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EUROLINE Paraneoplastic Neurologic Syndromes 12 Ag (IgG)
Test instruction

ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
DL 1111-1601-7 G DL 1111-6401-7 G DL 1111-5001-7 G	Amphiphysin, CV2, PNMA2 (Ma2/Ta), Ri, Yo, Hu, recoverin, SOX1, titin, zic4, GAD65 and Tr (DNER)	IgG	Ag-coated immunoblot strips	16 x 01 (16) 64 x 01 (64) 50 x 01 (50)

Indications: The EUROLINE test kit provides qualitative in vitro determination of human autoantibodies of the immunoglobulin class IgG to 12 different antigens: **amphiphysin, CV2, PNMA2 (Ma2/Ta), Ri, Yo, Hu, recoverin, SOX1, titin, zic4, GAD65 and Tr (DNER)** in serum or plasma to support the diagnosis of paraneoplastic neurological syndromes (PNS).

Application: The determination of autoantibodies is of major importance in the diagnosis of paraneoplastic syndromes (PNS). In suspected cases of PNS, all established and well characterised antibodies should therefore be investigated. The laboratory analysis should be based on two different test methods (immunoblot/line assay and immunohistochemistry). The EUROLINE Paraneoplastic Neurologic Syndromes 12 Ag (IgG) is the first test to allow automated analysis of 12 specific neurological antibodies, including well characterised antibodies, on one test strip.

Principles of the test: The test kit contains test strips coated with parallel lines of highly purified antigens and antigen fragments. In the first reaction step, the immunoblot strips are incubated with diluted patient samples. In the case of positive samples, the specific IgG antibodies (also IgA and IgM) will bind to the corresponding antigenic site. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalysing a colour reaction.
The format DL 1111-5001-7 G belongs to the Immunoblot-PreQ system. The test strips are already placed into the incubation trays (EUROTray).

Contents of the test kit:

Component	Format	Format	Format	Symbol
1. Test strips coated with the antigens Amphiphysin, CV2, PNMA2 (Ma2/Ta), Ri, Yo, Hu, recoverin, SOX1, titin, zic4, GAD65 and Tr (DNER)	16 strips	4 x 16 strips	5 x 10 strips in EUROTrays	STRIPS
2. Positive control (IgG, human), 100x concentrate	1 x 0.02 ml	4 x 0.02 ml	5 x 0.1 ml	POS CONTROL 100x
3. Enzyme conjugate Alkaline phosphatase-labelled anti-human IgG (goat), 10x concentrate	1 x 3 ml	4 x 3 ml	---	CONJUGATE 10x
4. Enzyme conjugate Alkaline phosphatase-labelled anti-human IgG (goat), ready for use	---	---	4 x 30 ml	CONJUGATE
5. Sample buffer ready for use	1 x 100 ml	3 x 100 ml	2 x 100 ml	SAMPLE BUFFER
6. Wash buffer 10x concentrate	1 x 50 ml	1 x 100 ml	1 x 100 ml	WASH BUFFER 10x
7. Substrate solution Nitroblue tetrazolium chloride/5-Bromo-4-chloro-3-indolylphosphate (NBT/BCIP), ready for use	1 x 30 ml	4 x 30 ml	4 x 30 ml	SUBSTRATE
8. Incubation tray	2 x 8 channels	---	---	
9. Test instruction	1 booklet	1 booklet	1 booklet	

LOT Lot description — CE — Storage temperature

IVD In vitro diagnostic medical device — Unopened usable until

Modifications to the former version are marked in grey.

The following components are not provided in the test kits but can be ordered at EUROIMMUN under the respective order numbers.
Performance of the test requires an **incubation tray**:
ZD 9895-0130 Incubation tray with 30 channels
ZD 9898-0144 Incubation tray with 44 channels (black, for the EUROBlotOne and EUROBlotCamera system)

If using Immunoblot-PreQ (DL 1111-5001-7 G), no additional incubation tray is needed.

For the creation of work protocols and the evaluation of incubated test strips using **EUROLineScan** green paper and adhesive foil are required:
ZD 9880-0101 Green paper (1 sheet)
ZD 9885-0116 Adhesive foil for approx. 16 test strips
ZD 9885-0130 Adhesive foil for approx. 30 test strips
If a **visual evaluation** is to be performed in individual cases, the required evaluation protocol can be ordered under:
ZD 1111-0101-7 G Visual evaluation protocol EUROLINE Paraneoplastic Neurologic Syndromes 12 Ag (IgG).

If using Immunoblot-PreQ (DL 1111-5001-7 G), the strips should stay in the EUROTray during evaluation. For the evaluation we generally recommend using a EUROIMMUN camera system connected to EUROLineScan software. Strips need to be dry before starting the evaluation.

Preparation and stability of the reagents

Note: This test kit may only be used by trained personnel. Test strips and incubation trays are intended for single use. All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. Unopened, reagents are stable until the indicated expiry date when stored at +2°C to +8°C. After initial opening, reagents are stable for 12 months or until the expiry date, unless stated otherwise in the instructions. Opened reagents must also be stored at +2°C to +8°C and protected from contamination.

