研究用試薬

# IIFT: Neurology Mosaics
# Instructions for use

| ORDER NO. | ANTIBODIES AGAINST | SUBSTRATE | SPECIES | FORMAT SLIDES x FIELDS |
|---|---|---|---|---|
| FA 111a-3 | hippocampus antigens | hippocampus | rat | |
| FA 111m-3 | cerebellum antigens | cerebellum | rat | |
| FA 112d-6 | glutamate receptor (type NMDA) | transfected cells | EU 90 | |
| FA 112d-51 | glutamate receptor (type AMPA1/2) | transfected cells | EU 90 | |
| FA 112k-53 | contactin-associated protein 2 (CASPR2) | transfected cells | EU 90 | 10 x 05 (050) |
| FA 112l-50 | dipeptidyl aminopeptidase-like protein 6 (DPPX) | transfected cells | EU 90 | 10 x 10 (100) |
| FA 112m-50 | | | | |
| FA 1439-1 | leucine-rich glioma-inactivated protein 1 (LGI1) | transfected cells | EU 90 | |
| FA 1439-50 | | | | |
| FA 1439-51 | $GABA_B$ receptor (GABARB1/B2) | transfected cells | EU 90 | |
| (see p. 24) | | | | |

**Indication:** This test kit provides qualitative or semiquantitative in vitro determination of human antibodies of immunoglobulin class IgG against neuronal antigens in patient samples to support the diagnosis of neurological diseases (encephalitis). 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. If cerebrospinal fluid is used as the sample material, the results for the parameters dipeptidyl aminopeptidase-like protein 6 (DPPX), leucine-rich glioma-inactivated protein 1 (LGI1) and glutamate receptor (type AMPA1/2) must be confirmed using a CE-labelled test.

**Test principle:** The test fields are incubated with diluted patient sample. If the reaction is positive, 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 test kit for 50 determinations (e.g. FA 112d-1005-6):**

| Description | Format | Symbol |
|---|---|---|
| 1. Slides, with a mosaic of BIOCHIPs (specifications: see page 24) | 10 slides | SLIDE |
| 2. FITC-labelled anti-human IgG (goat), ready for use | 1 x 1.5 ml | CONJUGATE |
| 3. Positive control: anti-glutamate receptor (type NMDA), human, ready for use | 1 x 0.1 ml | POS CONTROL |
| 4. Negative control: autoantibody-negative, human, ready for use | 1 x 0.1 ml | NEG CONTROL |
| 5. Salt for PBS pH 7.2 | 2 packs | PBS |
| 6. Tween 20 | 2 x 2.0 ml | TWEEN 20 |
| 7. Mounting medium, ready for use | 1 x 3.0 ml | GLYCEROL |
| 8. Cover glasses (62 mm x 23 mm) | 12 pieces | COVERGLASS |
| 9. Instruction booklet | 1 booklet | --- |

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

Single slides (e.g. EUROIMMUN order no. FB 112d-1005-6) are provided together with cover glasses. Additional positive control (e.g. EUROIMMUN order no. CA 112d-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 Reagent trays for slides containing up to 10 fields (5 x 5 mm).

**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 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.

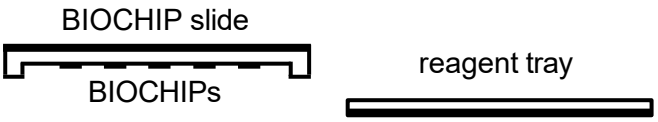
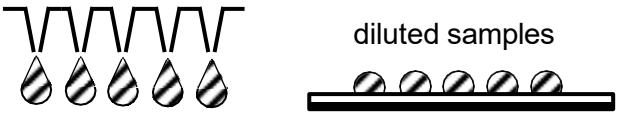
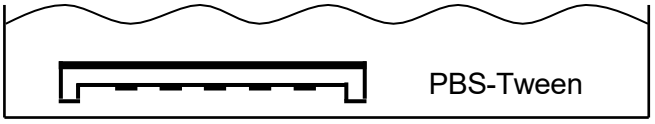
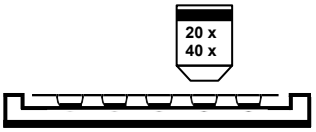
**Incubate:** Remove one BIOCHIP slide from the cuvette. Within five seconds blot only the back and the long sides with a paper towel and immediately put the BIOCHIP slide into the recesses of the reagent tray. Do not dry the areas between the reaction fields. Check for correct contact between the BIOCHIPs 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.

**研究用試薬**

| **TITERPLANE Technique** | |  |
|---|---|---|
| **Pipette:** | 30 µl per field |  |
| **Incubate:** | 30 min |  |
| **Wash:** | 1 s flush<br>5 min cuvette |  |
| **Pipette:** | 25 µl per field |  |
| **Incubate:** | 30 min |  |
| **Wash:** | 1 s flush<br>5 min cuvette |  |
| **Mount:** | max. 10 µl per field |  |
| **Evaluate:** fluorescence microscopy<br>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.

**研究用試薬**

## 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 them thoroughly.

- **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 minutes 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 no.: 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.

**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.

**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

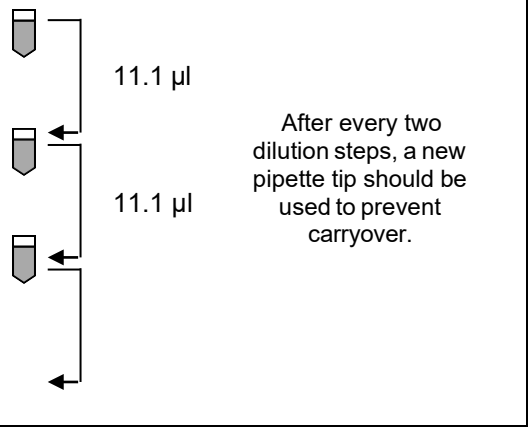
**Samples:** Human sera or EDTA, heparin or citrate plasma, cerebrospinal fluid (CSF) (limitations see page 24).

**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 PBS-Tween. For example, dilute 11.1 µl sample in 100 µl PBS-Tween and mix thoroughly, e.g. vortex for 4 seconds. EUROIMMUN recommends incubating samples from a dilution of 1:10 and 1:100. CSF samples are used undiluted.

**Recommended sample dilution for semiquantitative evaluation:** The dilution of samples to be investigated is performed using PBS-Tween. Add 100 µl of PBS-Tween 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 starting dilution of 1:10 and analysing CSF samples undiluted.

| Dilution | Dilution scheme |
|---|---|
| 1:10 | 100 µl PBS-Tween + 11.1 µl undiluted sample |
| 1:100 | 100 µl PBS-Tween + 11.1 µl 1:10 diluted sample |
| 1:1000 | 100 µl PBS-Tween + 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.

Autoantibodies against the **glutamate receptors (type NMDA)** react specifically with the corresponding transfected cells. They induce a fine granular cytoplasmic fluorescence with typical cellular protrusions. The staining of the cell nuclei is sample-specific and mostly weak. On neurological tissue sections fluorescence is observed in the stratum moleculare of the hippocampus (neuropil staining) and in the stratum granulosum of the cerebellum.

Autoantibodies against **glutamate receptors (type AMPA1 and AMPA2; GluR1/GluR2)** react with the stratum moleculare of the hippocampus (neuropil staining), the stratum moleculare and granulosum of the cerebellum and Purkinje cells. Antibodies against AMPA receptors react specifically with the corresponding transfected cells. They induce a smooth to fine-speckled fluorescence of the cell with an accentuation of the cell membrane, while the area of the cell nucleus is only slightly stained.

Antibodies against **contactin-associated protein 2 (CASPR2)** react specifically with the correspondingly transfected cells of the substrate. They cause a smooth to fine-speckled fluorescence of the cell with an accentuation of the cell membrane. The area of the cell nucleus is only weakly stained. On neurological tissue sections fluorescence is observed in the stratum moleculare of the hippocampus (neuropil staining) and the cerebellum. Concerning the hippocampus the outer molecular layer shows a stronger reactivity than the inner layer. Additionally, a blotchy fluorescence can be detected in the stratum granulosum of the cerebellum.

