研究用試薬

Anti-Myelin-Oligodendrocyte Glycoprotein (MOG) IIFT
Instructions for the indirect immunofluorescence test

ORDER NO.	ANTIBODIES AGAINST	SUBSTRATE	SPECIES	FORMAT SLIDES x FIELDS
FA 1156-1005-50 FA 1156-1010-50	Myelin oligodendrocyte glycoprotein (MOG)	transfected cells control transfection	EU 90	10 x 05 (050) 10 x 10 (100)

Indication: This test kit provides qualitative or semiquantitative in vitro determination of human antibodies of immunoglobulin class IgG against myelin oligodendrocyte glycoprotein (MOG) in patient samples to support the diagnosis of demyelinating diseases of the central nervous system. The fluorescence is either evaluated using the fluorescence microscope (specifications see chapter “Incubation”, section “Evaluation”) or, following automated image recording by the EUROPattern microscope at the computer screen, optionally supported by the EUROPattern Classifier software. The product is designed for use as IVD.

Test principle: The test fields are incubated with diluted patient sample. If a positive reaction is obtained, specific antibodies of classes IgA, IgG and IgM attach to the antigens. In a second step, the attached antibodies are stained with FITC-labelled anti-human antibodies and made visible with a fluorescence microscope.

Contents of a package for 50 determinations (e.g. FA 1156-1005-50):

Description	Format	Symbol
1. Slides, each containing 5 x 2 BIOCHIPs: the first coated with MOG-transfected cells, the second with control-transfected cells	10 slides	SLIDE
2. FITC-labelled anti-human IgG (goat), ready for use	1 x 1.5 ml	CONJUGATE
3. Positive control: autoantibodies against MOG, ready for use	1 x 0.1 ml	POS CONTROL
4. Negative control: autoantibody negative, ready for use	1 x 0.1 ml	NEG CONTROL
5. Sample buffer, ready for use	2 x 4.5 ml	SAMPLE BUFFER
6. Salt for PBS pH 7.2	2 packs	PBS
7. Tween 20	2 x 2.0 ml	TWEEN 20
8. Mounting medium, ready for use	1 x 3.0 ml	GLYCEROL
9. Cover glasses (62 mm x 23 mm)	12 pieces	COVERGLASS
10. Instruction booklet	1 booklet	---

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

Additional positive control (e.g. EUROIMMUN order no. CA 1156-0502) and negative control (e.g. EUROIMMUN order no. CA 1000-0502) can be ordered.
Performance of the test requires reagent trays TRAY, which are not provided in the test kits. They are available from EUROIMMUN under the following order no.:
- ZZ 9999-0110-R Reagent trays for slides containing up to 10 fields.

Storage and stability: The slides and the reagents should be stored at a temperature between +2° C and +8° C. Unopened, all test kit components are stable until the indicated expiry date.

Waste disposal: Patient samples, controls and slides are to be handled as potentially infectious materials. All reagents are to be disposed of in accordance with official disposal regulations.

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

Performing the test (reaction fields 5 x 5 mm)

The **TITERPLANE Technique** was developed by EUROIMMUN in order to standardise immunological analyses: Samples or conjugate are applied to the reaction fields of a reagent tray. The BIOCHIP slides are then placed into the recesses of the reagent tray, where all BIOCHIPs of the slide come into contact with the fluids, and the individual reactions commence simultaneously. Position and height of the droplets are exactly defined by the geometry of the system. As the fluids are confined to a closed space, there is no need to use a conventional “humidity chamber”. It is possible to incubate any number of samples next to each other and simultaneously under identical conditions.

Prepare: The preparation of the reagents and of the serum and plasma samples is described on **page 4** of this test instruction.

Pipette: Apply **30 µl of diluted sample** to each reaction field of the reagent tray, avoiding air bubbles. Transfer all samples to be tested before starting the incubation (up to 200 droplets). Use a polystyrene pipetting template.

Incubate: Start reactions by fitting the BIOCHIP slides into the corresponding recesses of the reagent tray. Ensure that each sample makes contact with its BIOCHIP and that the individual samples do not come into contact with each other.
Incubate for **30 minutes** at room temperature (+18 °C to +25 °C).