- **Coated test strips:** Ready for use. Open the package with the test strips only when the strips have reached room temperature (+18°C to +25°C) to prevent condensation on the strips. After removal of the strips/Immunoblot-PreQ the package should be sealed tightly and stored at +2°C to +8°C.
- **Positive control:** The control is a 100x concentrate. For the preparation of the ready for use control the amount required should be removed from the bottle using a clean pipette tip and diluted 1:101 with sample buffer. Example: add 15 µl of control to 1.5 ml of sample buffer and mix thoroughly. The ready for use diluted control should be used at the same working day.
- **Enzyme conjugate:** The enzyme conjugate is supplied as a 10x concentrate. For the preparation of the ready for use enzyme conjugate the amount required should be removed from the bottle using a clean pipette tip and diluted 1:10 with sample buffer. For one test strip, dilute 0.15 ml enzyme conjugate with 1.35 ml sample buffer. The ready for use diluted enzyme conjugate should be used at the same working day.
- **Enzyme conjugate:** Ready for use.
  Note: Only for DL 1111-5001-7 G!
- **Sample buffer:** Ready for use.
- **Wash buffer:** The wash buffer is supplied as a 10x concentrate. For the preparation of the ready for use wash buffer the amount required should be removed from the bottle using a clean pipette tip and diluted 1:10 with distilled water. For one test strip, dilute 1 ml in 9 ml of distilled water. The ready for use diluted wash buffer should be used at the same working day.
- **Substrate solution:** Ready for use. Close bottle immediately after use, as the contents are sensitive to light.

Storage and stability: The test kit must be stored at a temperature between +2°C to +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Waste disposal: Patient samples, controls and incubated test strips should be handled as infectious waste. Other reagents do not need to be collected separately, unless stated otherwise in official regulations.

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Warning: The control of human origin has tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2. Nonetheless all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain sodium azide in a non-declarable concentration. Avoid skin contact.

Preparation and stability of the patient samples

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

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Sample dilution: The **patient samples** for analysis are diluted **1:101** with sample buffer using a clean pipette tip. For example, add 15 µl of sample to 1.5 ml sample buffer and mix well by vortexing. Sample pipettes are not suitable for mixing.

Incubation

If using Immunoblot-PreQ (DL 1111-5001-7 G), manual incubation is not possible. Please see below for options of automated incubation.

Pretreat:	Remove the required amount of test strips from the package and place them each in an empty channel (Make sure that the surface of the test strips is not damaged!). The number on the test strip should be visible. Fill the channels of the incubation tray according to the number of serum samples that should be tested with 1.5 ml sample buffer each. Use of Immunoblot-PreQ: Set up the required antigen profiles according to the work protocol and insert into the incubation device. Incubate for **5 minutes** at room temperature (+18°C to +25°C) on a rocking shaker. Afterwards aspirate off all the liquid.
Incubate: (1st step)	Fill each channel with 1.5 ml of the diluted serum samples using a clean pipette tip. Incubate for **30 minutes** at room temperature (+18°C to +25°C) on a rocking shaker.
Wash:	Aspirate off the liquid from each channel and wash **3 x 5 minutes** each with 1.5 ml working strength wash buffer on a rocking shaker.
Incubate: (2nd step)	Pipette 1.5 ml diluted enzyme conjugate (alkaline phosphatase-labelled anti-human IgG) into each channel. Incubate for **30 minutes** at room temperature (+18°C to +25°C) on a rocking shaker.
Wash:	Aspirate off the liquid from each channel. Wash as described above.
Incubate: (3rd step)	Pipette 1.5 ml substrate solution into the channels of the incubation tray. Incubate for **10 minutes** at room temperature (+18°C to +25°C) on a rocking shaker.
Stop:	Aspirate off the liquid from each channel and wash each strip **3 x 1 minute** with distilled water.
Evaluate:	Place test strip on the evaluation protocol, air dry and evaluate. Immunoblot-PreQ: The evaluation of the test strips is realised exclusively via the EUROIMMUN camera systems.