Antibodies directed against **leucine-rich glioma-inactivated protein 1 (LGI1)** react specifically with the transfected cells. They induce a smooth to fine-speckled fluorescence with an accentuation of the cell membrane. The area of the cell nucleus is only weakly stained. On frozen sections of hippocampus (neuropil staining) and cerebellum a fine-granular fluorescence is observed in the stratum moleculare. On hippocampus the outer molecular layer shows a stronger reaction than the inner. Additionally, a blotchy fluorescence can be detected in the stratum granulosum of the cerebellum.

Autoantibodies against **$GABA_B$ receptors (GABARB1/B2)** show a coarse granular fluorescence in the stratum moleculare of the hippocampus (neuropil staining) and the cerebellum. There is additionally a blotchy fluorescence in the stratum granulosum of the cerebellum. On specifically transfected cells, antibodies against $GABA_B$ receptors show a smooth to fine-speckled fluorescence with an accentuation of the cell membrane, while the area of the cell nucleus is only slightly stained.

Antibodies against **dipeptidyl aminopeptidase-like protein 6 (DPPX)** react with the transfected cells of the test substrate. They produce a spread, smooth to fine-speckled fluorescence with an accentuation of the cell membrane. The area of the cell nucleus is only slightly stained. On frozen tissue sections of hippocampus and cerebellum, a granular fluorescence of the stratum moleculare (neuropil staining) can be observed. Additionally, the stratum granulosum of the cerebellum reacts with a blotchy fluorescence.

Some cells are not transfected and show no specific fluorescence.

If the cell nuclei or the cytoplasm of all cells are stained, antinuclear antibodies or antibodies against mitochondria and 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: sera or plasma samples**

| Substrate | Cut-off | Evaluation | |
|---|---|---|---|
| | | negative | positive |
| Hippocampus rat, Cerebellum rat | 1:10 | No specific fluorescence at a dilution of 1:10. No antibodies against neuronal antigens in the patient sample. | Specific fluorescence at a dilution of 1:10. For antibodies against NMDA-R, AMPAR2, AMPAR1 / R2, CASPR2, LGI1, GABARB1 / B2, DPPX: If corresponding symptoms are present it indicates various forms of autoimmune encephalitis (see Clinical Significance). |
| NMDA-R | 1:10 | No specific fluorescence at a dilution of 1:10. No antibodies against antigens of specifically transfected cells in the patient sample. | Specific fluorescence at a dilution of 1:10. Indication of anti-NMDA receptor encephalitis. |
| AMPAR1/R2 | 1:10 | | Specific fluorescence at a dilution of 1:10. Indication of limbic encephalitis. |
| GABARB1/B2 | 1:10 | | Specific fluorescence at a dilution of 1:10. Indication of limbic encephalitis. |
| CASPR2 | 1:100* | | Specific fluorescence at a dilution of 1:100. Indication of limbic encephalitis, neuromyotonia and Morvan syndrome. |
| LGI1 | 1:10 | | Specific fluorescence at a dilution of 1:10. Indication of limbic encephalitis. |
| DPPX | 1:10 | | Specific fluorescence at a dilution of 1:10. Indication of limbic encephalitis. |

* For substrate combinations in mosaics and profiles, a 1:10 dilution should first be incubated. If the substrate **contactin-associated protein 2 (CASPR2)** shows a specific fluorescence at this dilution, then the sample should be incubated in a 1:100 dilution to confirm the specific result. If the sample shows no specific fluorescence in the dilution of 1:100, the result lies in the grey scale range (titer 1:10 or 1:32) and is to be regarded as borderline. The clinical relevance has to be clarified in the individual case and checked for plausibility in the clinical context.

**Recommended qualitative evaluation of CSF samples:**
The normal value for neurological autoantibodies in CSF is negative. A positive reaction in the undiluted sample should be evaluated as positive. Positive detection is not a proof of intrathecal synthesis, but indicates e.g. NMDA receptor encephalitis in negative serum results.

**Recommended semiquantitative evaluation:** The titer is defined as the sample dilution factor for which specific fluorescence is just identifiable. This should be compared with the reaction obtained using 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

**Antigens:** For the determination of various neuronal autoantibodies by indirect immunofluorescence, **rat cerebellum and rat hippocampus** are used as standard substrate.

Specific **transfected cells** (EU 90) are used as standard substrates for the monospecific detection of neuronal antibodies. EUROIMMUN provides a large range of transfected cell test substrates, for example, for the detection of antibodies against glutamate receptors (type NMDA and AMPA1/2), contactin-associated protein 2 (CASPR2), leucine-rich glioma-inactivated protein 1 (LGI1), dipeptidyl aminopeptidase-like protein 6 (DPPX) and $GABA_B$ receptor (GABARB1/B2). Use of these substrates in combination with neuronal rat tissue, e.g. cerebellum and hippocampus, enables comprehensive diagnosis of different forms of autoimmune encephalitis.

**Measurement range:** The dilution starting point for this measurement system is 1:10 (sera or plasma) or 1:1 (undiluted; CSF). 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 is no scientific literature known to EUROIMMUN in which cross-reactivity was described. The following panels of patients with other neurological diseases were analysed:

| Substrate | Ig class | Specificity of samples | n | Prevalence | |
|---|---|---|---|---|---|
| | | | | Positive | % |
| **NMDA-R** Hippocampus rat *(Serum/plasma)* | IgG | MS (multiple sclerosis) | 58 | 0 | 0% |
| **NMDA-R** Cerebellum rat *(Serum/plasma)* | IgG | MS (multiple sclerosis) | 58 | 0 | 0% |
| **NMDA-R** Transfected cells *(Serum/plasma)* | IgG | LGI1 | 5 | 0 | 0% |
| | | Aquaporin-4 | 11 | 0 | 0% |
| | | CASPR2 | 5 | 0 | 0% |
| | | GAD65 | 5 | 0 | 0% |
| | | DPPX | 12 | 0 | 0% |
| **GABARB1/B2** Transfected cells *(Serum/plasma)* | IgG | DPPX | 7 | 0 | 0% |
| | | LGI1 | 4 | 0 | 0% |
| | | CASPR2 | 12 | 0 | 0% |
| **GABARB1/B2** Transfected cells *(CSF)* | IgG | LGI1 | 4 | 0 | 0% |
| **LGI1** Transfected cells *(Serum/plasma)* | IgG | Glutamate receptor (type NMDA) | 9 | 0 | 0% |
| | | Aquaporin-4 | 11 | 0 | 0% |
| | | CASPR2 | 5 | 0 | 0% |
| | | GAD65 | 5 | 0 | 0% |
| | | GABARB1/B2 | 4 | 0 | 0% |
| | | DPPX | 7 | 0 | 0% |
| **CASPR2** Transfected cells *(Serum/plasma)* | IgG | Glutamate receptor (type NMDA) | 10 | 0 | 0% |
| | | Aquaporin-4 | 11 | 0 | 0% |
| | | LGI1 | 9 | 0 | 0% |
| | | GAD65 | 5 | 0 | 0% |
| | | DPPX | 7 | 0 | 0% |
| | | GABARB1/B2 | 4 | 0 | 0% |
| **CASPR2** Transfected cells *(CSF)* | IgG | GABARB1/B2 | 4 | 0 | 0% |
| | | LGI1 | 4 | 0 | 0% |
| **AMPAR1/R2** Transfected cells *(Serum/plasma)* | IgG | LGI1 | 7 | 0 | 0% |
| | | Aquaporin-4 | 8 | 0 | 0% |
| | | CASPR2 | 6 | 0 | 0% |
| | | Glutamate receptor (type NMDA) | 11 | 0 | 0% |
| | | DPPX | 5 | 0 | 0% |
| **DPPX** Transfected cells *(Serum/plasma)* | IgG | LGI1 | 8 | 0 | 0% |
| | | Aquaporin-4 | 10 | 0 | 0% |
| | | CASPR2 | 4 | 0 | 0% |
| | | Glutamate receptor (type NMDA) | 10 | 0 | 0% |
| | | GAD65 | 5 | 0 | 0% |
| | | mGluR1 | 2 | 0 | 0% |

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

**研究用試薬**

**Hook effect:** In high-titer CSF samples with the substrate “NMDA-R transfected cells”, a Hook effect (prozone effect) was observed in rare cases in low dilutions. EUROIMMUN therefore recommends testing the samples at two different dilutions (1:1 and 1:10).

