhibitors is ineffective for this form. Selection of therapy should therefore take into account the antibody status together with the patient's age at disease onset, thymus pathology and disease activity.[1,4]

Classic test systems for MG autoantibody diagnostics

The radioimmunoassay (RIA) is currently the gold standard for detection of autoantibodies against AChR and MuSK. However, the need to work with radioactive reagents is a major disadvantage for laboratory diagnostics. A non-radioactive alternative for quantitative measurement of anti-AChR autoantibodies is the Anti-Acetylcholine Receptor ELISA (IgG). The microplate wells of the ELISA are coated with recombinant adult and foetal acetylcholine receptors (AChR-E und AChR-G, respectively). The ELISA demonstrates similar sensitivity and specificity to RIA but requires less time and effort to perform.[5]

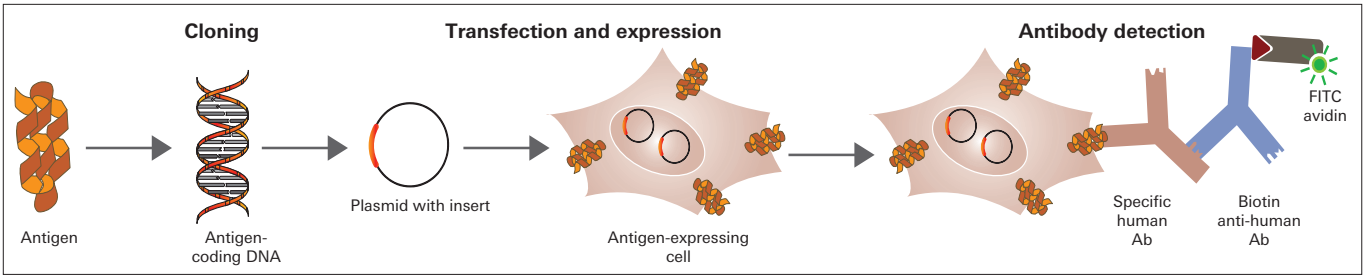


Anti-Acetylcholine Receptor ELISA (IgG)

New potential gold standard for MG autoantibody diagnostics

Monospecific antibody determination using transfected cells

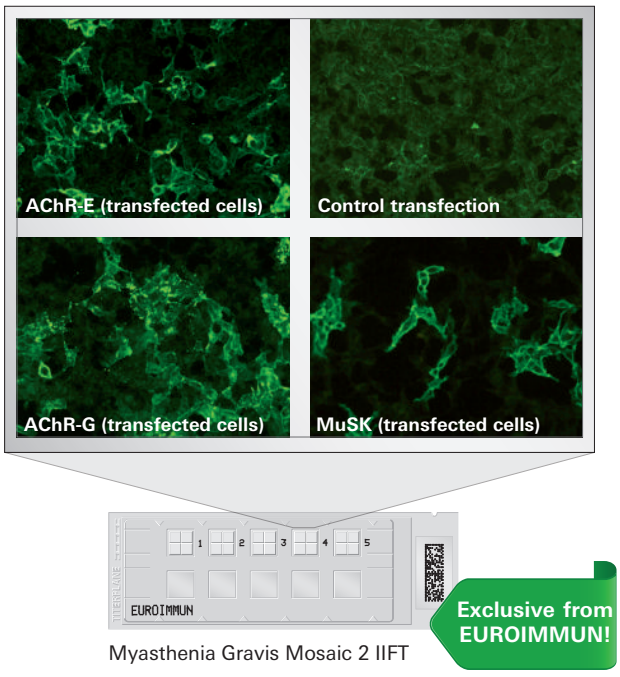
The cell-based assay (CBA) is an innovative technology for detection of autoantibodies by indirect immunofluorescence. In this method, DNA coding for the target antigen is inserted into a plasmid and introduced into vector cells (transfection). The transfected cells express the target antigen and are used directly as a substrate for monospecific detection of the corresponding autoantibodies in human serum or plasma samples. Compared to the tissue substrates traditionally used in indirect immunofluorescence assays (IFA), which contain many different antigens and often require a lot of experience in interpreting fluorescence patterns, the antigen-expressing cells are easier to evaluate.



Myasthenia Gravis Mosaic 2 IIFT

With the BIOCHIP Mosaic from EUROIMMUN, autoantibodies against AChR-E, AChR-G and MuSK can be detected using specifically transfected cells (EU 90). In a retrospective study on the detection of anti-AChR autoantibodies, the BIOCHIP Mosaic showed a specificity equivalent to that of RIA: 99.6% versus 100%. The study encompassed 618 patients with suspected MG. The CBA yielded a significantly higher sensitivity than the RIA (76.7% versus 72.7%) and detected anti-AChR autoantibodies in 21% of MG cases that were seronegative in RIA.[6] In a comparative study with an in-house CBA based on live cells, the BIOCHIP Mosaic yielded equivalent results. In 292 serum samples (192 MG patients, 100 control subjects) that were precharacterised using RIA, both tests showed a specificity of 100% for detection of anti-AChR and anti-MuSK autoantibodies. In the panel of autoantibody-positive sera, the Mosaic demonstrated a sensitivity of 98.5% for the detection of anti-AChR and 100% for the detection of anti-MuSK autoantibodies (in-house CBA: 100% for both). Moreover, the Mosaic detected autoantibodies in 10 out of the 86 samples that were characterised as negative by the RIA (in-house CBA: 16 cases).[7]