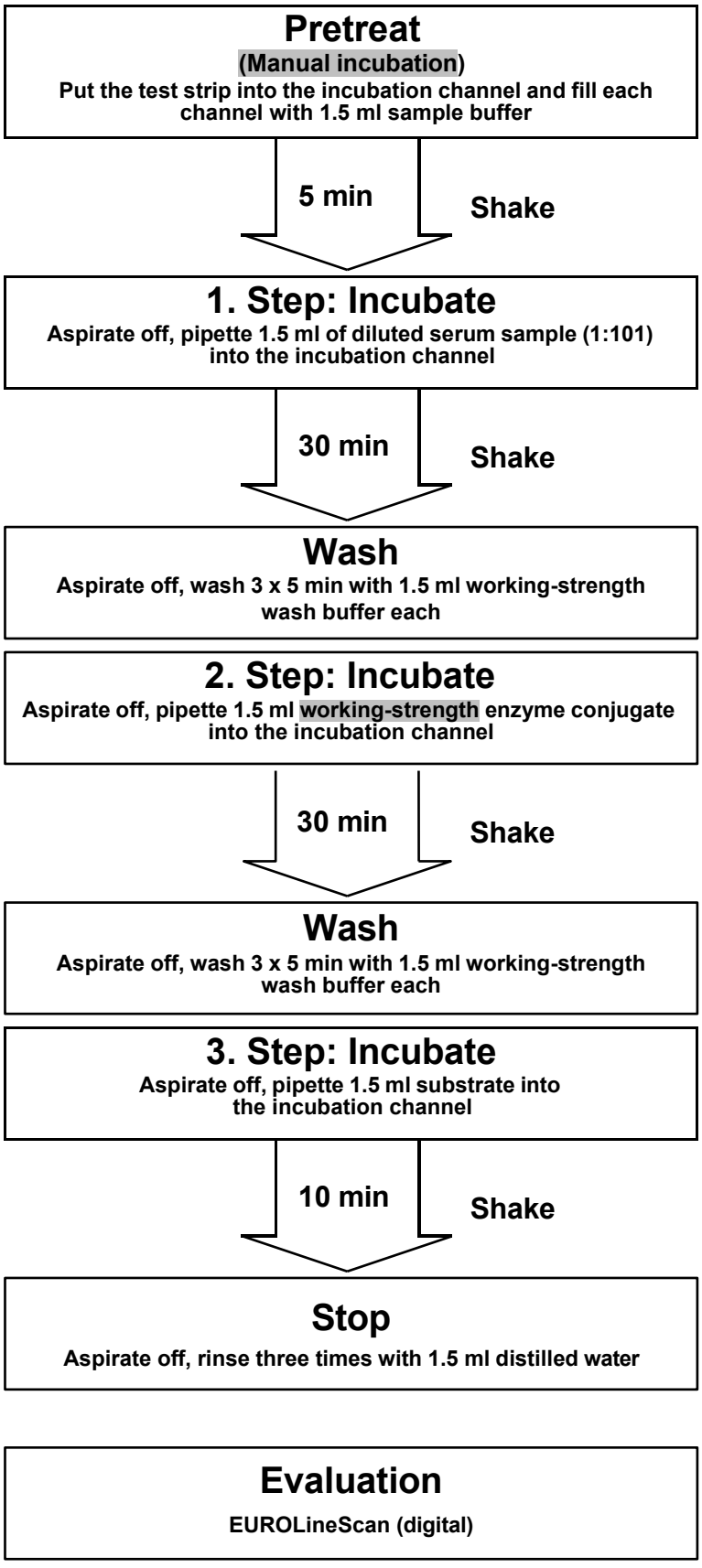
For automated incubation with the **EUROBlotMaster** select the program **Euro01 AAb EL30**.

For automated incubation with the **EUROBlotOne** select the program **Euro 01/02**.

For automated incubation of Immunoblot-PreQ with the **EUROBlotOne** see instruction manual EUROBlotOne (YG_0153_A_UK_CXX).

EUROLINE Paraneoplastic Neurologic Syndromes 12 Ag (IgG)

Incubation protocol



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Interpretation of results

Interpretation: According to the guidelines of the German Neurological Society, autoantibodies associated with paraneoplastic neurological syndromes should always be determined using at least 2 unrelated methods. Results yielded with the EUROLINE Paraneoplastic Neurologic Syndromes 12 Ag must be compared with the results obtained with indirect immunofluorescence. Only those results which show a plausible correspondence of both the two test systems and the neurological indication should be evaluated.

Handling: For the evaluation of incubated test strips we generally recommend using the **EUROLineScan** software. After stopping the reaction using deionised or distilled water, place the incubated test strips onto the adhesive foil of the green work protocol using a pair of tweezers. The position of the test strips can be corrected while they are wet. As soon as all test strips have been placed onto the protocol, they should be pressed hard using filter paper and left to air-dry. After they have dried, the test strips will be stuck to the adhesive foil. The dry test strips are then scanned using a flatbed scanner (EUROIMMUN) and evaluated with **EUROLineScan**. Alternatively, imaging and evaluation is possible directly from the incubation trays (EUROBlotCamera and EUROBlotOne). For general information about the EUROLineScan program please refer to the EUROLineScan user manual (YG_0006_A_UK_CXX, EUROIMMUN). The code for entering the **test** into EUROLineScan is **Neuro_PNS12**.

If a visual evaluation must be performed, place the incubated test strips onto the respective work protocol for visual evaluation. This protocol is available at EUROIMMUN under the order no. ZD 1111-0101-7 G.

If using Immunoblot-PreQ (DL 1111-5001-7 G), the strips should stay in the EUROTray during evaluation. For the evaluation we generally recommend using a EUROIMMUN camera system connected to EUROLineScan software. Strips need to be dry before starting the evaluation.

Note: Correct performance of the incubation is indicated by an intense staining of the control band.

Antigens and their arrangement on the strips: The EUROLINE test strips have been coated with the following antigens:

Amphiphysin: Amphiphysin I protein manufactured and purified using biochemical methods.

CV2: CV2 protein manufactured and purified using biochemical methods.

PNMA2 (Ma2/Ta): PNMA2 Protein (Ma2/Ta) manufactured and purified using biochemical methods.

Ri: NOVA1 protein (Ri, 55 kDa) manufactured and purified using biochemical methods.

Yo: Yo protein (cdr62) manufactured and purified using biochemical methods.

Hu: HuD protein manufactured and purified using biochemical methods.

Recoverin: Recoverin protein manufactured and purified using biochemical methods.