**Reference range:** Titer 1: <10 or 1:<100** (sera, plasma; IgG)
CSF, undiluted; negative (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 |
|---|---|---|---|---|---|
| **Hippocampus rat** *(Serum/plasma)* | NMDA-R | IgG | 0% | Serum, plasma: 1:10 / 1:100**<br>CSF: 1:1 undiluted | 200 |
| **Hippocampus rat** *(CSF)* | NMDA-R | IgG | 0% | | 71 |
| **Cerebellum rat** *(Serum/plasma)* | NMDA-R | IgG | 0% | | 200 |
| **Cerebellum rat** *(CSF)* | NMDA-R | IgG | 0% | | 71 |
| **NMDA-R** Transfected cells *(Serum/plasma)* | NMDA-R | IgG | 0.8% | | 400 |
| **NMDA-R** Transfected cells *(CSF)* | NMDA-R | IgG | 1.7% | | 60* |
| **GABARB1/B2** Transfected cells *(Serum/plasma)* | GABARB1/B2 | IgG | 0% | | 400 |
| **GABARB1/B2** Transfected cells *(CSF)* | GABARB1/B2 | IgG | 0% | | 124* |
| **LGI1** Transfected cells *(Serum/plasma)* | LGI1 | IgG | 0.5% | | 206 |
| **CASPR2**** Transfected cells *(Serum/plasma)* | CASPR2 | IgG | 0.2% | | 595 |
| **CASPR2** Transfected cells *(CSF)* | CASPR2 | IgG | 0% | | 124* |
| **AMPAR1/R2** Transfected cells *(Serum/plasma)* | AMPAR1/R2 | IgG | 0% | | 254 |
| **DPPX** Transfected cells *(Serum/plasma)* | DPPX | IgG | 0% | | 400 |

* Negative CSF samples from patients without information on the clinical picture.
** For the evaluation of the reference range, results with a titer 1:<100 were considered negative.

**研究用試薬**

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

| Substrate | Antibodies against | Conjugate | Prevalence | Cut-off | Number of samples |
|---|---|---|---|---|---|
| **NMDA-R** Transfected cells *(Serum/plasma)* | NMDA-R | IgG | 1.0% | Serum, plasma: 1:10 / 1:100** | 104 |
| **GABARB1/B2** Transfected cells *(Serum/plasma)* | GABARB1/B2 | IgG | 0% | | 104 |
| **LGI1** Transfected cells *(Serum/plasma)* | LGI1 | IgG | 0% | | 104 |
| **CASPR2**** Transfected cells *(Serum/plasma)* | CASPR2 | IgG | 0% | | 104 |

** For the evaluation of the reference range, results with a titer 1:<100 were considered negative.

**Method comparison – Specificity and sensitivity:**

**Anti-Glutamate receptor (type NMDA) IIFT *(serum/plasma)***

| **Overview of the tested samples / reference test system:** | **n** |
|---|---|
| Samples of patients with anti-NMDA receptor encephalitis (n = 29) and a control group (16 patients with other encephalopathies, 1 patient with cerebellar degeneration, 1 patient with retinopathy; origin: Prof. J. Dalmau, USA). The samples were serologically precharacterised as positive or negative by in-house cell-based assay*. | 47 |
| **Number of samples** | 47 |

* Literature reference: *Prüss H, Dalmau J, Harms L, Holtje M, Ahnert-Hilger G, Borowski K, Stoecker W, Wandinger KP.* ***Retrospective analysis of NMDA receptor antibodies in encephalitis of unknown origin.*** *Neurology 75 (2010) 1735-1739.*

| n = 47 | | In-house Anti-NMDA-R CBA | |
|---|---|---|---|
| | | positive | negative |
| **EUROIMMUN** | positive | 29 | 0 |
| **Anti-Glutamate receptor (type NMDA) IIFT** | negative | 0 | 18 |

| | |
|---|---|
| **Specificity** | **100%** |
| **Sensitivity** | **100%** |

**Anti-Glutamate receptor (type NMDA) IIFT (*CSF*)**

| **Overview of the tested samples / reference test system:** | **n** |
|---|---|
| Samples of patients with anti-NMDA receptor encephalitis (n = 10), and a control group (13 patients with other encephalopathies; origin: Prof. J. Dalmau, USA). The samples were precharacterised as antibody positive or negative by in-house cell-based assay*. | 23 |
| **Number of samples** | 23 |

* Literature reference: *Pruss H, Dalmau J, Harms L, Holtje M, Ahnert-Hilger G, Borowski K, Stoecker W, Wandinger KP.* ***Retrospective analysis of NMDA receptor antibodies in encephalitis of unknown origin.*** *Neurology 75 (2010) 1735-1739.*

| n = 23 | | In-house Anti-NMDA-R CBA | |
|---|---|---|---|
| | | positive | negative |
| **EUROIMMUN** | positive | 10 | 0 |
| **Anti-Glutamate receptor (type NMDA) IIFT** | negative | 0 | 13 |

| | |
|---|---|
| **Specificity** | **100%** |
| **Sensitivity** | **100%** |

**研究用試薬**

### Anti-GABARB1/B2 IIFT *(serum/plasma)*

| Overview of the tested samples / reference test system: | n |
|---|---|
| Samples of patients with limbic encephalitis, positive serological precharacterisation by in-house methods (origin: Prof. Dalmau, Hospital of the University of Philadelphia, Dept. of Neurology, Philadelphia, USA). | 17 |
| **Number of samples** | **17** |

| n = 17 | | Reference | |
|---|---|---|---|
| | | positive | negative |
| **EUROIMMUN** | positive | 14 | 0 |
| **Anti-GABARB1/B2 IIFT** | negative | 3 | 0 |

| | |
|---|---|
| **Specificity** | **---** |
| **Sensitivity** | **82.4%** |

### Anti-GABARB1/B2 IIFT *(CSF)*

| Overview of the tested samples / reference test system: | n |
|---|---|
| Samples of patients with limbic encephalitis, antibody positive precharacterisation by in-house methods (origin: Prof. Dalmau Hospital of the University of Philadelphia, Dept. of Neurology, Philadelphia, USA). 6/8 of the associated serum samples were anti-$GABA_B$ receptor positive, no serum material was available for two CSF samples. | 10 |
| **Number of samples** | **10** |

| n = 10 | | Reference | |
|---|---|---|---|
| | | positive | negative |
| **EUROIMMUN** | positive | 6 | 0 |
| **Anti-GABARB1/B2 IIFT** | negative | 4 | 0 |

| | |
|---|---|
| **Specificity** | **---** |
| **Sensitivity** | **60%** |

### Anti-LGI1 IIFT *(serum/plasma)*

| Overview of the tested samples / reference test system: | n |
|---|---|
| Samples of patients with positive or negative serological precharacterisation. The precharacterisation was carried out by means of different in-house methods (in-house-CBA and tissue section) (origin: Prof. Angela Vincent, University of Oxford, United Kingdom). | 21 |
| **Number of samples** | **21** |

| n = 21 | | In-house IFT | |
|---|---|---|---|
| | | positive | negative |
| **EUROIMMUN** | positive | 8 | 0 |
| **Anti-LGI1 IIFT** | negative | 0 | 13 |

