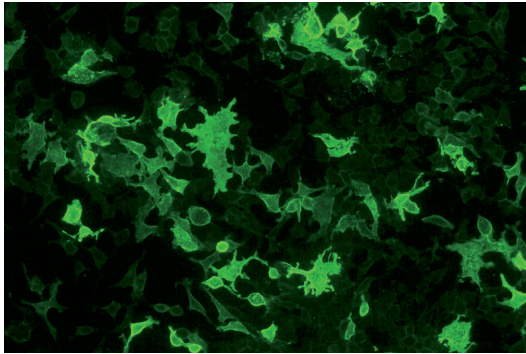
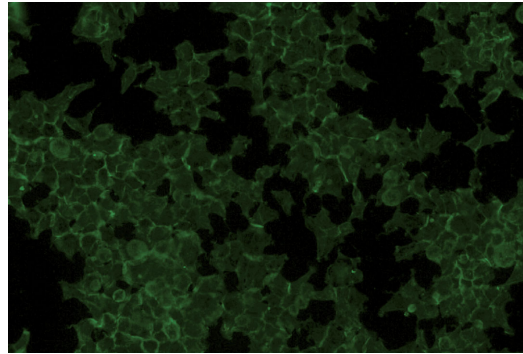


Anti-Aquaporin-4 IIFT



AQP-4 (transfected cells)



Non-transfected cells

Anti-aquaporin-4

Indication: Test system for the in vitro determination of antibodies against aquaporin-4 in human serum, plasma or cerebrospinal liquid (CSF) for the diagnosis of the following disease: neuromyelitis optica (NMO, opticospinal encephalomyelitis, Devic's syndrome).

Clinical significance: The inflammatory autoimmune disease NMO is a rare form (around 1%) of the group of acquired demyelinating diseases of the central nervous system (CNS) with degradation of the insulating sheath of at least one optical nerve (neuritis nervi optici) and at the same time or a few months later the spinal cord (myelitis).

Highly specific serum autoantibody markers are found very frequently in NMO, while they are not detected in multiple sclerosis (MS) patients or in healthy subjects. Antibodies known as NMO-IgG from their initial description cause a characteristic colouring of the Virchow-Robin's space along the small arterioles in the grey and white matter in immunofluorescence (IIFT) on CNS tissue. The protein aquaporin-4 (AQP-4) was later identified as the target antigen.

NMO was previously considered a localised form of multiple sclerosis (MS). With today's knowledge it is known to be a separate disease with respect to its pathogenesis. The disease symptoms of NMO are, on the one hand, acute visual disorders up to blindness (amaurosis) in one or both eyes which develop within hours or days, and on the other hand, symptoms of paraplegic syndrome, sometimes with advancing severity, sensitivity disorders, muscle weakness or paralysis of the extremities and loss of control of intestines and bladder, which develop acutely or within 1 to 14 days. Histologically, demyelinating lesions (similar to MS) are found, which can recover well. However, damage often remains because of tissue destruction (necrosis).

Without adequate therapy half of patients become blind in one or both eyes or cannot walk without supports within 5 years. Prognosis depends on the number and severity of flare-ups during the first two years. The 5-year survival rate is given as 70%, with the cause of death usually being neuropathic breathing insufficiency.

Application of the Anti-Aquaporin-4 IIFT: Anti-AQP-4 antibodies are detectable with IIFT using CNS tissue in around 70% of patients with NMO. They can also be determined using radioimmunoprecipitation assay (RIPA) and ELISA. 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 aquaporin-4 is achieved with test systems based on recombinantly transfected cells as antigen substrate. In the EUROIMMUN IIFT antibodies against AQP-4 are detected with a sensitivity of 80% and a specificity of 100% using a cell line which has been molecular biologically modified to produce large quantities of AQP-4.

Thus, antibodies against AQP-4 (synonym: NMO-IgG) can be determined for the first time in an easy standardised method in any laboratory that knows how to perform IFT. The major significance of the antibody detection is that antibodies against AQP-4 (NMO-IgG) allow serological differentiation of prognostically poor NMO from classic MS, which can influence therapy decisions. Together with CNS tissue sections, AQP-4-transfected cells provide a particularly powerful IIFT Mosaic, which additionally allows the identification of other potential reactivities against neuronal and glial antigens in the serum, e.g. paraneoplastic antibodies.