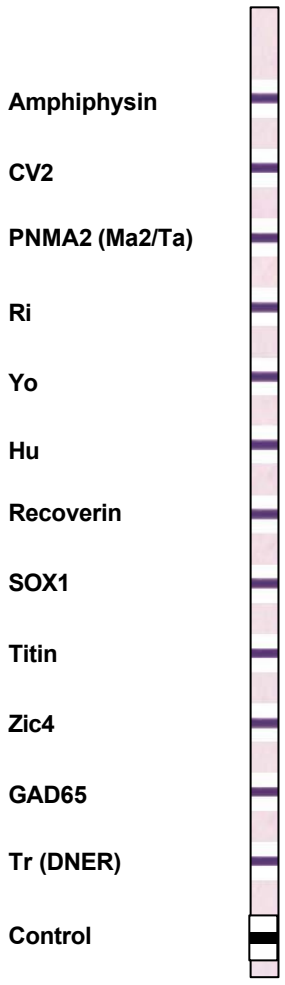
SOX1: SOX1 protein manufactured and purified using biochemical methods.

Titin: Titin protein (MGT-30 peptide) manufactured and purified using biochemical methods.

Zic4: Zic4 protein manufactured and purified using biochemical methods.

GAD65: GAD65 protein manufactured and purified using biochemical methods.

Tr (DNER): Tr protein manufactured and purified using biochemical methods.



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Neuronal antigens and their location:

Amphiphysin:	membrane of synaptic vesicles
CV2:	cytoplasm of oligodendrocytes
PNMA2 (Ma2/Ta):	neurons and testis
Ri/ANNA-2:	cell nuclei of neurons in the central nervous system
Yo/PCA-1:	purkinje cell cytoplasm (cerebellum)
Hu/ANNA-1:	cell nuclei and neurons of the central and peripheral nervous system
Recoverin:	photoreceptors of the retina
SOX1:	localised in cell nuclei of Bergmann glia in the Purkinje cell layer
Titin:	intracellular filamentous protein of striated muscles
Zic4:	neuronal cell nuclei (mainly granular layer of the cerebellum)
GAD65:	granular layer of the cerebellum
Tr (DNER):	cytoplasm of Purkinje cells (cerebellum)

EUROIMMUN recommends interpreting results based on the signal intensity:

Signal **Visual evaluation**	**Signal intensity** **EUROLineScan** **Flatbed scanner**	**Result**	
No signal	0-5	o	Negative
Very weak band	6-10	(+)	Borderline
Medium to strong band	11-25 or 26-50	+, ++	Positive
Very strong band with an intensity comparable to the control band	>50	+++	Strong positive

Results in the **borderline range** (+) should be evaluated as increased but negative. The table above contains **values** for the evaluation using a flatbed scanner. The **values** for other instruments supported by EUROLineScan can be found in the EUROLineScan program. To do so mark the corresponding assay in the test list (main menu “Help” → “Test”) and click on details and select **the corresponding instrument** in **“image source”**.

Caution! Exception: Borderline results for GAD65 should be interpreted as positive to achieve the highest possible sensitivity.

An indirect immunofluorescence test should always be performed in parallel with the EUROLINE for the determination of antibodies against neuronal antigens. On the one hand, this provides a check on plausibility as a safeguard against false-positive results, on the other hand, immunofluorescence permits the detection of a wider range of antibodies.

A **EUROIMMUN BIOCHIP Mosaic for neurology** is particularly suitable as indirect immunofluorescence test, using frozen sections of the following primate tissues: peripheral nerves, cerebellum, cerebrum, foetal intestine and liver (optional HEp-2 for the differentiation of systemic ANA).

Note: Not all of the target antigens of autoantibodies associated with paraneoplastic syndromes have yet been identified, purified and sufficiently validated.
The EUROLINE Paraneoplastic Neurologic Syndromes 12 Ag is not suitable for the detection of antibodies against GAD65 in diabetes mellitus. A negative result in the EUROLINE does not exclude the presence of Anti-GAD65.

For the medical diagnosis, the clinical symptoms of the patient and, if available, further findings should always be taken into account alongside the serological result. A negative serological result does not exclude the presence of a disease.

Test characteristics

Measurement range: The EUROLINE is a qualitative method. No measurement range is provided.

Cross reactions: The high analytical specificity of the test system is guaranteed by the quality of the antigen substrates used (antigens and antigen sources). This EUROLINE specifically detects IgG class antibodies against Amphiphysin, CV2, PNMA2 (Ma2/Ta), Ri, Yo, Hu, recoverin, SOX1, titin, zic4, GAD65 and Tr (DNER). No cross reactions with other autoantibodies have been found.

Interference: Haemolytic, lipaemic and icteric sera up to a concentration of 5 mg/ml haemoglobin, of 20 mg/ml triglycerides and of 0.4 mg/ml bilirubin showed no effect on the analytical results of the present EUROLINE.

Inter- and intra-assay variation: The inter-assay variation was determined by multiple analyses of characterised samples over several days. The intra-assay variation was determined by multiple analyses of characterised samples on one day. In every case, the intensity of the bands was within the specified range. This EUROLINE displays excellent inter- and intra-assay reproducibility.

Sensitivity and specificity: 101 serologically precharacterised sera from patients with paraneoplastic syndrome (PNS) and sera from at least 100 blood donors serving as the negative control group were tested for antibodies against neuronal antigens.