| | |
|---|---|
| **Specificity** | **100%** |
| **Sensitivity** | **100%** |

**研究用試薬**

**Anti-CASPR2 IIFT *(serum/plasma)***

| Overview of the tested samples / reference test system: | n |
|---|---|
| 1. Preselected samples of patients with limbic encephalitis, which were serologically precharacterised by in-house IFT (origin: Prof. Angela Vincent, University of Oxford, United Kingdom). | 9 |
| 2. Samples of patients with various neurological disorders (incl. limbic encephalitis, epilepsy) and healthy controls, which were serologically precharacterised by in-house IFT (origin: Prof. Angela Vincent, University of Oxford, United Kingdom). | 22 |
| **Number of samples** | **31** |

| n = 31 | | In-house IFT | |
|---|---|---|---|
| | | positive | negative |
| **EUROIMMUN** | positive | 14 | 0 |
| **Anti-CASPR2 IIFT** | negative | 0 | 17 |

| | |
|---|---|
| **Specificity** | **100%** |
| **Sensitivity** | **100%** |

**Anti-Glutamate receptor (type AMPA1/2) IIFT *(serum/plasma)***

| Overview of the tested samples / reference test system: | n |
|---|---|
| Preselected samples of patients with positive or negative serological precharacterisation. The precharacterisation was carried out using an in-house IFT (origin: Prof. Josep Dalmau, Hospital Clinic, Dept. of Neurology, Center of Neuroimmunology and Paraneoplastic Disorders, Barcelona, Spain). | 13 |
| **Number of samples** | 13 |

| n = 13 | | In-house IFT | |
|---|---|---|---|
| | | positive | negative |
| **EUROIMMUN** | positive | 5 | 0 |
| **Anti-AMPAR1/R2 IIFT** | negative | 0 | 8 |

| | |
|---|---|
| **Specificity** | **100%** |
| **Sensitivity** | **100%** |

**Anti-DPPX IIFT *(serum/plasma)***

| Overview of the tested samples / reference test system: | n |
|---|---|
| 1. Samples of patients with positive or negative serological precharacterisation were investigated. The precharacterisation was done using an in-house method (origin: Prof. Josep Dalmau, Hospital Clinic, Dept. of Neurology, Center of Neuroimmunology and Paraneoplastic Disorders, Barcelona Spain). | 8 |
| 2. Samples of patients with positive serological precharacterisation were investigated. The precharacterisation was done using an IFT (tissue sections; origin: Prof. Vanda Lennon, Department of Laboratory Medicine and Pathology, Neuroimmunology Laboratory, Mayo Clinic, USA). | 3 |
| **Number of samples** | 11 |

| n = 11 | | Reference | |
|---|---|---|---|
| | | positive | negative |
| **EUROIMMUN** | positive | 8 | 0 |
| **Anti-DPPX IIFT** | negative | 0 | 3 |

| | |
|---|---|
| **Specificity** | **100%** |
| **Sensitivity** | **100%** |

**研究用試薬**

**Clinical specificity and sensitivity:**

| Substrate | Ig class | Sample characterisation<br>Clinical patient panels (origin of samples) | n | Prevalence | |
|---|---|---|---|---|---|
| | | | | Positive | % |
| **NMDA-R**<br>Transfected cells<br>*(Serum/plasma)* | IgG | Anti-NMDA-R encephalitis<br>(Germany) | 206 | 172 | 83.5% |
| | | Control group (other encephalitides)<br>(Germany) | 12 | 0 | 0% |
| | | Panel of healthy blood donors<br>(Germany) | 100 | 0 | 0% |
| **NMDA-R**<br>Transfected cells<br>*(CSF)* | IgG | Anti-NMDA-R encephalitis<br>(Germany) | 206 | 205 | 99.5% |
| | | Control group (other encephalitides)<br>(Germany) | 33 | 0 | 0% |
| **NMDA-R**<br>Hippocampus rat<br>*(Serum/plasma)* | IgG | Anti-NMDA-R encephalitis<br>(USA) | 29 | 29 | 100% |
| | | Other encephalitides<br>(USA) | 18 | 4 | 22.2% |
| | | Panel of healthy blood donors<br>(Germany) | 100 | 0 | 0% |
| **NMDA-R**<br>Hippocampus rat<br>*(CSF)* | IgG | Anti-NMDA-R encephalitis<br>(USA) | 10 | 10 | 100% |
| | | Other encephalitides<br>(USA) | 13 | 3 | 23.1% |
| **NMDA-R**<br>Cerebellum rat<br>*(Serum/plasma)* | IgG | Anti-NMDA-R-encephalitis<br>(USA) | 29 | 29 | 100% |
| | | Other encephalitides<br>(USA) | 18 | 4 | 22.2% |
| | | Panel of healthy blood donors<br>(Germany) | 100 | 0 | 0% |
| **NMDA-R**<br>Cerebellum rat<br>*(CSF)* | IgG | Anti-NMDA-R encephalitis<br>(USA) | 10 | 10 | 100% |
| | | Other encephalitides<br>(USA) | 13 | 3 | 23.1% |
| **GABARB1/B2**<br>Transfected cells<br>*(Serum/plasma)* | IgG | Samples from patients with known psychological disease picture: schizophrenia, stable schizophrenia, unipolar depression, borderline syndrome and samples of controls, matching with respect to age, sex and body mass<br>(Germany) | 726 | 0 | 0% |
| | | Hashimoto's thyroiditis<br>(Germany) | 45 | 0 | 0% |
| | | Panel of healthy blood donors<br>(Germany) | 48 | 0 | 0% |
| **LGI1**<br>Transfected cells<br>*(Serum/plasma)* | IgG | Limbic encephalitis<br>(UK) | 14 | 14 | 100% |
| | | Panel of healthy blood donors<br>(Germany) | 152 | 0 | 0% |
| | | Hashimoto's thyroiditis<br>(Germany) | 45 | 0 | 0% |
| | | Samples from patients with known psychological disease picture: schizophrenia, stable schizophrenia, unipolar depression, borderline syndrome and samples of controls, matching with respect to age, sex and body mass<br>(Germany) | 726 | 0 | 0% |

| Substrate | Ig class | Sample characterisation<br>Clinical patient panels (origin of samples) | n | Prevalence | |
|---|---|---|---|---|---|
| | | | | Positive | % |
| **CASPR2**<br>Transfected cells<br>*(Serum/plasma)* | IgG | Samples from patients with known psychological disease picture: schizophrenia, stable schizophrenia, unipolar depression, borderline syndrome<br>Samples of controls, matching with respect to age, sex and body mass<br>(Germany) | 726 | 0 | 0% |
| | | Hashimoto's thyroiditis<br>(Germany) | 45 | 0 | 0% |
| | | Panel of healthy blood donors<br>(Germany) | 156 | 0 | 0% |
| **CASPR2**<br>Transfected cells<br>*(CSF)* | IgG | Patients with pathologically and clinically secured autoimmune encephalitis (origin: Netherlands). For these patients, only results from CSF samples are available.* | 21 | 1 | 4.8% |
| **AMPAR1/R2**<br>Transfected cells<br>*(Serum/plasma)* | IgG | Samples from patients with known psychological disease picture: schizophrenia, stable schizophrenia, unipolar depression, borderline syndrome<br>Samples of controls, matching with respect to age, sex and body mass<br>(Germany) | 726 | 0 | 0% |
| | | Hashimoto's thyroiditis<br>(Germany) | 45 | 0 | 0% |
| | | Panel of healthy blood donors<br>(Germany) | 18 | 0 | 0% |
| **DPPX**<br>Transfected cells<br>*(Serum/plasma)* | IgG | Samples from patients with known psychological disease picture: schizophrenia, stable schizophrenia, unipolar depression, borderline syndrome<br>Samples of controls, matching with respect to age, sex and body mass<br>(Germany) | 726 | 0 | 0% |
| | | Hashimoto's thyroiditis<br>(Germany) | 45 | 0 | 0% |
| | | Panel of healthy blood donors<br>(Germany) | 5 | 0 | 0% |