Wash: Rinse the BIOCHIP slides with a flush of PBS-Tween using a beaker and immerse them immediately afterwards in a cuvette containing PBS-Tween for at least **5 minutes**. Shake with a rotary shaker if available. Wash max. 16 slides, then replace PBS-Tween with new buffer.

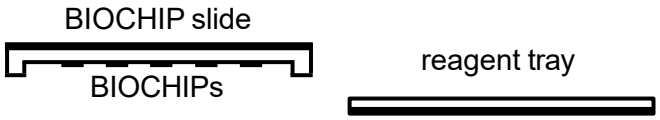
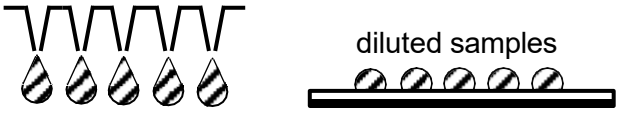
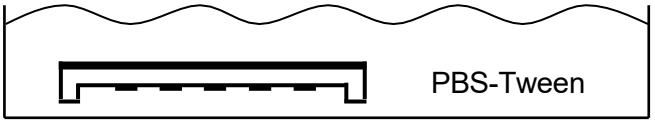
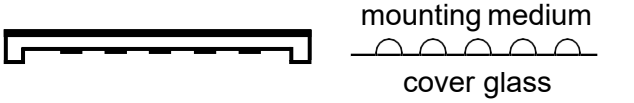
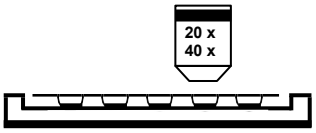
Pipette: Apply **25 µl of conjugate** to each reaction field of a clean reagent tray. Add all droplets before continuing incubation. Use a stepper pipette. The conjugate should be mixed thoroughly before use. To save time, conjugate can be pipetted onto separate reagent trays during the incubation with the diluted sample.

Incubate: Remove one BIOCHIP slide from the cuvette. Within five seconds blot only the back and the long sides with a paper towel and immediately put the BIOCHIP slide into the recesses of the reagent tray. Do not dry the areas between the reaction fields. Check for correct contact between the BIOCHIPs and liquids. Then continue with the next BIOCHIP slide. From now on, protect the slides from direct sunlight.
Incubate for **30 minutes** at room temperature (+18 °C to +25 °C).

Wash: Fill cuvette with new PBS-Tween. Rinse the BIOCHIP slides with a flush of PBS-Tween using a beaker and put them into the cuvette filled with the new PBS-Tween for at least **5 minutes**. Shake with a rotary shaker if available. Wash max. 16 slides, then replace PBS-Tween with new buffer.

Mount: Pipette the mounting medium onto the cover glass (up to 10 µl per reaction field; use the polystyrene mounting tray). Remove one BIOCHIP slide from PBS-Tween and dry the back and all four sides with a paper towel. Put the BIOCHIP slide, with the BIOCHIPs facing downwards, onto the prepared cover glass. Check immediately that the cover glass is properly fitted into the recesses of the slide. Correct the position if necessary.

Evaluate: Evaluate the fluorescence on the microscope or on the automatically recorded images (optionally following the result suggestion by the software).
The evaluation can be performed using a manual fluorescence microscope or the EUROPattern Microscope Live. Every result issued by the software must be verified by trained laboratory personnel.
Manual microscopy:
General recommendation: objective 20x (tissue sections, infected and transfected cells), 40x (cell substrates).
Excitation filter: 450 – 490 nm, colour separator: 510 nm, blocking filter: 515 nm
Light source: mercury vapour lamp, 100 W, EUROIMMUN LED, EUROStar Bluelight
EUROPattern Microscope Live:
The image recording settings are defined by the microscope and the software.