Antibodies	n	Sensitivity
anti-amphiphysin	2	100%
anti-CV2	4	100%
anti-PNMA2 (Ma2/Ta)	9	89%
anti-Ri (Nova1)	8	100%
anti-Yo (cdr62)	20	100%
anti-Hu (HuD)	27	100%
anti-recoverin	3	100%
anti-SOX1	8	100%
anti-titin	14	100%
anti-zic4	6	100%

The specificity of the test system which was calculated from the samples of blood donors, is at least 99%.

The sensitivity and specificity of anti-Tr (DNER) antibodies were determined by analysing 14 sera from patients with cerebellar degeneration (anti-Tr positive and PCA positive in the IFT) and 212 sera from control panels (blood donors (n = 150), autoimmune diseases (n = 40), cerebellar degeneration, anti-Tr negative (n = 22)). With a specificity of 100% in the control panels, 13 of the 14 precharacterised sera were identified (sensitivity 93%).

Method comparison anti-GAD65: Patients with stiff-person syndrome (SPS, n = 44), progressive encephalomyelitis with rigidity and myoclonia (PERM, n = 25), and other diseases (n = 31) (Neurology Clinic, University of Heidelberg) were investigated in a study using a CE-notified indirect immunofluorescence test with GAD65-transfected cells (EUROIMMUN AG) and this present EUROLINE.

Samples from patients with SPS, PERM and other diseases		GAD65 transfected cells (IIFT)	
		positive	negative
Anti-GAD65 in the EUROLINE	positive	40	0
	borderline	8	0
	negative	5	47

In comparison to the IIFT, the sensitivity for anti-GAD65 in the EUROLINE amounted to 90.6%, with a specificity of 100%.

The investigation of a panel of 150 healthy blood donors (UKSH Lübeck) for anti-GAD65 using the EUROLINE showed a prevalence of 0.7%.

Additionally, there are methods for the investigation of CSF which are validated for the EUROLINE Neuronal Antigens Profiles.
For this, please request the test instruction DL_1111-XLG_A_UK_ZXX respective.

Clinical significance

Serological diagnostics in neurological diseases using highly specific and sensitive test procedures for detection of associated neuronal autoantibodies (AAb) supplement the clinical disease criteria and contribute greatly to establishing a diagnosis.

PNS AAb represent a special group of AAb in the area of neurology, which are associated with paraneoplastic neurological syndromes (PNS). PNS are defined as neurological clinical syndromes that accompany malignant tumours, but are not directly caused by them or their metastases, are not of vascular or infectious origin and do not result from therapy-related side effects. They are caused by tumour-released immunological mechanisms, which take their effect distally from the primary and/or metastatic tumour site.

In literature, two types of nomenclature are used; one is based on the first two letters of the index patient's name (e.g. Hu for Hull, Yo for Young, Ma for Margret), the other on the initial letters of the immunohistochemical colouration (ANNA = antinuclear neuronal antibodies). We use the nomenclature of Posner (anti-Hu, -Yo, -Ma etc.), since this is antigen-based and independent of the test procedure.

Depending on the type of tumour, tumour cells express antigens, e.g. amphiphysin, CV2/CRMP5, PNMA2 (Ma2/Ta), Ri, Yo or Hu, which can induce the formation of specific AAb. These AAb bind to the respective antigens localised in the nervous tissue and are thus probably involved in neurological disorders, the so-called PNS.

It has been known since 1985, when the AAb anti-Hu was described for the first time, that more than two thirds of all PNS patients exhibit specific AAb in the serum. The antigen-antibody reactions not only prove the paraneoplastic aetiology to almost 100%, but they are also closely related to specific tumours which up until that time were undetected in these patients. PNS AAb detection is therefore very useful in the diagnosis of tumours in their early stages.

PNS occur in approx. 15% of malignant diseases, particularly in lung and gastric tumours. Paraneoplastic otoneuro-ophthalmological syndromes normally result from brain stem encephalitis and/or cerebellar degeneration. They can develop from different immune responses to onconeural proteins (e. g. anti-Hu, anti-Ri, anti-Yo) or to unknown antigens.