* Literature reference: Maat P, de Beukelaar JW, Jansen C, Schuur M, van Duijn CM, van Coevorden MH, de Graff E, Titulaer M, Rozemuller AJ, Sillevis Smitt P. **Pathologically confirmed autoimmune encephalitis in suspected Creutzfeldt-Jakob disease**. Neurol Neuroimmunol Neuroinflamm. 2015 Nov 12;2(6):e178

**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 111a-####-3 | IIFT Mosaic: Cerebellum (Rat) / Hippocampus (Rat) | – | – |
| FA 111m-####-3 | IIFT: Glutamate Receptor Mosaic 3 | – | – |
| FA 112d-####-6 | IIFT: Autoimmune Encephalitis Mosaic 6 | EPM Live | EPM Live |
| FA 112d-####-51 | Anti-Glutamate receptor (type NMDA) IIFT | EPM Live | EPM Live |
| FA 112k-####-53 | Anti-Glutamate Receptor (Type AMPA1/2) IIFT | EPM Live | EPM Live |
| FA 112l-####-50 | Anti-$GABA_B$ receptor IIFT | EPM Live | EPM Live |
| FA 112m-####-50 | Anti-DPPX IIFT | EPM Live | EPM Live |
| FA 1439-####-1 | IIFT: Anti-VGKC associated proteins Mosaic 1 | EPM Live | EPM Live |
| FA 1439-####-50 | Anti-Contactin-associated protein 2 IIFT | EPM Live | EPM Live |
| FA 1439-####-51 | Anti-Leucine-rich glioma-inactivated protein 1 (LGI1) IIFT | EPM Live | EPM Live |

## Clinical significance

Autoantibodies against neuronal surface antigens are found in patients with autoimmune encephalopathies. The antibodies are directed against glutamate receptors (type NMDA and type AMPA), $GABA_B$ receptors, voltage-gated potassium channels (VGKC) or VGKC-associated proteins (LGI1, CASPR2, TAG-1/contactin-2). Since these antigens play a direct or indirect role in synaptic signal transduction and plasticity, the associated autoimmunities manifest with seizures and neuropsychiatric symptoms. The resulting conditions include special forms of autoimmune limbic encephalitis, neuromyotonia or Morvan's syndrome.

These severe, potentially lethal syndromes can have a non-paraneoplastic or paraneoplastic aetiology. The frequency of underlying tumours ranges from 10 to 70%, depending on the type of antibody. The antibodies most likely play a causal role in the pathogenesis.

Since appropriate therapy (immunomodulatory intervention, tumour resection) results in considerable regression of symptoms in most patients, early diagnosis is important for a favourable prognosis.

The diagnosis of autoimmune encephalitis is generally based on a combination of the characteristic clinical picture, supporting findings from brain MRT, EEG and CSF analysis if necessary, and antibody determination in serum/CSF.
The specific autoimmune reactions have five characteristics:

1. The epitopes are extracellular.
2. Transfected cells containing the target antigen show an antigen-antibody reaction.
3. The antibodies change the structure and function of the attacked neuronal antigens.
4. The effects are often reversible.
5. The clinical symptoms resemble those in pharmacological or genetic antigen changes.

Monospecific recombinant assays are the method of choice for serological and CSF diagnostics and can be combined with conventional immunohistochemical detection procedures.

The following conditions must be excluded by differential diagnostics: infectious encephalitis (especially HSV), other autoimmune aetiologies (e.g. limbic encephalitis with autoantibodies against Hu, Ma2, CV2, amphiphysin) and clinically similar diseases of the central and/or peripheral nervous system. It should be taken into account that overlap syndromes and combinations of different syndromes can also occur. When a positive serological result is obtained, a comprehensive tumour investigation should be undertaken.

| Name | Alternative description | Antigen (MW) | Function | Neurological syndrome | Frequently associated tumours |
|---|---|---|---|---|---|
| Anti-glutamate receptor (type NMDA) | | Extracellular domains of NR1 subunit of receptor (approx. 105 kDa) | Cation channel, synaptic (glutamatergic) signal transmission, synaptic plasticity | Anti-glutamate receptor (type NMDA) encephalitis (approx. 60% as PNS) | Ovarian teratoma, Testicular teratoma |
| Anti-glutamate receptor (type AMPA) | Anti-GluR1/ Anti-GluR2 | GluR1 and GluR2 subunits of receptor (each approx. 100 kDa) | Cation channel, synaptic (glutamatergic) signal transmission, synaptic plasticity | Limbic encephalitis (approx. 70% as PNS) | Bronchial carcinoma, breast carcinoma, thymoma |
| Anti-$GABA_B$ receptor | Anti-GABARB1/B2 | Genuine $GABA_{B1}$ and $_{B2}$ subunits of human receptor (approx. 108 and 106 kDa) | Synaptic (GABAergic) signal transmission, synaptic plasticity | Limbic encephalitis (approx. 50% as PNS) | SCLC |
| Anti-LGI1 | | LGI1 (approx. 60 kDa) | Component of trans-synaptic complex involved in synaptic signal transmission | Limbic encephalitis (approx. 10% as PNS) | Thyroid carcinoma, SCLC, kidney cell carcinoma, ovarian teratoma, thymoma |
| Anti-CASPR2 | | CASPR2 (approx. 180 kDa) | Component of ad-hesion complex for VGKC localisation in juxtaparanodes of myelinated axons | Neuromyotonia, Morvan's syndrome, limbic encephalitis (30% as PNS) | Thymoma |
| Anti-DPPX | | Dipeptidyl aminopeptidase-like protein 6 | Regulator of membrane excitability in hippocampal CA1 pyramid cells | Anti-DPPX-associated autoimmune encephalitis | |

**Abbreviations:**

| | |
|---|---|
| AMPA | α-amino-3-hydroxy-5-methyl-4-isoxazol-propionic acid |
| CASPR2 | Contactin-associated protein 2 |
| DPPX | Dipeptidyl aminopeptidase-like protein 6 |
| GABA | γ-amino-butyric acid |
| LGI1 | Leucine-rich glioma-inactivated protein 1 |
| NMDA | N-methyl-D-aspartate |
| PNS | Paraneoplastic neurological syndrome |
| SCLC | Small-cell lung cancer |

**Autoantibodies against glutamate receptors (type NMDA)** are specific markers for anti-NMDA receptor encephalitis, an inflammatory encephalopathic autoimmune disease which was first described in 2007 and is currently a still widely underdiagnosed disease. The antibodies are directed against an extracellular epitope of the receptor subunit NR1 and can be determined in patient serum or CSF by immunohistochemical detection methods or recombinant assays.

The occurrence of these specific antibodies, immunopathological findings and the possibility of immunotherapeutic intervention suggest an immune-mediated pathogenesis for anti- NMDA receptor encephalitis. The antibody-mediated dysfunction of glutamatergic synapses also supports this assumption. In cell culture experiments on hippocampal neurons it could be demonstrated that the binding of the antibodies induced a reversible, titer-dependent reduction in glutamate receptors (type NMDA) on the neuronal cell surface. Furthermore, a pharmacological blockade of the receptors with NMDA antagonists causes clinical symptoms similar to those of anti- NMDA receptor encephalitis, in particular psychosis.

Anti- NMDA receptor encephalitis proceeds with a virtually stereotypical clinical course occurring in phases. A flu-like prodromal phase with subfebrile temperature, headache and fatigue occurs in 70% of cases. This is followed by a psychotic stage with severe behavioural and personality changes, delusions, disturbed thoughts and hallucinations, which occurs in 100% of patients. Because of these features a large proportion of patients end up in psychiatric therapy, and in many cases a drug-induced psychosis is initially diagnosed. In the following phase consciousness disorders, hypoventilation, epileptic attacks, autonomous instability and dyskinesia develop. Due to the severity of this disease (coma, status epilepticus, etc.) affected individuals must often be treated in intensive care for long periods of time.