TITERPLANE Technique		
Pipette:	30 µl per field	
Incubate:	30 min	
Wash:	1 s flush 5 min cuvette	
Pipette:	25 µl per field	
Incubate:	30 min	
Wash:	1 s flush 5 min cuvette	
Mount:	max. 10 µl per field	
Evaluate: fluorescence microscopy EUROPattern Microscope Live		

Automated incubation: The test kit can be incubated by using automated devices, e.g. IF Sprinter, Sprinter XL, EUROLabLiquidHandler or others. The incubation and washing conditions correspond to the specific programming. The test settings for EUROIMMUN devices are validated in combination with the kit. Any other combination has to be validated by the user. For details please refer to the device manual.

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Preparation and stability of reagents

Note: After initial opening, the reagents are stable until the expiry date when stored between +2° C and +8 °C and protected from contamination, unless stated otherwise below.

- **Slides:** Ready for use. Remove the protective cover only when the slides have reached room temperature (+18 °C up to +25 °C; condensed water can damage the substrate). Do not touch the BIOCHIPs. If the protective cover is damaged, the slide must not be used for diagnostics. The slide must be disposed of after single use, even if not all incubation fields have been used.

- **FITC-labelled secondary antibody:** Ready for use. Before using for the first time, mix thoroughly. The conjugate is sensitive to light. Protect from sunlight.

- **Positive and negative controls:** Ready for use. Before using for the first time, mix thoroughly.

- **Sample buffer:** Ready for use.

- **PBS-Tween:** 1 pack of “Salt for PBS” should be dissolved in 1 liter of distilled water (optimal: aqua pro infusione, aqua ad injectabilia) and mixed with 2 ml of Tween 20 (stir for 20 min until homogeneous). The prepared PBS-Tween can be stored at +2 °C to +8 °C, generally for 1 week. PBS-Tween should not be used if the solution becomes cloudy or contamination appears.

- **Mounting medium:** Ready for use.

- **Reagent trays:** The reaction fields of the reagent tray must be hydrophilic and the surrounding area hydrophobic. If necessary, leave in 2% Deconex 11 universal (EUROIMMUN order number: ZZ 9912-0101) for 12 hours. Afterwards rinse generously with water and dry. Cleaning: Rub reagent trays with 5% Extran MA 01 (EUROIMMUN order number: ZZ 9911-0130) and rinse with plenty of water. To disinfect: Spray reagent trays generously with Mikrozid AF (EUROIMMUN order number: ZZ 9921-0125), turn over and leave for 5 minutes. Afterwards, rinse generously with water and dry.

Warning: The BIOCHIPs coated with antigen substrates have been treated with a disinfecting fixing agent. Neither HBsAg nor antibodies against HIV-1, HIV-2, and HCV could be detected in the control sera using appropriate ELISA or indirect immunofluorescence tests. **Nevertheless, all test system components should be handled as potentially infectious materials.** Some of the reagents also contain sodium azide in a non-declarable concentration. Avoid skin contact.

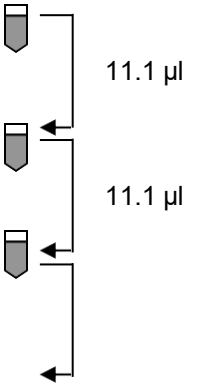
Preparation and stability of samples

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

Stability: The patient samples to be investigated can generally be stored up to 14 days at a temperature between +2° C and +8 °C. Diluted samples must be incubated within one working day.

Recommended sample dilution for qualitative evaluation: The sample to be investigated is diluted 1:10 in sample buffer. For example, dilute 11.1 µl sample in 100 µl sample buffer and mix thoroughly, e.g., vortex for 4 seconds.

Recommended sample dilution for semiquantitative evaluation: The dilution of samples to be investigated is performed using sample buffer. Add 100 µl of sample buffer to each tube and mix with 11.1 µl of the next highest concentration, e.g., vortex for 2 seconds. EUROIMMUN recommends incubating samples from a dilution of 1:10.

