Data Sheet: ravo PNS Line Assays

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 neuronal autoantibodies can be detected in a majority of patients with PNS (see table).
According to the current diagnostic criteria (Graus et al. Updated Diagnostic Criteria for Paraneoplastic Neurological Syndromes. Neurol Neuroimmunol Neuroinflamm, Vol 8, July 2021) neuronal autoantibodies are divided into 3 groups according to the frequency of cancer associated:
Group 1: High-risk antibodies which are associated > 70% with cancer
Group 2: Intermediate-risk antibodies which are associated between 30% and 70% with cancer
Group 3: Lower-risk antibodies which are associated < 30% with cancer

The ravo Line Assays detect the following antineuronal antibodies (see table).

High-risk antibodies:	Anti HuD (ANNA-1), Yo (PCA-1), Ri (ANNA-2), CV2/CRMP5, Amphiphysin, Ma1, Ma2 (TA), SOX1, Tr (DNER)
Low-risk antibodies:	GAD65

Additionally antibodies to Zic4, PKCγ, Recoverin and Titin are detected.

Principle of the assays:

Membrane strips which are coated with highly purified and specific recombinant antigens HuD, Yo, Ri, CV2 (CRMP5), Amphiphysin, Ma1, Ma2, SOX1, Tr (DNER), Zic4, Titin (MGT30), Recoverin, PKCγ and GAD65 are incubated with a specimen of patient serum, plasma or cerebrospinal fluid.
Specific antibodies in the specimen will bind to the antigens. Nonspecific molecules in serum specimens will be removed by washing the strips. Bound antibodies are detected by conjugated anti-IgG using a corresponding specific substrate.

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** (Sensory and autonomic neuropathy, Encephalomyelitis, Polyradiculoneuropathy)	Breast cancer Small-cell-lung cancer
Anti-Ma1 and Anti-Ma2- (Ta-) Antibodies	• **Limbic Encephalitis** • **Brainstem encephalitis*** • **Cerebellar ataxia*** • **Diencephalitis**	Testicular cancer Lung-cancers
Anti-SOX1-Antibodies	• **Lambert Eaton Myasthenia gravis**	Small-cell-lung cancer
Anti-Tr (DNER)-Antibodies	• **Cerebellar truncal and limb ataxia**	Hodgkin Lymphoma
Anti-Zic4-Antibodies**	• **Cerebellar degeneration**	Small-cell-lung cancer
Anti-Titin-Antibodies	• **Myasthenia gravis**	Thymoma
Anti-Recoverin-Antibodies	• **Cancer Associated Retinopathy (CAR)**	Small-cell-lung cancer
Anti-PKCγ-Antibodies	• **Cerebellar degeneration**	Non-small-cell-lung cancer, Adenocarcinoma
Anti-GAD65-Antibodies	• **Stiff-Person-Syndrom** • **Limbic Encephalitis** • **Cerebellar ataxia**	Non paraneoplastic

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

** often associated with anti-HuD- and anti-CV2- (CRMP5-) and to lesser extent to anti-Ri-antibodies

Glossary:

The terms HuD, Yo, Ri, Tr, Ma and Ta refer to the initials of patients for whom these autoantibodies have been described for the first time.

HuD = ANNA-1	Antineuronal nuclear antibody-1
Yo = PCA1	Purkinje cell antibody 1
Ri = ANNA-2	Antineuronal nuclear antibody-2
CV2 (CRMP5)	Collapsing response.mediator protein 5
Ma1 = PNMA1	Paraneoplastic antigen Ma1
Ma2 = PNMA2 = Ta	Paraneoplastic antigen Ma2
SOX1	SRY-Box Transcription Factor 1
Tr (DNER)	Delta/Notch-like Epidermal Growth Factor-Related Receptor)
PKCγ	Protein Kinase C γ
Zic4	Zinc finger protein 4
GAD65	Glutamic acid decarboxylase 65

Automation of test performance:
PNS9 DIVER, PNS11 DVER and PNS14 DIVER

The manual versions of the ravo PNS Line Assays have been adapted to the BlueDiver instrument (BDI) and BlueDiver instrument II (BDI II) in close co-operation with the Belgian company D-tek.