EUROIMMUN IIFT Autoimmune Diagnostics

tissue/cell substrates:
adrenal gland, monkey
bladder, rat
cartilage (trachea), monkey*
cerebellum, monkey
cerebrum, monkey
Crithidia luciliae
DNS-bound lactoferrin
erythrocytes, human*
eye, monkey*
granulocytes, human (ethanol-fixed)
granulocytes, human (formaldehyde-fixed)
granulocytes, human (methanol-fixed)
heart, monkey
HEp-2 cells
HEp-20 cells
HUVEC
hypothalamus, monkey*
intestine, monkey
kidney, monkey
kidney, mouse
kidney, rat
lacrimal gland, monkey
lip, monkey*
liver, monkey
liver, mouse
liver, rat
lobus temporalis, monkey*
lung, monkey
lymph nodes, monkey*
lymphocytes, human*
mammary gland, monkey*
mouth mucosa, monkey*
nerve, monkey
oesophagus, monkey
oesophagus, rat
ovary, monkey
pancreas, monkey
parathyroid gland, monkey
parotid gland, monkey
pituitary gland, monkey
placenta, monkey*
prostate, monkey
Saccharomyces cerevisiae
skeletal muscle, monkey
spermatozoa, human
spleen, monkey*
spinal cord, monkey
stomach, monkey
stomach, mouse
stomach, rat
synovium, monkey
testis, monkey
thrombocytes, human
thymus, monkey
thyroid gland, monkey
tongue, monkey
VSM47 cells
umbilical cord, human

EUROPLUS™ substrates:
AIH (LC-1 + SLA/LP)
BP180
GBM
gliadin
intrinsic factor
myeloperoxidase (MPO)
PBC (AMA M2 + Sp100)
proteinase 3 (PR3)
ribosomal P-proteins + Jo-1
SS-A + SS-B
SS-B + ribosomal P-proteins + Jo-1
SS-B + Sol-70 + Jo-1
thyroglobulin (TG)

transfected cells:
aquaporin-4
BP230
desmoglein 1 + 3
NMDA receptor
rPAG 1 + 2 (pancreas antigen 1 + 2)

BIOCHIP Mosaics™:
ANA global test: HEp-20/monkey liver
Autoantibody Profile: combination of 30 different tissues per slide
CIBD Profile: monkey pancreas/intest. goblet cells (culture)/granulocytes (EOH)/Saccharomyces cerevisiae
Basic Profile: HEp-20/monkey liver/rat kidney/rat stomach
EUROPLUS andomysium + gliadin: monkey intestine/monkey liver/gliadin
Granulocyte Mosaic: granulocytes (EOH)/granulocytes (HCHO)/HEp-20/monkey liver
Liver Mosaic: HEp-20/monkey liver/rat liver/rat kidney/rat stomach/monkey heart
Neuronal Antibody Screen: monkey cerebellum/monkey nerve/monkey intestine
Polyendocrinopathy Mosaic: monkey thyroid/monkey pancreas/monkey adrenal/monkey ovary/monkey testis/monkey stomach

Other mosaics also available
Special substrate combinations on request

* Currently not available as IVD in the EU.

Made in Germany

**EUROIMMUN IIFT
Infectious Serology**

Viruses:
Adenoviruses
Chikungunya virus
Coxsackieviruses
Crimean Congo fever virus (CCHFV)*
Cytomegalovirus (CMV)
Dengue viruses types 1-4 (DENV)
ECHO virus
Epstein-Barr virus capsid antigen (EBV-CA)
Epstein-Barr virus early antigen (EBV-EA)
Epstein-Barr virus nuclear antigen (EBNA)
Hantaviruses (types Hantaan, Puumala, Seoul, Saaremaa, Dobrava, Sin Nombre, Andes*)
Herpes simplex virus types 1 and 2 (HSV-1/2)
Human herpes virus type 6 (HHV-6)
HIV-1 and -2*
Influenza virus A (Shangdong, Singapore, Beijing)
Influenza virus B (Panama)
Japanese encephalitis virus (JEV)*
Measles virus
Mumps virus
Parainfluenza viruses types 1-4
Respiratory syncytial virus (RSV)
Rubella virus*
Sandfly fever virus*
(types Sicilian, Naples, Toscana, Cyprus)
SARS Coronavirus (SARS-CoV)
Tick-borne encephalitis (TBE) virus
Varicella zoster virus (VZV)
West Nile virus (WNV)
Yellow fever virus (YFV)

Bacteria:
Afipia felis*
Bartonella henselae
Bartonella quintana
Bordetella parapertussis
Bordetella pertussis
Borrelia afzelii
Borrelia burgdorferi
Borrelia garinii
Campylobacter coli*
Campylobacter jejuni*
Chlamydia pneumoniae
Chlamydia psittaci
Chlamydia trachomatis
Haemophilus influenzae*
Helicobacter pylori
Klebsiella pneumoniae*
Legionella bozemanii*
Legionella dumoffii*
Legionella gormanii*
Legionella jordanis*
Legionella longbeachae*
Legionella micdadei*
Legionella pneumophila serotypes 1-14
Listeria monocytogenes 1/2 a, 4b*
Mycoplasma hominis
Mycoplasma pneumoniae
Treponema pallidum
Treponema phagedenis
Ureaplasma urealyticum
Yersinia enterocolitica*