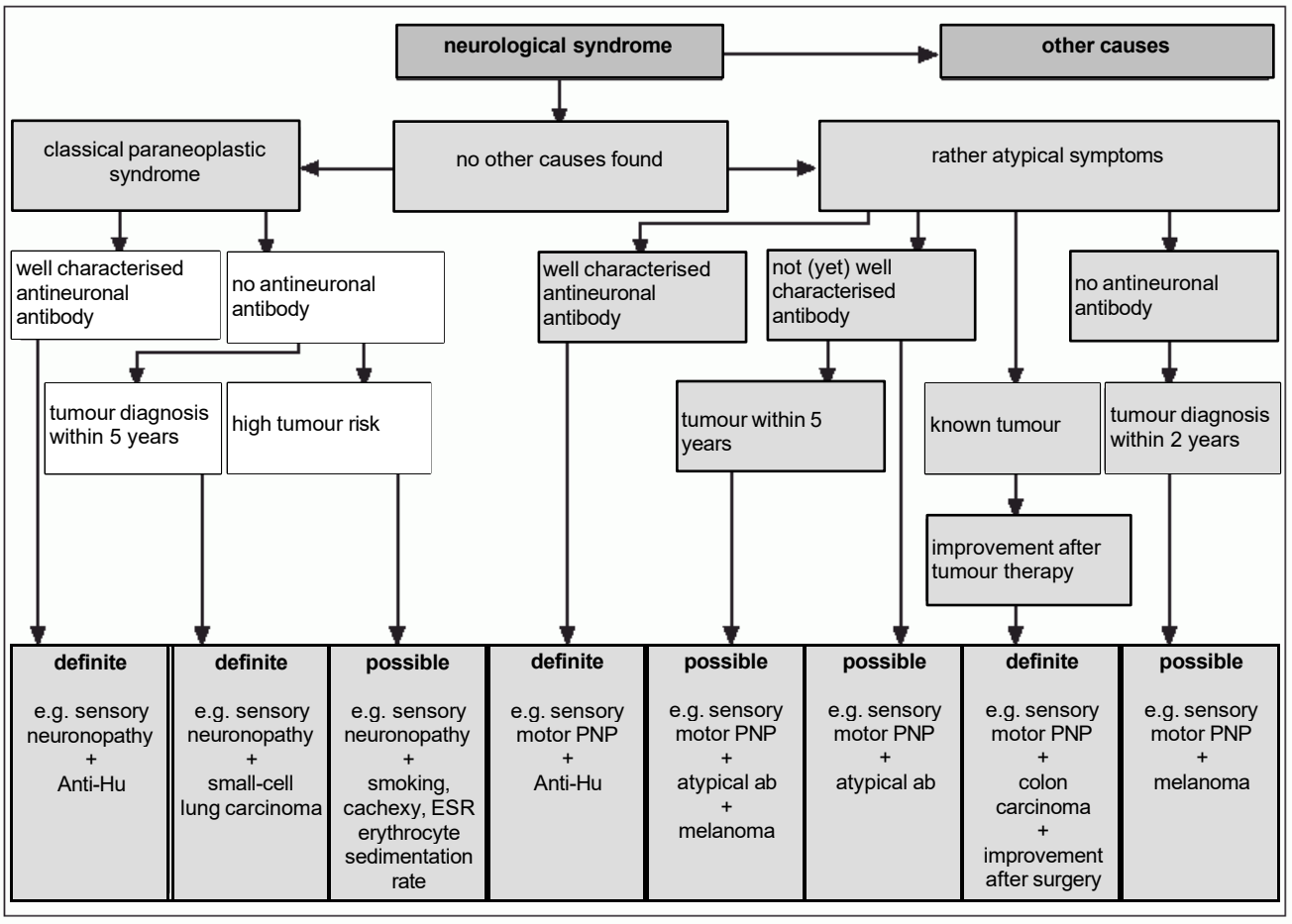
PNS occur most frequently in association with small-cell lung carcinoma (SCLC), breast or ovarian carcinoma. Lambert-Eaton myasthenic syndrome (LEMS), which is the most frequent PNS and can be diagnosed by determining Purkinje cell antibodies, has the highest predictive value with respect to an underlying tumour (here SCLC). In most PNS, particularly in paraneoplastic limbic encephalitis (PLE), paraneoplastic cerebellar degeneration (PCD), paraneoplastic opsoclonus myoclonus ataxia (POMA), stiff-person syndrome (stiff-man syndrome) and retinopathy (subacute amaurosis), the detection of specific antibodies has a much higher predictive value with respect to the presence of a tumour than the clinical symptoms by themselves.

PCD, for example, is caused by autoimmune mechanisms occurring during malignant diseases, most often in the very early stage of ovarian, breast or lung carcinoma and in Hodgkin's lymphoma. It is assumed that an immune reaction against identical antigens in the cerebellum and the tumour is taking place since paraneoplastic antibodies such as anti-Yo, anti-Hu, anti-Ri or anti-Tr can be detected both in the blood and cerebrospinal fluid. The association between cancer illnesses and cerebellar changes is shown by the fact that in IIFA anti-Yo positive serum reacts with Purkinje cells of the cerebellum as well as with carcinoma cells.

PNS not only accompany malignant tumours but may also occur in autoimmune diseases such as insulin-dependent diabetes mellitus (IDDM, diabetes mellitus type I), as demonstrated by the parallel occurrence of specific AAb against grey matter (GMAb) and AAb against islet cells (anti-ICA, ICAb). The determination of AAb against cerebellar glutamic acid decarboxylase (against the enzyme GAD) is important for the early diagnosis of diabetes mellitus type I and identification of individuals at risk, and for the diagnosis of neurological diseases such as stiff-person syndrome with Hodgkin’s lymphoma as associated tumour.

The European network for paraneoplastic neurological diseases (PNS Euronetwork) has published diagnostic criteria. These lead to two levels of diagnostic certitude, namely a **definitive** or a **possible** paraneoplastic syndrome.

Flow chart for the diagnosis of PNS



According to the guidelines of the German Society of Neurology, AAb in PNS should always be determined using at least two unrelated methods. In addition to indirect immunofluorescence with BIOCHIP Mosaics for neurology, the EUROLINE Profile Neuronal Antigens can be used to compare and confirm test results. Results should only be used for diagnosis when both test results are congruent in qualitative and/or quantitative determination and are in line with the clinical symptoms.

In the following table the basic PNS-relevant AAb are listed together with the neurological disease and the associated tumour.