Dilution	Dilution scheme	
1:10	100 µl sample buffer + 11.1 µl undiluted sample	 11.1 µl 11.1 µl After every two dilution steps, a new pipette tip should be used to prevent carryover.
1:100	100 µl sample buffer + 11.1 µl 1:10 diluted sample	
1:1000	100 µl sample buffer + 11.1 µl 1:100 diluted sample	
⋮	⋮	

Evaluation

Fluorescence pattern (positive reaction):
The fluorescence is either evaluated through the ocular or, following automated image recording by the EUROPattern microscope at the computer screen, optionally supported by the EUROPattern Classifier software (see table “EUROPattern”). Every result issued by the software must be verified by trained laboratory personnel.

Antibodies against **myelin oligodendrocyte glycoprotein** react with the transfected cells of the substrate. They produce a flat, smooth to coarse-granular fluorescence of the cell with an accent of the cell membrane. The area of the cell nucleus is only slightly stained.
At high titers, MOG-specific antibodies manifest as a granular pattern in the lamina alba of the cerebellum.

All cells are stained, i.e. also control transfected cells, antibodies against other cell antigens are present.

If the positive control shows no specific fluorescence pattern or the negative control shows a clear specific fluorescence, the results are not to be used and the test is to be repeated.

A large range of fluorescence images can be found on the EUROIMMUN website (www.euroimmun.com).

Recommended qualitative evaluation:

Anti-MOG reactivity (IgG)	**Evaluation**
No reaction at 1:10	Negative. No antibodies against MOG detected in the serum/plasma sample.
Positive reaction at 1:10	Positive. Indication of neuromyelitis optica or associated spectrum disorders.

Recommended semiquantitative evaluation: The titer is defined as the sample dilution factor for which specific fluorescence is just identifiable. This should be compared to the reaction obtained with an equivalently diluted negative serum.

Antibody titers can be determined according to the following table from the fluorescence of the different sample dilutions:

Fluorescence at				Antibody titer
1:10	1:100	1:1000	1:10,000	
weak	negative	negative	negative	1:10
moderate	negative	negative	negative	1:32
strong	weak	negative	negative	1:100
strong	moderate	negative	negative	1:320
strong	strong	weak	negative	1:1000
strong	strong	moderate	negative	1:3200
strong	strong	strong	weak	1:10,000
⋮	⋮	⋮	⋮	⋮

Limitations of the procedure

1. A diagnosis should not be made based on a single test result. The clinical symptoms of the patient should always be taken into account along with the serological results by the physician.
2. Adaptation of this assay for use with automated sample processors and other liquid handling devices, in whole or in part, may yield differences in test results from those obtained using the manual procedure. It is the responsibility of each laboratory to validate that their automated procedure yields test results within acceptable limits.
3. Mishandling of slides during the staining procedure, especially allowing slides to dry between steps, may result in a “washed out” pattern appearance and/or a high level of background staining.
4. Coplin jars used for slide washing should be free from all residues. Use of Coplin jars containing residues may cause staining artefacts.
5. The light source, filters and optical unit of the fluorescence microscope can influence the sensitivity of the assay. Using traditional mercury vapour lamp systems, the performance of the microscope depends on correct maintenance, especially alignment of the lamp and replacement of the lamp after the recommended period of time. The EUROIMMUN fluorescence microscopes with LED Bluelight as the light source offer many advantages. For further information, please contact EUROIMMUN.

Test characteristics

Antigen: For the detection of antibodies against **myelin oligodendrocyte glycoprotein (MOG)**, specifically transfected cells EU 90 are used as the standard substrate.

Measurement range: The dilution starting point for this measurement system is 1:10. Samples can be further diluted by a factor of 10 so that the dilution series is 1:100, 1:1000, 1:10,000 etc. There is no upper limit to the measurement range.

Reproducibility: Inter-lot, intra-assay and inter-assay reproducibility are ensured.

Cross-reactivity: There are no literature data on the cross-reactivity available to EUROIMMUN. The following patient panels were investigated.

Antibodies against/ Substrate	Ig class	Sample specificity (antibodies against)	n	Prevalence	
				positive	%
MOG (transfected cells) *Serum/plasma*	IgG	Myelin	10	0	0%
		SS-A and SS-B	8	0	0%
		SS-A	2	0	0%
		Scl-70	10	0	0%
		Jo-1	10	0	0%
		dsDNA	10	0	0%
		IgLON5	8	0	0%

No cross-reactions were observed.