About half of patients show irregularities in cerebral MRT. The EEG is pathologically altered in over 90% of persons with the disease. Investigation of CSF reveals mild lymphocytic pleocytosis in 90% of cases, intrathecal protein increase in 33% and oligoclonal bands in 25%. In the majority of mostly young female patients ovarian tumours (teratoma) are found, which amongst other things contain nerve cells. These cases involve a paraneoplastic syndrome (PNS) in anti- NMDA receptor encephalitis. The probability of an associated tumour disease is, on average, around 60%, although this is dependent on age and gender. Anti- NMDA receptor encephalitis is increasingly diagnosed not just in young women, but also in older female patients, in women without teratoma, in men (some with teratoma of the testis) and in children.

Prognosis for patients is improved with appropriate immunomodulatory therapy, and, in paraneoplastic syndrome, tumour detection and resection as early as possible. Convalescence can take a long time (up to several years) and result in regression of frontotemporal atrophy and hypoperfusion. Even in severe cases of anti- NMDA receptor encephalitis during pregnancy, immediate therapy measures can secure a favourable outcome for both mother and baby. In general, significant remission of symptoms is achieved in around 75% of patients. However, 25% of affected persons die or suffer from severe neurological deficits. Survivors have memory loss for the duration of the illness, and there is a risk of relapses of the encephalitis syndrome, the latter in particular when the tumour is removed too late or not at all or if no tumour could be found.

In general, antibodies against glutamate receptors (type NMDA; NR1) should be determined in all patients with encephalitis but no evidence of a causative organism and in suspected cases of limbic encephalitis or schizophrenic psychosis.

Around 10% of persons affected by acute schizophrenia are NMDA-positive with predominantly specific IgA and/or IgM. In particular disease forms (catatonic or disorganised schizophrenia) specific IgG may be detected in addition. When a positive serological result is obtained, a comprehensive teratoma investigation should always be undertaken. Alongside serum analysis, parallel investigation of CSF is of great significance, since in most patients intrathecal synthesis of anti- NMDA receptor antibodies is in the foreground. If immunomodulatory therapy has already been started, the antibody titer can be significantly decreased and may therefore no longer be detectable. Clinical improvement accompanies a reduction in antibody titer.

**Antibodies against the GluR1/GluR2 subunits of glutamate receptors (type AMPA)** are found in patients (>90% women, average age 60) with a special form of autoimmune-mediated limbic encephalitis. This is caused by an inflammatory brain disease which predominantly affects the mediotemporal structures (e.g. hippocampus) and the orbitofrontal cortex. Associated symptoms include progressive memory deficits, confusion, disorientation, lethargy, aggressive behaviour, hallucinations, epileptic fits and nystagmus. In some cases the clinical picture is limited to acute psychotic symptoms. Around 70% of affected individuals have a bronchial carcinoma, breast carcinoma or thymoma (with GluR1/GluR2 expression), which suggests a paraneoplastic aetiology of the encephalitis syndrome. Consequently, the detection of GluR1/GluR2 antibodies can be the first indication of an underlying tumour. In one of the patient panels so far investigated an overlap with other systemic autoimmune diseases (e.g. stiff-person syndrome, diabetes mellitus, Raynaud's syndrome, hypothyroidism) was observed in 50% of cases. 30% of patients also exhibited autoantibodies against GAD, CV2/CRMP5, VGCC and/or SOX1 in parallel to the tumour. In cerebral MRT 89% of patients show abnormal signal intensities in the region of the medial temporal lobes, while abnormalities in EEG occur in 75%. In addition, there are nearly always pathological changes in CSF (90% lymphocytic pleocytosis 70% intrathecal protein increase).

Immunotherapy (e.g. plasmapheresis, intravenous immunoglobulin, corticosteroids) and, in the case of a paraneoplastic syndrome, tumour resection/chemotherapy generally result in an improvement in symptoms. Noticeable, however, is a tendency (56%) to a recurrent course. In these cases neurological relapses can occur once or several times even in the absence of or after removal of the neoplasia. Without appropriate treatment or in cases of poor response to therapy, this form of limbic encephalitis can be fatal.
An antibody-mediated pathogenesis is suggested by both the success of immunomodulatory intervention and the observation that in cultured hippocampal neurones the application of antibodies leads to a reversible reduction in the receptor density on the synapses. Some individual cases suggest a correlation between the anti-GluR1/GluR2 antibody titer and the disease activity. The majority of patients investigated so far demonstrate an isolated reactivity to GluR1 (30%) or GluR2 (60%). The simultaneous presence of antibodies against both subunits is less frequent (10%). Therefore, in serum and CSF diagnostics both types of antibody (anti-GluR1, anti-GluR2) should be determined using monospecific test systems.

In general, antibodies against glutamate receptors (type AMPA; GluR1/GluR2) should be determined in all patients with encephalitis but no evidence of a causative organism and in suspected cases of limbic encephalitis. Where applicable, the analysis of these antibodies should also be taken into account in patients with rapidly progressive behavioural abnormalities suggestive of acute psychosis. Since intrathecal antibody synthesis may predominate, the parallel analysis of serum and CSF is advisable. When a positive serological result is obtained, a comprehensive tumour investigation should always be undertaken.

In general, **anti-$GABA_B$ receptor antibodies** should be determined in all patients with encephalitis but no evidence of a causative organism and in suspected cases of limbic encephalitis. Serum and CSF samples should be analysed in parallel, since intrathecal synthesis may occur even when the serum titer is low or absent. When a positive serological result is obtained, a comprehensive tumour investigation should be undertaken.

Autoantibodies against $GABA_B$ receptors were first identified in 15 patients with suspected paraneoplastic or immune-mediated limbic encephalitis. Almost all information available so far about the clinical significance of these antibodies originates from this case study. All patients (53% men, 47% women, average age 62 years) showed epileptic seizures, confusion and memory deficits.
In 87% of cases the seizures were the primary clinical symptom. In addition, strange behavioural patterns, psychoses, delusions, hallucinations, sleep and consciousness disorders, status epilepticus, coma and lethality were also observed. 47% of affected individuals had a tumour (mostly small-cell lung carcinoma), indicating a paraneoplastic aetiology of the neurological syndrome. In almost half of the patients, autoantibodies against GAD, TPO, VGCC and/or SOX1 were detected in addition to anti-$GABA_B$ receptor antibodies. 73% had abnormalities in cerebral MRT (mostly elevated FLAIR/T2 signal in the medial temporal lobes) and more than 90% showed epileptic potential in EEG, corresponding to the limbic dysfunction. 90% of CSF findings were abnormal due to the presence of lymphocytic pleocytosis (80%), elevated intrathecal protein (80%) and oligoclonal bands (10%). Immunotherapy and, if applicable, tumour resection/chemotherapy almost always resulted in alleviation of symptoms. An improved prognosis is achieved by early recognition of the syndrome and adequate treatment.

Antibody-mediated inhibition or destruction of $GABA_B$ receptors is considered a likely cause of the associated form of limbic encephalitis. This assumption is backed up by the success of immunomodulatory intervention and also the observation that $GABA_{B1}$ and $GABA_{B2}$ null mutants (mouse model) show symptoms corresponding to limbic encephalitis (e.g. spontaneous epileptic seizures, memory deficits, anxiety, increased sensitivity to pain, excessive movement). Antibodies against the $GABA_{B1}$ subunit were detected in patient serum and/or CSF in 100% of cases investigated so far, while anti-$GABA_{B2}$ antibodies were detected in only one case (7%). Therefore, the relevant epitopes are localised primarily in the $GABA_{B1}$ subunit.

**Antibodies against VGKC-associated proteins (LGI1, CASPR2)**
Recently it has been found that in most cases diagnosed as VGKC antibody-associated syndromes (autoimmune voltage-gated potassium channelopathies), the antibody epitopes are located to 80% on channel-bound proteins rather than the potassium channel subunits themselves. The actual target antigens are LGI1 (57.3%), CASPR2 (19.8%) and TAG-1/contactin-2 (5.2%). The antibodies from the remaining patients are either directed against subunits of the potassium channels (3.1%) or against currently unknown targets or are undetectable (18.8%).

