



PNS9 DIVER Blot IgG

Order Code: PNS9DIV-24

BlueDiver Protocol: 02

Version 07/2015

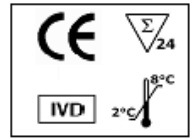
Please pay attention to the differences in comparison to the previous version 10/2014

Page 4: Kit contents: **positive control** added

Testing of cerebrospinal fluid CSF (pages 4-7)



IFU – Instructions for Use
PNS9DIV-24/p. 2 of 8



1 INTENDED USE AND SUMMARY

PNS9 DIVER BIOT IgG is a line assay kit intended for the detection of IgG autoantibodies against the following antigens in human sera/plasma and cerebrospinal fluid CSF: GAD 65, SOX1, Ma2, Ma1, Amphiphysin, CV2 (CRMP5), Ri, Yo and HuD.

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 (1-11, table).

SOX1: In up to 50 % of patients with Lambert-Eaton myasthenic syndrome (LEMS) – a disorder mediated by voltage-gated calcium channels (VGCC) - cancer is detected, almost always a small cell lung cancer (SCLC). Recently it has been shown that in 43 % of patients with LEMS and SCLC the detectable antibodies (AGNA = anti-glial nuclear antibodies) are directed to SOX1. SOX1 is a protein which plays a role in neuronal development and which is expressed in SCLC (12). In single cases autoantibodies to SOX1 have been detected in patients with paraneoplastic limbic encephalitis. Antibodies to SOX1 can also be detected in other paraneoplastic neurological syndromes in association with the well characterized antineuronal autoantibodies.

GAD65: Antibodies to GAD (glutamat-decarboxylase) are considered as serological markers for Stiff-Person-Syndrome a rare neurological autoimmune disease that is characterized by rigidity and episodic spasms of muscles as a result of continuous motor unit activity. The majority of patients have high titers of antibodies to both isoforms, GAD65 and GAD67. These enzymes catalyse the conversion of glutamate to GABA (γ -aminobutyric acid), a major inhibitory neurotransmitter of the CNS.

High titer GAD antibodies have recently been described to define a form of non-paraneoplastic Limbic Encephalitis (13).

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
Anti-SOX1-Antibodies	Lambert Eaton Myasthenia gravis	Small-cell-lung cancer
Anti-GAD65-Antibodies	Stiff-Person-Syndrom Limbic Encephalitis	Non paraneoplastic

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

2 PRINCIPLE OF THE TEST

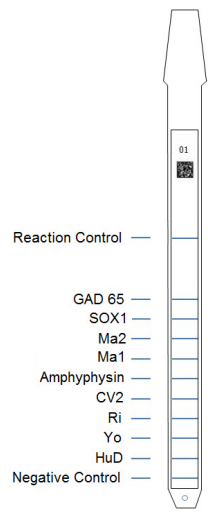
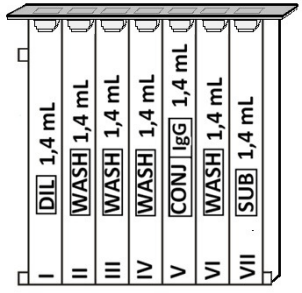
This kit is intended to be performed on the *BlueDiver Instrument* (hereafter BDI). The test is based on the principle of an Enzyme Immunoassay. The test strips are composed of a membrane fixed on a specific plastic support. During the automated test procedure, the BDI sequentially incubates the strips in the wells of ready-to-use reagent cartridges. Briefly: the strips are first incubated with diluted patients' sera. Human antibodies, if present, bind to the corresponding specific antigen(s) on the membrane. Unbound or excess antibodies are removed by washing. Upon further incubation into AP-conjugated goat antibodies against human IgG, the enzyme conjugate binds to the antigen-antibody complexes. After removal of excess conjugate by washing, the strips are finally incubated into a substrate solution. Enzyme activity, if present, leads to the development of purple lines on the membrane pads. The intensity of the coloration is directly proportional to the amount of antibody present in the sample.



3 KIT CONTENTS

Abbreviations in alphabetic order:

AP = Alkaline Phosphatase; BCIP = Bromo-Chloro-Indolyl-Phosphate; BSA = Bovine Serum Albumine; MgCl₂ = Magnesium Chloride; ZnCl₂ = Zinc Chloride; NBT = NitroBlue Tetrazolium; PBS = Phosphate Buffered Saline; TBS = Tris Buffer Saline

		STRIP	CARTRIDGE
Line strips	3 x 8 units on plastic supports breakable individually; sealed in aluminium pouch <u>11 lines on each:</u> 1 positive control (C+) 9 antigens 1 negative control (C-)		
Positive control	50 µl		
Cartridge	24 units having each 7 compartments; sealed.		
Diluent buffer	I st position, 1 x 1,4 ml contains: H ₂ O • PBS • Tween 20 • Preservative (ProClin300) • BSA • Antifoam emulsion		
Wash buffer	II nd , III rd , IV th and VI th position, 4 x 1,4 ml contains: H ₂ O • PBS • Tween 20 • Preservative (ProClin300) • Antifoam emulsion		
Conjugate	V th position, 1 x 1,4 ml contains: H ₂ O • TBS • NaCl • ZnCl ₂ • MgCl ₂ • AP-conjugated goat anti-human IgG • Stabilizer • Preservative (ProClin300) • BSA • Antifoam emulsion. Cartridge for CSF: blue dye		
Substrate	VII th position, 1 x 1,4 ml (pale yellow solution) contains: H ₂ O • NBT • BCIP		
Other material	Absorbent paper (for drying of tips pt 8.1.16), sealed Together with line strips in aluminium pouch		
Documents	IFU, Certificate of Analysis		

4 MATERIAL REQUIRED BUT NOT PROVIDED

BDI, Micropipettes / Laboratory gloves

5 STORAGE AND STABILITY

The test kit must be stored at a temperature between +2°C to +8°C. Do not freeze. After initial opening of the kit, unused reagent cartridges must be stored at 2-8°C protected from (sun)light preferably inside the original kit box. Unused strips have to be placed back into the provided pouches, sealed and stored at 2-8°C preferably *inside* the original kit box. When stored properly, all test kit components are stable until the indicated expiry date.