Due to its high sensitivity and easy implementation in the laboratory routine, the CBA has already been proposed as a first-line test for MG serology.[8]



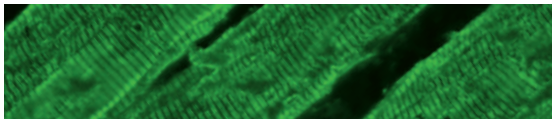
Myasthenia Gravis Mosaic 2 IIFT

Automation solutions

The Myasthenia Gravis Mosaic 2 IIFT, as well as the Anti-MuSK IIFT and Anti-AChR IIFT, offer a further advantage. They can be processed either manually or fully automatically on the EUROIMMUN instruments IF Sprinter, Sprinter XL and EUROLabWorkstation IFA. The immunofluorescence microscopy can also be performed fully automatically using the EUROPattern Microscope or the EUROPattern Microscope Live.

Supplementary diagnostic tests

Autoantibodies against striated muscle can occur in MG with thymoma. In most cases the target antigen is titin.[9] In a tissue-based assay (TBA) with monkey skeletal muscle, the autoantibodies produce a fluorescence pattern with staining of the cytoplasm of the skeletal muscle cells and a typical striation. For monospecific detection of autoantibodies against titin, EUROIMMUN offers various EUROLINE immunoblots, which provide titin together with other neural target structures such as SOX1 for differential diagnostics. Autoantibodies against SOX1 can occur in paraneoplastic syndrome with small-cell lung carcinoma (SCLC) in LEMS.[10]



Skeletal muscle (monkey)

EUROLINE Anti-SOX1, -Titin (IgG)

Ordering information

Test system	Test name	Antibodies against	Substrate	Ig class	Order number
IIFT	Myasthenia Gravis Mosaic 2 IIFT	Adult acetylcholine receptor (AChR-E) Foetal acetylcholine receptor (AChR-G) Muscle-specific kinase (MuSK)	4 BIOCHIPs per field: transfected cells transfected cells transfected cells control transfection	IgG	FA 1435-####-2-R
	Anti-MuSK IIFT	MuSK	2 BIOCHIPs per field: transfected cells control transfection	IgG	FA 1434-####-90-R
	Anti-AChR IIFT	AChR-E (adult), AChR-G (foetal)	3 BIOCHIPs per field: transfected cells transfected cells control transfection	IgG	FA 1435-####-90-R
	Skeletal Muscle (Monkey)	Skeletal muscle	M. iliopsoas	IgG	FA 1430-1005
ELISA	Anti-Acetylcholine Receptor ELISA (IgG)	AChR	Antigen-coated microplate wells (recombinant AChR: combination of adult and foetal receptors)	IgG	EA 1435-9601 G
EUROLINE	EUROLINE Anti-SOX1, -Titin (IgG)	SOX1 Titin	Antigen-coated test strips	IgG	DL 1111-1601-6 G
	EUROLINE Paraneoplastic Neurological Syndromes 12 Ag (IgG)	Amphiphysin, CV2, PNMA2 (Ma2/Ta), Ri, Yo, Hu, recoverin, SOX1, titin, Zic4, GAD65, Tr (DNER)	Antigen-coated test strips	IgG	DL 1111-####-7 G

References

[1]Wiendl H, et al. **Diagnostik und Therapie myasthener Syndrome, S2k-Leitlinie, 2022, DGN.** German Society for Neurology (eds.), Guidelines for diagnostics and therapy in neurology. www.dgn.org/leitlinien (2022) [2]Carr AS, et al. **A systematic review of population based epidemiological studies in Myasthenia Gravis.** BMC Neurol. 10:46 (2010). [3]Huang K, et al. **Autoimmune channelopathies at neuromuscular junction.** Front Neurol. 10:516 (2019). [4]Wiendl H, et al. **Guideline for the management of myasthenic syndromes.** Ther Adv Neurol Disord. 16:17562864231213240. (2023). [5]Kawaguchi N, et al. **Performance evaluation of the EUROIMMUN anti-acetylcholine receptor enzyme-linked immunoassay. Clin** Exp Neuroimmunol. (2023). [6]Mirian A, et al. **Comparison of fixed cell-based assay to radioimmunoprecipitation assay for acetylcholine receptor antibodies detection in myasthenia gravis.** J Neurol Sci 432:120084 (2022). [7]Spagni G, et al. **Comparison of fixed and live cell-based assay for the detection of AChR and MuSK antibodies in myasthenia gravis.** Neurol Neuroimmunol Neuroinflamm. 10(1):e200038. (2022) [8]Budhram A. **Fixed cell-based assays for autoantibody detection in myasthenia gravis: a diagnostic breakthrough.** Lancet Reg Health West Pac. 38:100876. (2023). [9]Aarli JA, et al. **Patients with myasthenia gravis and thymoma have in their sera IgG autoantibodies against titin.** Clin Exp Immunol. 82(2):284-8 (1990). [10]Sabater L, et al. **SOX1 antibodies are markers of paraneoplastic Lambert-Eaton myasthenic syndrome.** Neurology. 70(12):924-8 (2008).

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