Interference: Haemolytic, lipaemic and icteric samples showed no influences on analysis results.

Reference range: Titer 1: <10 (IgG)

The following antibody prevalences were determined using a panel of samples from healthy blood donors (origin: Germany):

Substrate	Antibodies against	Conjugate	Prevalence	Cut-off	Number of samples
MOG (transfected cells) *Serum/plasma*	Myelin oligodendrocyte glycoprotein (MOG)	IgG	1.9%	1:10	206

Method comparison: specificity and sensitivity: Specificity and sensitivity of the Anti-MOG IIFT were investigated in an internal study with a total of 167 patient samples, serologically pre-characterised externally using in-house assays (60 samples with positive pre-characterisation, 107 samples with negative pre-characterisation; origin: Germany, Norway, Austria, Luxembourg).

Reference test system: In-house anti-MOG assay of the Neurological Research Laboratory, University Clinic for Neurology, Medical University Innsbruck, Austria.

Overview on the investigated samples:	n = 167
1a. Samples with information on the clinical picture of the patients (origin: Norway)	14
1b. Samples without information on the clinical picture (origin: Germany, Luxembourg)	44
1c. Samples without information on the clinical picture (origin: Austria)	50
2a. Control panel: anti-aquaporin-4 positive samples (origin: Germany)	9
2b. Samples from apparently healthy blood donors (origin: Germany)	50

n = 167		In-house anti-MOG assay Innsbruck	
		positive	negative
EUROIMMUN Anti-Myelin-Oligodendrocyte Glycoprotein (MOG) IIFT	positive	57	17
	negative	3	90

Specificity	**84.1%**
Sensitivity	**95.0%**
Positive predictive value	**77.0%**
Kappa value	**0.75**

Clinical sensitivity:
A panel (Origin: Germany, China, Norway) consisting of n = 72 clinically characterised patients with different diseases from the group of neuromyelitis optica spectrum disorders (NMOSD) and individual clinical symptoms was investigated for antibodies against MOG.

Panel	n	Ig class	Anti-MOG IIFT	
			positive	prevalence
Optic neuritis (ON), different forms (thereof n = 22 with negative AQP-4 antibody finding)	23	IgG	17	74%
Neuromyelitis optica (NMO) with negative AQP-4 antibody finding	1	IgG	0	0%
NMOSD with accompanying collagenosis	45	IgG	0	0%
Neuropathy/paralysis/spinal canal inflammation	3	IgG	0	0%

EUROPattern (software evaluation) from classifier version 2.3.21
The fluorescence is either evaluated using the fluorescence microscope (specifications see chapter "Incubation", Section "Evaluation") or, following automated image recording by the EUROPattern microscope at the computer screen, optionally supported by the EUROPattern Classifier software. The performance data are stored in the EUROPattern software.

Order no	Product	Automated image recording	EUROPattern Classifier
FA 1156-####-50	Anti-Myelin-Oligodendrocyte Glycoprotein (MOG) IIFT	EPM Live	EPM Live

Clinical significance

The Anti-Myelin Oligodendrocyte Glycoprotein IIFT provides determination of autoantibodies against **myelin oligodendrocyte glycoprotein** (MOG). This specific and sensitive test is based on transfected cells expressing the full-length protein. MOG is expressed exclusively in the central nervous system (CNS) on the outer layer of the myelin sheath and the plasma membrane of oligodendrocytes. Although the 28 kDa protein comprises only a fraction of the myelin sheath (<1%), it is an important surface marker which is involved in the myelination of CNS nerves among other things. The determination of autoantibodies against MOG is of particular significance in the diagnosis of acquired demyelinating diseases of the CNS especially in children, as well as in adults with neuromyelitis optica (NMO) and NMO spectrum diseases (NMOSD). These rare neuroinflammatory diseases differ typically in the age of onset, clinical course, disease severity, neuroradiological and/or pathological characteristics, and changes in cerebrospinal fluid (CSF). Nevertheless, a clear diagnosis is often only made retrospectively, since differential diagnosis is difficult, especially at disease onset, due to the partly overlapping presentation and similar findings in MRI, serum and/or CSF. Diagnostic delimitation of acute demyelinating syndrome from multiple sclerosis (MS) is particularly important, since treatment for MS and diseases such as NMO/NMOSD differ fundamentally.