**Autoantibodies against LGI1** were first detected monospecifically in 2010. In 89 to 100% of cases the patients had limbic encephalitis (approx. 65% men, average age 60 years). Morvan's syndrome, isolated neuromyotonia, isolated epilepsy and other neurological syndromes were diagnosed in isolated cases. The main symptoms were epileptic seizures (82 to 89%), memory deficits (85 to 100%) and confusion/disorientation (75%). Additional observations were high T2 signal intensities in one of the medial temporal lobes in cerebral MRT (56 to 84%), EEG abnormalities (76%), hyponatraemia (60 to 62%), myoclonia (40%), abnormal CSF findings with elevated intrathecal protein or lymphocytic pleocytosis (41%) and sleep disorders (29%). More rarely dysautonomy (15%), pain (11%), neuromyotonia (4%) or weight loss (4%) were found. The frequency of tumours (paraneoplastic syndrome) was relatively low at 0 to 11%. Associated tumours included thyroid carcinoma, small-cell lung carcinoma, kidney cell carcinoma, ovarian teratoma and thymoma. Immunotherapy (e.g. intravenous immunoglobulin, plasmapheresis, glycocorticoids) and tumour removal if applicable resulted in complete or substantial regression of symptoms in 80% of cases (relapses possible), while in individual cases neurological deficits remained. The lethality was 2 to 6%.

The effectiveness of immunotherapeutic intervention suggests that LGI1 antibodies have a pathogenic role. Antibody-mediated impairment of LGI1 function could lead to increased excitability, which could result in limbic encephalitis symptoms due to the predominantly hippocampal localisation of the antigen. In support of this, mutations in the LGI1 gene that cause a secretory or functional disorder in LGI1 are associated with a hereditary epileptic syndrome (ADLTE or ADPEAF). Moreover, loss of the LGI1 gene results in lethal epilepsy.

Anti-LGI1 antibodies should be investigated in particular in serum/CSF of patients with encephalitis but no evidence of a causative organism and in suspected cases of limbic encephalitis. A positive serological result is an indication for investigation for a possible tumour.

**Autoantibodies against CASPR2** were also first described in 2010 in a group of 19 patients (84% men, 16% women). Neuromyopathy or Morvan's syndrome was diagnosed in 53% of these cases, limbic encephalitis in 37% and epilepsy alone in 11%. Symptoms included neuromyotonia, memory deficits, seizures (53%), confusion/disorientation (42%), pain (37%), insomnia (32%), dysautonomy (32%), weight loss (32%) and hyponatraemia (11%). MRT signal increases in one of the medial temporal lobes were found in around a quarter of patients. About a third of cases could be attributed to paraneoplastic syndrome, mostly in connection with thymoma. In patients without tumours, immunomodulatory intervention and symptomatic treatment generally resulted in an improvement in symptoms and prognosis. Paraneoplastic cases were associated with low or no therapy success, poor prognosis and frequently (67%) with a fatal course.

Four anti-CASPR2-positive patients were identified in a further study. Of these patients one suffered from Morvan's syndrome, one from neuromyotonia, one from limbic encephalitis and one from encephalitis with seizures. The patient with limbic encephalitis also exhibited anti-LGI1 antibodies. No tumour could be identified in any of the four cases. Notably, the frequency of anti-CASPR2-positive sera in patients with neuromyotonia or limbic encephalitis was only 2 to 3% in this study.

With regard to the pathophysiology, it is assumed that CASPR2 autoantibodies cause a quantitative decrease in the CASPR2-VGKC complexes on the axons of the peripheral nerves, leading to neurological syndromes.

The determination of antibodies against CASPR2 is advisable in patients with encephalitis but no evidence of a causative organism and in suspected cases of autoimmune acquired neuromyopathy, Morvan's syndrome or limbic encephalitis. Anti-CASPR2-positive patients should be investigated for the presence of a neoplasia.

**Autoantibodies against DPPX**

DPPX (dipeptidyl aminopeptidase-like protein 6) is a marker for autoimmune encephalitis. DPPX, which is mainly produced in brain tissue, interacts with the voltage-gated potassium channel Kv4. By co-expression of DPPX with Kv4-subunits, proteins interacting with the A type potassium channel (KChIPs) are synthesised. DPPX is an important regulator of membrane excitability in hippocampal CA1 pyramid cells. Autoimmunological reactions against DPPX lead to autoimmune encephalitis.

The diagnosis of autoimmune encephalitis is based on a combination of the characteristic clinical picture, supporting findings from brain MRT, EEG and CSF analysis if necessary, and antibody determination in serum/CSF. The main symptoms of anti-DPPX-associated encephalitis are restlessness, forgetfulness, confusion, hallucinations, muscle spasms, tremor and pleocytosis (in CSF). Overlapping and combinations of different symptoms must be taken into account. A positive serological result should not exclude a tumour investigation.

## Literature


- Bettler B, Kaupmann K, Mosbacher J, Gassmann M. **Molecular structure and physiological functions of GABA(B) receptors.** Physiol Rev 84 (2004) 835-867.
- Boronat A, Gelfand JM, Gresa-Arribas N, Jeong HY, Walsh M, Roberts K, Martinez-Hernandez E, Rosenfeld MR, Balice-Gordon R, Graus F, Rudy B, Dalmau J. **Encephalitis and antibodies to dipeptidyl-peptidase-like protein-6, a subunit of Kv4.2 potassium channels.** Ann Neurol 73 (2013) 120-128.
- EUROIMMUN AG. Saschenbrecker S, Rentzsch K, Probst C, Komorowski L, Stoecker W. **Auto-antikörperdiagnostik in der Neurologie mittels nativer und rekombinanter Antigen-substrate.** Der Nervenarzt 84 (2013) 471-476.
- EUROIMMUN AG, Wandinger KP. **Klinik und Labordiagnostik paraneoplastischer neurologischer Syndrome.** MTA Dialog 4 (2008) 266-270.
- EUROIMMUN AG. Stöcker W, Schlumberger W. **Alle Beiträge zum Thema Autoimmundiagnostik.** In: Gressner A, Arndt T. (Hrsg.). Lexikon der Medizinischen Laboratoriumsdiagnostik. 2. Aufl., Springer Medizin Verlag, Heidelberg (2012).
- Finke C, Kopp UA, Prüss H, Dalmau J, Wandinger* KP, Ploner CJ. (*EUROIMMUN AG). **Cognitive deficits following anti-NMDA receptor encephalitis.** J Neurol Neurosurg Psychiatry 83 (2012) 195-198.
- Finke C, Prüss H, Scheel M, Ostendorf F, Harms L, Borowski* K, Wandinger* KP, Ploner CJ. (*EUROIMMUN AG). **Anti-NMDA receptor antibodies in a case of MELAS syndrome.** J Neurol 259 (2012) 582-584.
- Fukata Y, Lovero KL, Iwanaga T, Watanabe A, Yokoi N, Tabuchi K, Shigemoto R, Nicoll RA, Fukata M. **Disruption of LGI1-linked synaptic complex causes abnormal synaptic transmission and epilepsy.** Proc Natl Acad Sci U.S.A. 107 (2010) 3799-3804.
- Kim J, Nadal MS, Clemens AM, Baron M, Jung SC, Misumi Y, Rudy B, Hoffman DA. **Kv4 accessory protein DPPX (DPP6) is a critical regulator of membrane excitability in hippocampal CA1 pyramidal neurons.** J Neurophysiol 100 (2008) 1835-1847.
- Kumar MA, Jain A, Dechant VE, Saito T, Rafael T, Aizawa H, Dysart KC, Katayama T, Ito Y, Araki N, Abe T, Balice-Gordon R, Dalmau J. **Anti-N-methyl-D-aspartate receptor encephalitis during pregnancy.** Arch Neurol 67 (2010) 884-887.