Table:
PNS-relevant autoantibodies against <u>intracellular neuronal antigens</u> with associated neurological diseases and possibly associated tumours

Autoantibody (Synonym(s))	**Antigen** (Molecular mass(es)) Localisation (**L**)	**Associated diseases** Most frequent syndromes (**FS**) Other syndromes (**OS**)	**Associated tumours** Most frequent tumours (**FT**) Other tumours (**OT**)
Anti-Hu (ANNA-1, Anti-neuronal nuclear auto-antibody, type 1)	**Hu protein**, basic RNA-binding protein (38 kDa) **L:** Cell nuclei of neurons in the central and peripheral nervous system	**FS:** Encephalitides with focus in brain stem, cerebellum and limbic system; myelitis; polyneuropathy (autonomous, sensory, sensory-motor) **OS:** PCD, extrapyramidal motor syndromes, LEMS, focal epilepsy, POMA, chronic gastrointestinal pseudoobstruction, retinopathy	**FT:** SCLC, neuroblastoma **OT:** Prostate carcinoma, bladder carcinoma, ovarian carcinoma, breast carcinoma, pancreas carcinoma, gastrointestinal malignant tumours
Anti-Ri (ANNA-2, Anti-neuronal nuclear auto-antibody, type 2)	**NOVA,** Ri/NOVA1, RNA-binding proteins (55 kDa and 80 kDa) **L:** Cell nuclei of neurons in the central nervous system	**FS:** POMA **OS:** PCD, rhombencephalitis	**FT:** SCLC, breast carcinoma **OT:** Hodgkin‘s lymphoma, non-Hodgkin’s lymphoma, fast-growing brain stem tumour, ovarian carcinoma
Anti-Yo (PCA-1, Purkinje cell antibody 1)	**CDR2, CDR62,** Yo antigen, signal transduction proteins (34 kDa and 62 kDa) **L:** Cytoplasm (rough ER, Golgi apparatus, cytoplasm membrane) of Purkinje cells of the cerebellum	**FS:** PCD **OS:** POMA	**FT:** Ovarian, breast, uterine carcinoma **OT:** Adenocarcinoma of the oesophagus, gall bladder carcinoma, prostate carcinoma, Hodgkin’s lymphoma, non-Hodgkin’s lymphoma, thymoma
PCA-2	**Purkinje cell protein** (280 kDa)	**FS:** Encephalitis, neuropathy	**FT:** SCLC
Anti-Tr (PCA-Tr)	**DNER** (Delta/Notch-like Epidermal Growth Factor-Related Receptor)	**FS:** PCD	**FT:** Hodgkin‘s lymphoma
Anti-SOX1	**SOX1 protein**	**FS:** LEMS, PCD	**FT:** SCLC
Anti-PNMA1 (Ma1)	**Ma protein** (37 kDa)	**FS:** Rhombencephalitis (brain stem), limbic encephalitis	**FT:** Breast carcinoma **OT:** Various tumours
Anti-PNMA2 (Ma2/Ta)	**Ma protein** (40 kDa) **L:** Neuronal nucleoli	**FS:** Rhombencephalitis, PLE, brain stem encephalopathy **OS:** POMA, PCD, focal epilepsy, extrapyramidal motor syndromes, retinopathy	**FT:** Germinal carcinoma of the testis (seminoma) **OT:** Non-small-cell lung carcinoma, parotid gland carcinoma, breast carcinoma, ovarian carcinoma, colon carcinoma, kidney carcinoma, lymphoma

Autoantibody (Synonym(s))	**Antigen** (Molecular mass(es)) Localisation (**L**)	**Associated diseases** Most frequent syndromes (**FS**) Other syndromes (**OS**)	**Associated tumours** Most frequent tumours (**FT**) Other tumours (**OT**)
Anti-GAD	**Glutamic acid decarboxylase** (65 kDa and 67 kDa)	**FS:** SPS	**FT:** SCLC breast carcinoma, colon carcinoma
Anti-amphiphysin	**Amphiphysin** (128 kDa protein) **L:** Membrane of the synaptic vesicles	**FS:** SPS **OS:** Myasthenic syndrome, polyneuropathy (autonomous, sensory, sensory-motor), encephalomyelitis with rigidity, cerebellar ataxia, POMA	**FT:** Breast carcinoma, SCLC **OT:** Thymoma, Hodgkin's lymphoma, colon carcinoma
Anti-CV-2 (Anti-CRMP5)	**CV2/CRMP5** (66 kDa protein) **L:** Cytoplasm of oligodendrocytes	**FS:** Limbic encephalitis **OS:** PCD, polyneuropathy (autonomous, sensory, sensory-motor), retinopathy, uveitis, myasthenic syndrome, chronic gastrointestinal pseudoobstruction, chorea, POMA, romb-encephalitis, focal epilepsy	**FT:** SCLC, thymoma **OT:** Uterine sarcoma
Anti-AQP-4 (NMO IgG)	**Aquaporin-4 protein**	Neuromyelitis optica (NMO), LETM, rec. ON	-
Anti-NMDA receptors	Extracellular domain of the NR1 subunit of the receptor	Anti-glutamate receptor (type NMDA) encephalitis	Teratoma (ovary, testis)
Anti-AMPA receptors (GluR1 and GluR2)	GluR1 and GluR2 subunits of the receptor (each approx. 100 kDa)	Limbic encephalitis	Breast carcinoma, thymoma, lung carcinoma
Anti-mGluR1	Metabotropic glutamate receptor (approx. 140 kDa)	Cerebellar degeneration	Hodgkin's lymphoma
$GABA_B$ receptors	Extracellular domain of the $GABA_B$ subunit of the receptor	Limbic encephalitis	SCLC
Anti-LG1	Protein (60 kDa) associated with voltage-gated potassium channels (VGKC)	Limbic encephalitis	SCLC, ovarian teratoma, thymoma, various tumours
Anti-CASPR2	Protein associated with voltage-gated potassium channels (VGKC)	Limbic encephalitis, neuromyotonia, Morvan's syndrome	Thymoma, uterine carcinoma
Anti-recoverin	**Recoverin** (23 kDa protein)	**FS:** Retinopathy	**FT:** SCLC
Anti-titin	**Titin**	**FS:** Myasthenia gravis	**FT:** Thymoma