Multiple sclerosis (MS, disseminated encephalomyelitis) is the most frequent inflammatory demyelinating disease of the CNS. MS is a chronic disease of the complete CNS which results from autoimmunological reactions against myelin sheaths of the axons. These lead to diverse neurological symptoms. At disease onset, visual disorders and paresthesia are often observed. MS often begins with an isolated symptom. This is referred to as **clinically isolated syndrome** (CIS).

In newer literature, the role of autoantibodies against MOG in MS patients is controversially discussed. In initial studies the detection of autoantibodies against MOG was performed using various monospecific methods based on recombinantly expressed extracellular domains of MOG either as linear or renatured epitope. The presentation of the epitopes used, in particular their heterogenicity with respect to correct membrane topology and/or aberrant glycolysation, has resulted in partly contradictory study data, with differing frequency of MOG antibodies in MS patients and control panels. An important advance in the understanding of autoantibodies against MOG was achieved in comparative studies of patients with **acute disseminated encephalomyelitis** (ADEM) and patients with MS. ADEM represents an important differential diagnosis to CIS. In contrast to MS, ADEM is more frequent in childhood (<10 years), occurs in close chronological coincidence with infections or vaccinations, and generally has a monophasic course and an overall favourable long-term prognosis. Early anti-inflammatory treatment with corticosteroids or plasmapharesis has a good chance of success in ADEM. In contrast, MS, NMO, recurrent opticus neuritis (ON) and longitudinal extensive transverse myelitis (LETM) are persistent, chronic diseases requiring long-term immunomodulatory or immunosuppressive therapy. Several studies using recombinant transfected cells showed that high MOG titers (IgG) occur in a portion of patients with ADEM or CIS but rarely in adults with MS. Intrathecal synthesis of MOG antibodies has been shown in CIS, but not in ADEM. According to the most recent knowledge, 30 to 40% of paediatric patients with inflammatory demyelinating diseases of the CNS exhibit autoantibodies against MOG at disease onset. Longitudinal studies of serum MOG autoantibodies showed that in patients with episodes of acute demyelination (ADEM, CIS) the autoantibodies often occur only transiently. A fall in the titer is associated with a more favourable prognosis for ADEM. Persistent MOG autoantibodies are rare, but have been reported in paediatric patients with multiphasic ADEM, MS or recurrent opticus neuritides. They appear to be associated with active, progressive disease.

Neuromyelitis optica (NMO) is a chronic autoimmune disease of the CNS with different levels of disease severity (NMO spectrum). Autoantibodies against aquaporin-4 (AQP-4), a water channel expressed on astrocytes, are an established biomarker for the disease. They can be detected in 60 to 90% of patients who fulfil the clinical diagnostic criteria for NMO. The detection of autoantibodies against AQP-4 is specific for NMO (specificity for NMO: 91 to 100%) and also for recurrent opticus neuritides and LETM as abortive or incomplete forms of NMO.

With the Anti-AQP-4 IIFT autoantibodies are detected in around 70% of patients with NMO. Determination using radioimmunoprecipitation assay (RIPA) and ELISA is also possible. However, due to the low sensitivity of RIPA and ELISA (approximately 56%) there is a high risk of false-negative results. The most reliable detection of antibodies against AQP-4 is achieved with test systems based on recombinantly transfected cells as antigen substrate. The major significance of the autoantibody detection is that autoantibodies against AQP-4 (NMO IgG) allow serological differentiation of prognostically poor NMO from other acute demyelinating diseases of the CNS, such as classic MS, which can significantly influence therapy decisions. Together with CNS tissue sections, AQP-4-transfected cells provide a particularly powerful IIFT Mosaic, which additionally allows the investigation of other potential reactivities against neuronal and glial antigens in the patient sample, e.g. paraneoplastic antibodies.