- Lai M, Hughes EG, Peng X, Zhou L, Gleichman AJ, Shu H, Mata S, Kremens D, Vitaliani R, Geschwind MD, Bataller L, Kalb RG, Davis R, Graus F, Lynch DR, Balice-Gordon R, Dalmau J. **AMPA receptor antibodies in limbic encephalitis alter synaptic receptor location.** Ann Neurol 65 (2009) 424-434.
- Lancaster E, Lai M, Peng X, Hughes E, Constantinescu R, Raizer J, Friedman D, Skeen MB, Grisold W, Kimura A, Ohta K, Iizuka T, Guzman M, Graus F, Moss SJ, Balice-Gordon R, Dalmau J. **Antibodies to the GABA(B) receptor in limbic encephalitis with seizures: case series and characterisation of the antigen.** Lancet Neurol 9 (2010) 67-76.
- Misawa T, Mizusawa H. **Anti-VGKC antibody-associated limbic encephalitis/Morvan syndrome.** Brain Nerve 62 (2010) 339-345.
- Mombereau C, Kaupmann K, Froestl W, Sansig G, Van der PH, Cryan JF. **Genetic and pharmacological evidence of a role for GABA(B) receptors in the modulation of anxiety- and antidepressant-like behavior.** Neuropsychopharmacology 29 (2004) 1050-1062.
- Nobile C, Michelucci R, Andreazza S, Pasini E, Tosatto SC, Striano P. **LGI1 mutations in autosomal dominant and sporadic lateral temporal epilepsy.** Hum Mutat 30 (2009) 530-536.
- Prüss H, Dalmau J, Harms L, Höltje M, Ahnert-Hilger G, Borowski* K, Stoecker* W, Wandinger* KP. (*EUROIMMUN AG). **Retrospective analysis of NMDA receptor antibodies in encephalitis of unknown origin.** Neurology 75 (2010) 1735-1739.
- Prüss H, Höltje M, Maier N, Gomez A, Buchert R, Harms L, Ahnert-Hilger G, Schmitz D, Terborg C, Kopp U, Klingbeil* C, Probst* C, Kohler S, Schwab JM, Stöcker* W, Dalmau J, Wandinger* KP. (*EUROIMMUN AG). **IgA NMDA receptor antibodies are markers of synaptic immunity in slow cognitive impairment.** Neurology 78 (2012) 1743-1753.
- Seikel E, Trimmer JS. **Convergent modulation of Kv4.2 channel alpha subunits by structurally distinct DPPX and KChIP auxiliary subunits.** Biochemistry 48 (2009) 5721-5730.
- Sirerol-Piquer MS, Ayerdi-Izquierdo A, Morante-Redolat JM, Herranz-Perez V, Favell K, Barker PA, Perez-Tur J. **The epilepsy gene LGI1 encodes a secreted glycoprotein that binds to the cell surface.** Hum Mol Genet 15 (2006) 3436-3445.
- Tan KM, Lennon VA, Klein CJ, Boeve BF, Pittock SJ. **Clinical spectrum of voltage-gated potassium channel autoimmunity.** Neurology 70 (2008) 1883-1890.
- Tuzun E, Dalmau J. **Limbic encephalitis and variants: classification, diagnosis and treatment.** Neurologist. 13 (2007) 261-271.
- Vincent A, Irani SR. **Caspr2 antibodies in patients with thymomas.** J Thorac Oncol 5 (2010) S277-S280.
- Wandinger* KP, Dalmau J, Borowski* K, Probst* C, Fechner* K, Stöcker* W. (*EUROIMMUN AG). **Recombinant immunofluorescence assay for the detection of anti-glutamate receptor (type NMDA) antibodies in the differential diagnosis of autoimmune encephalopathies.** In: Conrad K et al. (Hrsg.). From Pathogenesis to Therapy of Autoimmune diseases: Autoantigens, Autoantibodies, Autoimmunity. Pabst Science Publishers (2009) 434-435.
- Wandinger* KP, Klingbeil C*, Gneiss C, Waters P, Dalmau J, Saschenbrecker* S, Borowski K*, Deisenhammer F, Vincent A, Probst* C, Stoecker* W. (*EUROIMMUN AG). **Neue serologische Marker zur Differentialdiagnose der Autoimmun-Enzephalitis/New serological markers for the differential diagnosis of autoimmune limbic encephalitis.** J Lab Med 35 (2011) 329-342.
- Wandinger* KP, Saschenbrecker* S, Stoecker* W, Dalmau J. (*EUROIMMUN AG). **Anti-NMDA-receptor encephalitis: A severe, multistage, treatable disorder presenting with psychosis.** J Neuroimmunol 231 (2011) 86-91.

## Liability

The test kit, including original accessories, must only be used in accordance with the intended use. EUROIMMUN does not assume liability for any other use (e.g. non-compliance with the instructions for use and improper use) and for resulting damages.

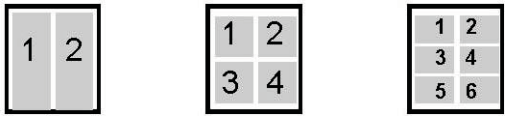
## Technical support

In case of technical problems you can obtain assistance via the EUROIMMUN website (https://www.euroimmun.de/en/contact/).

## Additional information

Regulatory information for customers in the European Union: Please observe the obligation to report any serious incidents occurring in connection with this test system to the competent authorities and to EUROIMMUN.

BIOCHIP position on the fields:




These instructions for use are valid for the following test kits (#### is a place holder for different test formats, e.g. 1005 = 10 slides with 5 fields):

| Order no. | Description | BIOCHIPs per field | | | | | | Field size (mm) |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | |
| FA 111a-####-3 | IIFT Mosaic: Cerebellum (Rat) / Hippocampus (Rat) | Cerebellum, rat | Hippocampus, rat | | | | | 5 x 5 |
| FA 111m-####-3 | IIFT: Glutamate Receptor Mosaic 3 | Hippocampus, rat | EU 90 | Cerebellum, rat | Glutamate receptor (type NMDA) | | | 5 x 5 |
| FA 112d-####-6 | IIFT: Autoimmune Encephalitis Mosaic 6 | Glutamate receptor (type NMDA) | Contactin-associated protein 2 (CASPR2) | Glutamate receptor (type AMPA1/2)* | Leucine-rich glioma-inactivated protein 1(LGI1)* | Dipeptidyl aminopeptidase-like protein 6 (DPPX)* | $GABA_B$ receptor | 5 x 5 |
| FA 112d-####-51 | Anti-Glutamate receptor (type NMDA) IIFT | Glutamate receptor (type NMDA) | EU 90 | | | | | 5 x 5 |
| FA 112k-####-53 | Anti-Glutamate Receptor (Type AMPA1/2) IIFT | Glutamate receptor (type AMPA1/2)* | EU 90 | | | | | 5 x 5 |
| FA 112l-####-50 | Anti-$GABA_B$ receptor IIFT | $GABA_B$ receptor | EU 90 | | | | | 5 x 5 |
| FA 112m-####-50 | Anti-DPPX IIFT | Dipeptidyl aminopeptidase-like protein 6 (DPPX)* | EU 90 | | | | | 5 x 5 |
| FA 1439-####-1 | IIFT: Anti-VGKC associated proteins Mosaic 1 | Leucine-rich glioma-inactivated protein 1 (LGI1)* | Contactin-associated protein 2 (CASPR2) | EU 90 | | | | 5 x 5 |
| FA 1439-####-50 | Anti-Contactin-associated protein 2 IIFT | Contactin-associated protein 2 (CASPR2) | EU 90 | | | | | 5 x 5 |
| FA 1439-####-51 | Anti-Leucine-rich glioma-inactivated protein 1 (LGI1) IIFT | Leucine-rich glioma-inactivated protein 1 (LGI1)* | EU 90 | | | | | 5 x 5 |

For clinical evaluation, the results must be confirmed with a CE-labelled test in the case of the labelled substrates:
* (confirmation required if sample material is CSF)

## 研究用試薬