ravo Diagnostika

CE

ravo PNS-Blot
Recombinant Immunoblot
for the Detection of the Paraneoplastic Autoantibodies
anti-HuD, Anti-Yo, anti-Ri, anti-CV2 (anti-CRMP5),
anti-Amphiphysin, anti-Ma1 and anti-Ma2

8, 16 or 24 Determinations

Version 06/2013
Please pay attention to the differences in comparison to the previous version 05/2012

Page 4 and 5: **No more additional** conjugate for the positive control required. **Only one** conjugate for the positive control, patient specimen (serum samples and cerebrospinal fluid).
Page 4: Reagents: more detailed description
Page 5: Procedure: More detailed description of wash steps.

Summary:

Paraneoplastic neurological syndromes (PNS) are a group of neurological disorders associated with a tumor and its metastasis that are not the cause of the syndromes. An autoimmune process is considered as the underlying pathophysiological mechanism. Specific antineuronal autoantibodies can be detected in a majority of patients with PNS. Demonstration of the well characterized autoantibodies **anti-HuD, anti-Yo, anti-Ri, anti-CV2/CRMP5, anti-Amphiphysin, anti-Ma1 and anti-Ma2** in the presence of paraneoplastic neurological symptoms provides strong diagnostic evidence of a – possibly occult – neoplasm. In two-thirds of the cases, a paraneoplastic neurological syndrome precedes the discovery of an underlying neoplasm by up to five years. Thus, demonstration of paraneoplastic antineuronal autoantibodies may lead to an early discovery of cancer (1-10, table).

Table:

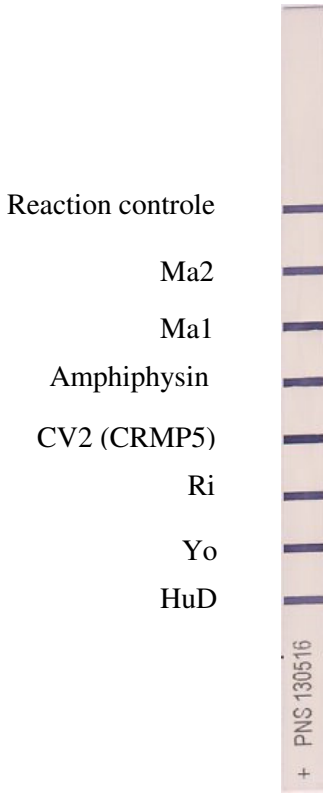
	Paraneoplastic neurological syndromes	**Most frequently associated tumors**
Anti-Hu-Antibodies (ANNA-1)	• **Sensory and autonomic neuropathy** • **Cerebellar ataxia** • **Encephalomyelitis** • **Limbic Encephalitis**	Small-cell-lung cancer Non-small-cell lung cancer Extrapulmonary small cell cancer
Anti-Yo-Antibodies (Purkinje-cell-antigen)	• **Cerebellar ataxia**	Breast cancer Ovarian cancer Uterus cancer
Anti-Ri-Antibodies (ANNA-2, anti-Nova-1)	• **Brainstem encephalitis (incl. Opsoclonus-Myoclonus-Syndrome)** • **Cerebellar ataxia**	Breast cancer Small-cell-lung cancer Medullary carcinoma of the thyroid gland
Anti-CV2-(CRMP5-) Antibodies	• **Sensory and sensorimotor neuropathy** • **Encephalomyelitis** • **Cerebellar ataxia** • **Limbic Encephalitis** • **Autonomic neuropathy** • **Chorea**	Small-cell-lung cancer Thymom
Anti-Amphiphysin-Antibodies	• **Stiff-person-syndrom** • **Various symptoms**	Breast cancer Small-cell-lung cancer
Anti-Ma1 and Anti-Ma2- (Ta-) Antibodies	• **Limbic Encephalitis** • **Brainstem encephalitis*** • **Cerebellar ataxia***	Testicular cancer Lung-cancers

* Brainstem encephalitis and cerebellar ataxia usually associated with tumors different from testicular and immunoreactivity against Ma2 and Ma1 proteins.

Generally, these autoantibodies have been identified by immunohistochemical techniques. Due to problems with specificity a positive result in immunohistochemistry needs to be confirmed by an immunoblot, employing crude extracts from neuronal tissue as antigen. Immunohistochemistry is also laborious and requires a high degree of experience for reliable interpretation.

Principle:

Nitrocellulose strips which are coated with the recombinant antigens HuD, Yo, Ri, CV2 (CRMP5), Amphiphysin, Ma1 and Ma2 are incubated with a specimen of patient serum. Specific antibodies in the specimen will bind to the antigens. Non specific molecules in serum specimens will be removed by washing the strips. Bound antibodies are detected by alkaline phosphate conjugated anti-human IgG using BCIP/NBT as substrate.