Titers of antibodies against AQP-4 are significantly higher in serum than in CSF. In contrast to anti-NMDA receptor encephalitis, in which intrathecal synthesis of autoantibodies occurs, the anti-AQP-4 antibodies in CSF originate from the peripheral lymphatic tissue. They only enter the CSF when a critical serum-CSF gradient has been reached. So far no patients with negative serum and positive CSF findings have been reported.

AQP-4 autoantibodies are detected in 64.7% of cases of **NMO spectrum diseases** (NMOSD), whereas anti-myelin-oligodendrocyte glycoprotein (MOG) autoantibodies are only found in 7.4%. In AQP-4-negative NMOSD cases the prevalence is 21.1%. AQP-4-seronegative patients with a positive serological result for MOG are more likely to have ON and LETM and often exhibit a monophasic course. These patients have a good prognosis, connected with less recurrences than patients with a positive anti-AQP-4 serological result or double-negative patients (AQP-4 and MOG seronegativity). A correlation between the titer and the clinical prognosis is not known.

Literature

- Chou IJ, Whitehouse WP, Wang HS, Tanasescu R, Constantinescu CS. **Diagnostic Modalities in Multiple Sclerosis: Perspectives in Children.** Biomed J 37 (2014) 50-59.
- EUROIMMUN AG. Stöcker W, Schlumberger W, Krüger C. **Alle Beiträge zum Thema Autoimmundiagnostik und Labordiagnostik der Infektionskrankheiten.** In: Gressner A, Arndt T (Hrsg.) Lexikon der Medizinischen Laboratoriumsdiagnostik. 2. Auflage. Springer Medizin Verlag, Heidelberg (2012).
- Mader S, Deisenhammer F, Reindl M. **Anti-AQP4-Antikörper als Biomarker zur Früherkennung von Neuromyelitis Optica-Spektrum-Erkrankungen / AQP4-IgG antibodies as biomarkers for early diagnosis of neuromyelitis optica spectrum disorders.** Laboratoriumsmedizin 34 Heft 6 (2010) 337-342.
- Matthews L, Marasco R, Jenkinson M, Küker W, Luppe S, Leite MI, Giorgio A, De Stefano N, Robertson N, Johansen-Berg H, Evangelou N, Palace J. **Distinction of seropositive NMO spectrum disorder and MS brain lesion distribution.** Neurology 80 (2013) 1330-1337.
- Sato DK, Callegaro D, Lana-Peixoto, Waters PJ, de Haidar FM, Takahashi T, Nakashima I, Apostolos-Pereira SL, Talim N, Simm RF, Martins AM, Misu T, Leite MI, Aoki M, Fujihara K. **Distinction between MOG antibodypositive and AQP4 antibody-positive NMO spectrum disorders.** Neurology 82 (2014) 474-481.
- Sepúlveda M, Armangué T, Sola-Valls N, Arrambide G, Meca-Lallana JE, Oreja-Guevara C, Mendibe M, Alvarez de Arcaya A, Aladro Y, Casanova B, Olascoaga J, Jiménez-Huete A, Fernández-Fournier M, Ramió-Torrentà L, Cobo-Calvo A, Viñals M, de Andrés C, Meca-Lallana V, Cervelló A, Calles C, Rubio MB, Ramo-Tello C, Caminero A, Munteis E, Antigüedad AR, Blanco Y, Villoslada P, Montalban X, Graus F, Saiz A. **Neuromyelitis optica spectrum disorders: Comparison according to the phenotype and serostatus.** Neurol Neuroimmunol Neuroinflamm. 2016 Apr 14;3(3):e225.#
- Kitley J, Waters P, Woodhall M, Leite MI, Murchison A, George J, Küker W, Chandratre S, Vincent A, Palace J. **Neuromyelitis optica spectrum disorders with aquaporin-4 and myelin-oligodendrocyte glycoprotein antibodies: a comparative study.** JAMA Neurol. 2014 Mar;71(3):276-83. doi: 10.1001/jamaneurol.2013.5857.
- Titulaer MJ, Höftberger R, Iizuka T, Leypoldt F, McCracken L, Cellucci T, Benson LA, Shu H, Irioka T, Hirano M, Singh G, Cobo Calvo A, Kaida K, Morales PS, Wirtz PW, Yamamoto T, Reindl M, Rosenfeld MR, Graus F, Saiz A, Dalmau J. **Overlapping demyelinating syndromes and anti-N-methyl-D-aspartate receptor encephalitis.** Ann Neurol. 2014 Mar;75(3):411-28.
- Höftberger R, Sepulveda M, Armangue T, Blanco Y, Rostásy K, Cobo Calvo A, Olascoaga J, Ramió-Torrentà L, Reindl M, Benito-León J, Casanova B, Arrambide G, Sabater L, Graus F, Dalmau J, Saiz A. **Antibodies to MOG and AQP4 in adults with neuromyelitis optica and suspected limited forms of the disease.** Mult Scler. 2015 Jun;21(7):866-74.
- Martinez-Hernandez E, Sepulveda M, Rostásy K, Höftberger R, Graus F, Harvey RJ, Saiz A, Dalmau J. **Antibodies to aquaporin 4, myelin-oligodendrocyte glycoprotein, and the glycine receptor α1 subunit in patients with isolated optic neuritis.** JAMA Neurol. 2015 Feb;72(2): 187-93.
- Hacohen Y, Absoud M, Deiva K, Hemingway C, Nytrova P, Woodhall M, Palace J, Wassmer E, Tardieu M, Vincent A, Lim M, Waters P. **Myelin oligodendrocyte glycoprotein antibodies are associated with non-MS course in children.** Neurol Neuroimmunol Neuroinflamm. 2015 Mar 12;2(2):e81.
- Pröbstel AK, Dornmair K, Bittner R, Sperl P, Jenne D, Magalhaes S, Villalobos A, Breithaupt C, Weissert R, Jacob U, Krumbholz M, Kuempfel T, Blaschek A, Stark W, Gärtner J, Pohl D, Rostasy K, Weber F, Forne I, Khademi M, Olsson T, Brilot F, Tantsis E, Dale RC, Wekerle H, Hohlfeld R, Banwell B, Bar-Or A, Meinl E, Derfuss T. **Antibodies to MOG are transient in childhood acute disseminated encephalomyelitis.** Neurology. 2011 Aug 9;77(6):580-8.