BlueDIVER Instrument **BlueDIVER Instrument II**

Advantages of the *ravo* PNS DIVER Assays:

- Detection of 9, 11 or 14 important neuronal autoantibodies on one strip using one serum dilution
- Automatic sample pipetting (**BDI II** only)
- Serum sample dilution 1:140
- Testing of cerobrospinal fluid
- Fully automated test performance
- No cross contamination between samples
- Barcode identification of strips and cartridges
- A drying module is included in the **BDI II**. Strips are dried within 6 minutes in an air-flow
- Automatic reading of results
- Full traceability, from sample barcode identification to final result interpretation

Sensitivity:

Antigen	Number of samples	Positive %
HuD	49	100 %
Yo	31	100 %
Ri	19	100 %
CV2 (CRMP5)	93	100 %
Amphiphysin	19	100 %
Ma1	5	100 %
Ma2	14	100 %
SOX1	14	100 %
Tr (DNER)	14	100 %
Zic4	4	100 %
Titin (MGT30)	8	100 %
Recoverin	4	100 %
PKCγ	*	*
GAD65	10	100 %

* Only a few cases have been described so far. The only human clinically confirmed serum sample available gives a clear positive result with the recombinant PKCγ as well as a commercially available rabbit anti-PKCγ antibody.

Specificity:

200 samples of blood donors have been tested to determine the specificity of the assay. The specificity of the assays is 98 %.

Interfering substances:

No interference was observed for the following interfering substances

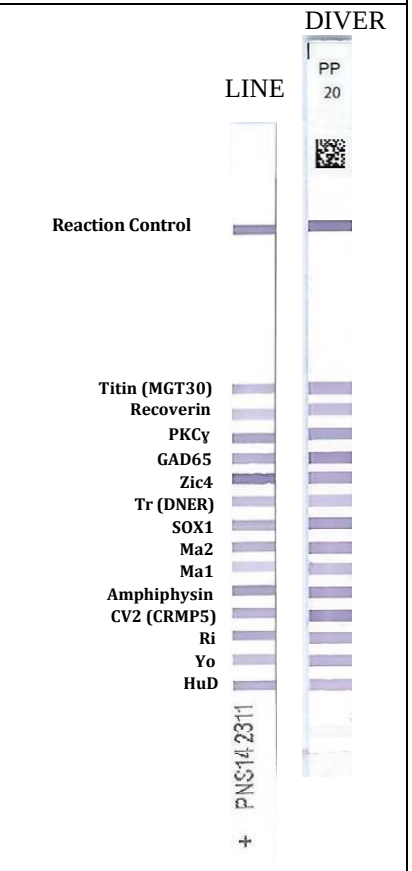
Interfering substance	Final concentration
Hemoglobin	2 mg /ml
Bilirubin	0,2 mg/ml
Triglycerides	32 mg/ml

External Quality Control: INSTAND e.V. and UK NEQAS

Instand e.V.:
In 2006 INSTAND e.V. introduced an external quality control for paraneoplastic autoantibodies. The respective distribution takes place once a year.
Since the beginning ravo participates in this external quality control.

UK NEQAS:
In November of 2010 UK NEQAS (United Kingdom National External Quality Assessment Service) introduced a pilot program for paraneoplastic autoantibodies. Respective samples are distributed six times per year.
Since the beginning ravo participates in this program.

Available PNS Line and DIVER Assays

Antigens	PNS Blot	PNS9 DIVER	PNS 11 Line Assay PNS11 DIVER	PNS 14 Line Assay and PNS14 DIVER	SOX1-Titin Line Assay*	Example: PNS 14 Line Assay and PNS14 DIVER
HuD (ANNA-1)	X	X	X	X		
Yo (PCA-1)	X	X	X	X		
Ri (ANNA-2, anti-Nova-1)	X	X	X	X		
CV2 (CRMP5-)	X	X	X	X		
Amphiphysin	X	X	X	X		
Ma1 (PNMA-1)	X	X	X	X		
Ma2 (Ta, PNMA-2)	X	X	X	X		
SOX1 (AGNA)		X	X	X	X	
Tr (DNER)			X	X		
Zic4			X	X		
Titin				X	X	
Recoverin				X		
PKCγ				X		
GAD65		X	X	X		

- ***Available on request only, minimum order quantity 25 testkits (24 determinations each)**

Automatic Reading using the B4C software from BioScitec

The manual PNS Line Assays can be read automatically using a scanner and the B4C software from the German company BioScitec. Further information and respective software licenses are available on request.

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