Reagents:

Component	Amount/Volume	Description
Nitrocellulose-Strips	8 (PNS001) 16 (PNS002) 24 (PNS003)	Nitrocellulose-Strips for the detection of IgG antibodies
Wash buffer	1 x 50 ml (PNS001) 2 x 50 ml (PNS002) 3 x 50 ml (PNS003)	Wash buffer, 20 x concentrated, blue screw cap
Dilution buffer for samples	1 x 25 ml (PNS001) 2 x 25 ml (PNS002) 3 x 25 ml (PNS003)	ready to use, contains 0,03% ProClin300, green screw cap
Positive control	1 x 2 ml (PNS001) 2 x 2 ml (PNS002) 3 x 2 ml (PNS003)	ready to use, contains ProClin300, purple screw cap
Conjugate for samples and positive control	1 x 20 ml (PNS001) 2 x 20 ml (PNS002) 3 x 20 ml (PNS003)	ready to use, Alkaline Phosphatase Conjugate contains 0,03% ProClin300, white screw cap
Substrate	1 x 20 ml (PNS001) 2 x 20 ml (PNS002) 3 x 20 ml (PNS003)	BCIP/NBT ready to use, black screw cap
Incubation trays	1 x (PNS001) 2 x (PNS002) 3 x (PNS003)	
Instruction for use	1 x	

Additional reagents available on request:

Negative Control Product No.:	2 ml PNSNKO	ready to use, contains 0,03% ProClin300 Colourless screw cap

Storage:
All kit components are stable until date of expiry stored at +4....8°C.

Reconstitution:

- Make sure all kit components are at room temperature before use.
- Dilute the wash buffer concentrate 1:20 with distilled water. **During storage at low temperatures, crystals may form in concentrated wash buffers, which can be dissolved by incubating the concentrate at 37°C for 30 minutes.** Let the solution cool down again to room temperature before use. Diluted wash buffer is stable for 4 weeks stored at +4 to +8°C.

- Dilute specimen **1: 2,000** in two steps in ready to use dilution buffer for samples
  1. Add 10 µl specimen to 90 µl ready to use dilution buffer, mix well. 2. See below
  The positive control is ready to use. No further dilution is necessary.

- The testing of cerebrospinal fluid (CSF) and the detection of intrathecal specific autoantibody synthesis is described on page 6

Procedure:

The nitrocellulose strips are labeled at the bottom (if not indicated otherwise). They must be incubated with the labels facing upwards and should be completely covered with fluid during all incubation steps.

Add the ready to use positive control (2ml) to one strip
Cover the other strips with 2 ml dilution buffer. Add 10 µl of the pre-diluted specimen and mix carefully (end-dilution: 1 : 2,000)
Incubate for 60 minutes at room temperature on a rocking table.

- wash with diluted wash buffer: Carefully remove the fluid using a pipet or pour away the fluid from each strip. Add ca. 2 ml of diluted wash buffer to each strip and shake for ca. 30 seconds. Repeat five times.

- Add 2 ml alkaline phosphatase IgG conjugate, ready to use, per strip.

Incubate for 30 minutes at room temperature on a rocking table.

- wash with diluted wash buffer: Carefully remove the fluid using a pipet or pour away the fluid from each strip. Add ca. 2 ml of diluted wash buffer to each strip and shake for ca. 30 seconds. Repeat five times.

- Incubate each strip in 2 ml ready to use substrate-solution.

Incubate for 25 minutes at room temperature until the bands become clearly visible. See control scan for comparison.

- Transfer the strips to distilled water to stop the reaction. Put the strips onto filter paper and let them dry. Interpret the results. Store the strips in the dark.

Testing of cerebrospinal fluid (CSF), detection of intrathecal specific autoantibody synthesis:

- Determination of the total IgG concentration of a serum-CSF-sample pair
- Dilute both, serum and CSF, in ready to use sample buffer to a concentration of 1 mg/liter as working solution (see example)
- Perform test in parallel as described above. Use 2 ml each of the respective calculated dilution.

Example:

1 mg/l	Determined Concentration of IgG	Dilution
CSF	97,3 mg/l	1: 97
Serum	10,8 g/l	1 : 10.000

Compare the intensity of the bands of the serum-CSF-pair.
A more intensive band for the CSF-sample hints at an intrathecal specific antibody synthesis. If the intensity of the serum-CSF-pair is almost equal, run of the test at an IgG- concentration of 0.1 mg/liter may be helpful for easier interpretation.

Interpretation:

Put the strips side by side to assign the proteins. Only strips from the same batch should be compared.
The positive control and control scan helps to assign the proteins, the documentation sheet to stick the strips on the latter for documentation.

Usually strong bands are observed in serum samples from patients with clinically defined paraneoplastic syndroms.
The meaning of weak reactions is still unknown with the exception of autoantibodies to HuD. For HuD- auto-antibodies weak reactions are described in 18% of patients suffering from small cell lung cancer without paraneoplastic neurological disease. Thus, in case of low concentrations of anti-Hu antibodies a thorough search for a possible underlying tumor is recommended. Concerning weak reactions with the other antigens there is nothing known at present about the incidence of underlying tumours so that no clear recommendation can be given.
At least a control of the antibody status is recommended in the course of the disease.

Ma1/Ma2 Reactivity

Ma1 - / Ma2 +:
If patient sera are reactive with the Ma2 antigen exclusively this hints at a testicular cancer as underlying malignancy.

Ma1 + /Ma2 + :
The presence of both antibodies specific for Ma1 and Ma2 antigens proofs a paraneoplastic etiology of a neurological disease but it does not hint at a special type of cancer.

Remarks:

- Avoid contact of the skin with substrate solution.

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Product-No: PNS001 (8 Determinations)
PNS002 (16 Determinations)
PNS003 (24 Determinations)

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