- Brilot F, Dale RC, Selter RC, Grummel V, Kalluri SR, Aslam M, Busch V, Zhou D, Cepok S, Hemmer B. **Antibodies to native myelin oligodendrocyte glycoprotein in children with imflammatory demyelinating central nervous system disease.** Ann Neurol. 2009 Dec;66(6):833-42.
- Majed M, Fryer JP, McKeon A, Lennon VA, Pittock SJ. **Clinical utility of testing AQP4-IgG in CSF.** Neurol Neuroimmunol Neuroinflamm June 2016 vol. 3 no. 3 e231.
- Wingerchuck DM, Banwell B, Bennett JL, Cabre P, Carroll W, Chitnis T, de Seze J, Greenberg B, Jacob A, Jarius S, Lana-Peixoto M, Levy M, Simon JH, Tenembaum S, Traboulsee AL, Waters P, Wellik KE, Weinshenker BG. **International consensus diagnostic criteria for neuromyelitis optica spectrum disorders.** Neurology. 2015 Jul 14;85(2):177-89.
- EUROIMMUN AG. Stöcker W, Rottmann N. **Verfahren und Analysevorrichtung zur mikroskopischen Untersuchung eines Gewebeschnittes oder eines Zellausstriches.** Deutsche und Internationale Patentanmeldung DE 10 2012 013 678 (angemeldet 2012) und WO2014/009066 (angemeldet 2013).
- EUROIMMUN AG. Stöcker W, Rottmann N. **Verfahren sowie Vorrichtung zur Inkubation von Patientenproben.** Deutsche und Internationale Patentanmeldung DE 10 2012 013 680 (angemeldet 2012) und WO2014/009067 (angemeldet 2013).