Antibodies against amphiphysin (128 kDa) are directed against the protein amphiphysin, which is responsible for vesicle endocytosis. The detection of antibodies against amphiphysin plays an important diagnostic role in paraneoplastic syndromes (e.g. Lambert-Eaton myasthenic syndrome, autonomous, sensitive, sensory-motor polyneuropathy, encephalomyelitis with rigidity, cerebellar syndrome, sensory neuronopathy, opsoclonus myoclonus ataxia, stiff-person syndrome), in small cell lung carcinoma, breast carcinoma, thymoma, colon carcinoma and in Hodgkin's lymphoma. Neuronal autoimmune responses can be linked to endocrinological reactions such as in stiff-person syndrome. Parallel occurrence of specific autoantibodies against grey matter (GMAb) and autoantibodies against islet cells (ICAb) is indicative of a connection between paraneoplastic syndrome and insulin dependent diabetes mellitus (IDDM).

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The detection of **antibodies against CV2** is of importance in the diagnosis of paraneoplastic syndromes (e.g. limbic encephalitis, focal epilepsy, opsoclonus myoclonus ataxia, rhombencephalitis, extrapyramidal motor syndromes, cerebellar degeneration, autonomous, sensitive, sensory-motor polyneuropathy), small cell lung carcinoma and thymoma.

The determination of **antibodies against PNMA2 (Ma2/Ta)** is relevant in the diagnosis of paraneoplastic syndromes (e.g., limbic encephalitis, focal epilepsy, opsoclonus myoclonus ataxia, rhombencephalitis, extrapyramidal motor syndromes, cerebellar degeneration), seminoma and other carcinomas.

The detection of **antibodies against Ri** is of diagnostic relevance in paraneoplastic syndromes (e.g. opsoclonus myoclonus ataxia, rhombencephalitis) and in rapidly growing brain stem tumours, small cell lung carcinoma, breast carcinoma and ovarian carcinoma.

The detection of **antibodies against Yo** plays an important role in the diagnosis of paraneoplastic syndromes (e.g. opsoclonus myoclonus ataxia, cerebellar degeneration), adenocarcinoma of the oesophagus, prostate carcinoma, uterine carcinoma, breast carcinoma, ovarian carcinoma and Hodgkin's lymphoma with antibodies against Purkinje cells, which decrease after successful lymphoma treatment.

The detection of **antibodies against Hu** is diagnostically relevant in paraneoplastic syndromes (e.g. retinopathy, encephalitides focussing on the brain stem, cerebellum and limbic system, focal epilepsy, opsoclonus myoclonus ataxia, rhombencephalitis, extrapyramidal motor syndromes, cerebellar degeneration, myelitis, autonomous, sensitive, sensory-motor polyneuropathy, mononeuropathy, motor neuron syndromes, Lambert-Eaton myasthenic syndrome), small cell lung carcinoma, prostate carcinoma and neuroblastoma.

The detection of **autoantibodies against recoverin** (retinal Ca^{2+} binding protein with a regulatory function in phototransduction) is diagnostically significant in tumour-associated retinopathy, a paraneoplastic syndrome that almost always occurs in connection with small-cell lung carcinoma (less frequent: endometrial carcinoma, thymoma, prostate carcinoma, etc).

Autoantibodies against SOX1 are frequently associated with Lambert-Eaton myasthenic syndrome, but also occur in paraneoplastic cerebellar degeneration as well as in paraneoplastic and nonparaneoplastic neuropathy. The detection of these antibodies may provide an indication of a causative neoplasia (small-cell lung carcinoma).

Autoantibodies against titin, a filamentous protein of striated muscle, occur in myasthenia gravis alongside acetylcholine receptor antibodies. In many patients with this neurological disease, the detection of titin antibodies is indicative of the additional presence of thymoma. The anti-titin serum titer is thought to correlate with the severity of myasthenia gravis.

Autoantibodies against Zic4, GAD65 and Tr (PCA-Tr, PCA2, DNER) are diagnostic markers for the serological diagnosis of paraneoplastic neurological syndromes (PNS). Antibodies against Tr and Zic4 occur with cerebellar degeneration. While anti-Tr is often associated with Hodgkin's lymphoma, anti-Zic4 is indicative for small-cell lung carcinoma. The detection of antibodies against GAD65 is relevant for the diagnosis of stiff-person syndrome and cerebellar ataxia.

研究用試薬

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