EUROPLUS™ substrates:
Borrelia VlsE (recombinant)
Borrelia OspC
EBV p19 + gp125

Yeasts:
Candida albicans
Candida glabrata*
Candida krusei*
Candida parapsilosis*
Candida tropicalis*

Parasites:
Echinococcus granulosus
Leishmania donovani
Plasmodium falciparum HRP-2/MSP-2 (rec.)*
Plasmodium vivax MSP/CSP (recombinant)*
Toxoplasma gondii

Profiles:
Accompanying hepatitis profile
Central nervous system profile
Exanthema profile
Fever profile South East Asia
Flavivirus profile
Gastrointestinal tract profile
Infectarthritis profile
Infectarthritis profile (The Tropics)
Lymphadenitis profile
Myocarditis profile
Ophthalmology profile
Otitis profile
Pregnancy profile
Respiratory tract profile
Sexually transmitted diseases (STD) profile
TORCH profile

* Currently not available as IVD in the EU.

Special substrate combinations
on request

Made in Germany
Version: 01/10
FA_1128_D_UK_A01

Test Characteristics

Anti-Aquaporin-4 IIFT

Test principle: The test system exclusively serves for the in vitro determination of human antibodies in human serum or plasma. The determination can be performed qualitatively or quantitatively. BIOCHIPS are incubated with diluted patient samples. In the case of positive reactions, specific antibodies of the classes IgA, IgG and IgM will bind to the antigens. In a second step, the attached antibodies are stained with fluorescein-labelled anti-human antibodies and made visible with the fluorescence microscope.

Test procedure: EUROIMMUN BIOCHIP slides are incubated using the proprietary TITERPLANE Technique. This technique enables multiple samples to be incubated next to each other and simultaneously under identical conditions. Results are evaluated by fluorescence microscopy.

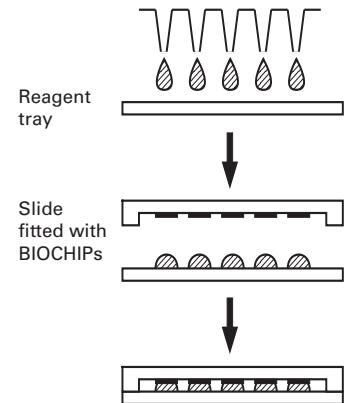
Intra-assay reproducibility: Ten determinations for each of two characterised samples were incubated in parallel. The deviation in the fluorescence intensity of the IIFT amounted to no more than ± 1 intensity level for all samples.

Inter-assay reproducibility: Two characterised samples were incubated in duplicate on at least 2 different days in 5 test runs. In quantitative evaluation of results, no deviation in the fluorescence intensity was found.

Inter-lot reproducibility: Three slide lots were tested with different characterised sera to estimate any deviations between individual lots. The deviation in the fluorescence intensity of the IIFT amounted to no more than ± 1 intensity level for all samples.

Sensitivity and specificity: The clinical specificity of the test system is 100% and the clinical sensitivity for NMO 78%. Samples from 51 patients with NMO-spectrum diseases (NMO and incomplete NMO forms: longitudinally extensive transverse myelitis, recurrent optic neuritis without and with non-extensive transverse myelitis), 89 patient controls with other neurological diseases and 100 healthy blood donors were investigated. Reference: Jarius et al., J Neurol Sci, doi: 10.1016/j.jns.2010.01.002 (2010)

Incubation with the TITERPLANE™ Technique



Panel	n	Anti-AQP-4 Ab pos.
NMO	32	78 %
Longitudinally extensive transverse myelitis	12	67 %
Recurrent optic neuritis	5	20 %
Recurrent optic neuritis/ non-extensive transverse myelitis	2	100 %
Total	51	71 %
Multiple sclerosis	66	0 %
Other neurological diseases	23	0 %
Blood donors	100	0 %
Total	189	0 %

Technical data:

Antigen substrate	Transfected cells and non-transfected cells (EU 90).
Sample dilution	Serum or plasma. Qualitative: 1:10, quantitative: 1:10/100/1000 etc. There is no upper limit to the measurement range.
Conjugate	IgG
Test procedure	30 min (sample)/30 min (conjugate). Room temperature.
Microscopy	Objective 40x, excitation filter: 488 nm, colour separator: 510 nm, blocking filter: 520 nm, light source: EUROIMMUN LED or mercury vapour lamp, 100 W.
Reagents	Ready for use, with the exception of the PBS Tween buffer.
Stability	Stable at +2°C to +8°C for 18 months after the date of manufacture.
Test kit format	10 or 20 slides, each containing 3, 5 or 10 test fields. Kits include all necessary reagents.
Order no.	FA 1128-####-50
Related products	FA 1111-17: cerebellum, monkey/cerebrum, monkey/optical nerve, monkey/AQP-4/EU 90