6 SAFETY PRECAUTIONS

All reagents are for in vitro diagnostic and professional use only. The test kit should be processed by trained technical staff only. The kit contains potentially hazardous components, thus contact with skin, eyes or mucosae has to be avoided.

Patient samples shall be handled with care as being a potential infection hazard.

A medical safety data sheet (MSDS) is available on request.

Waste disposal: Patient samples 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.

ravo and its authorized distributors shall not be liable for damages indirectly or consequentially brought about by changing or modifying the procedure indicated. In any case, GLP should be applied with all general and individual regulations to the use of this kit.

7 SAMPLE COLLECTION, HANDLING AND STORAGE

Blood samples can be collected in dry tubes or in tubes containing EDTA, heparin or citrate. After separation serum or plasma samples can generally be stored at 2-8°C for up to three days. Long term storage requires freezing at -20°C. Avoid repeated freezing/thawing cycles. After freezing always agitate samples before use to ensure homogeneity.

8 ASSAY PROCEDURE

BASIC INFORMATION, HANDLING AND TIPS:

TEST PROCESS principle:

After manual loading of the strips and reagent cartridges, the incubation and washing steps of the procedure are automatically processed by the BDI which ensures an efficient circulation of fluids over the strips by continuously agitating them up and down in the wells of ready-to-use reagent cartridges. The whole test procedure is run at room temperature.

STRIPS description:

The reactive (front) side of the strips displays a strip number and a 2-dimensional square barcode for traceability of the strips after removal from the BDI at the end of the test.



The non-reactive (back) side of the strips displays both alphanumeric and bar-coded information for identification of the strip type and lot number by the BDI.



The strips must be manually inserted into the dedicated clamp before starting the automated process (see Test Preparation hereafter, pt 8.1.4). During this operation, avoid touching the membrane zone of the strips with fingers. Always wear laboratory gloves and use the plastic parts (strip support) for handling or manipulation.

REAGENT CARTRIDGES description: (see image on page 1)

The reagent cartridges are composed of 7 different wells filled with ready-to-use reagents. The cartridges are sealed, and the reagent wells are hermetically separated. The sealing has to be removed before starting the test. Once opened, manipulate the cartridges with care in order to avoid reagent spilling and contamination from well to well.

The rear (back) side of the cartridges is labelled with both alphanumeric and bar-coded information for identification of the cartridge type and lot number by the BDI.

The cartridges must be manually loaded onto the dedicated cartridge holder before starting the automated process (see Test Preparation hereafter, pt 8.1.10). The front and rear (back) sides of the cartridges have, respectively, a bottom triangular and two (bottom + top) square plastic edges for secure position and orientation into the holder.

Testing of cerebrospinal fluid CSF (see 8.1.19.): please use the special CSF cartridges which are available on request.

STRIPS/CARTRIDGES associations

The strips and cartridges of a same test kit share the same lot number and are dedicated to be associated in lot-specific pairs. Do not associate, in a same pair, a strip and a cartridge with different lot numbers as this will be detected as an invalid setting by the BDI and will stop the process.

As far as each strip/cartridge pair is valid, the BDI can process strips/cartridges associations of different kits; However, only kits having the same protocol number (same incubation time and sequence) can be processed together in one same run (please refer to the protocol number indicated under the kit reference at the top of first page).

8.1 Test preparation

- Allow all kit components to equilibrate at room temperature (+18°C to +25°C) before use.
- A working list (either edited from Dr DOT software, or external) should always be prepared for easy loading and correct association of strips, cartridges and patient samples.
- Make sure that the cartridge holder is fixed in its emplacement in the BDI.
- Make sure that the BDI is plugged in.

The following steps sequence summarizes the loading and preparation of the BDI, test strips, reagent cartridges and patient samples before starting the test. For detailed information or in case of any problem met at one of the following steps, please refer to the Manual of Use of the BDI.

1. Switch ON the BDI and wait a few seconds until the date and time are displayed on the touch screen.
2. Confirm the correct Date and Time by pressing ✓ on the touch screen (in case of first use or for reset, refer to the manual of use of the BDI) → **"Initialize?"** is displayed on the screen.
3. Confirm Initialization by pressing ✓ on the touch screen → the horizontal arm of the instrument automatically moves forward to a central (stand-by) position → **"Load strips (24)"** is displayed on the screen.
4. (Do not set nor confirm the number of strips at this step). Remove the clamp from its emplacement on the arm by gently pulling it upwards and load the strips to be tested: handle the clamp with numbered side facing up (open position) and insert the strips, also with numbered (reactive) side facing up, by slipping the upper plastic part (tongue) into the dedicated holes of the clamp. Apply a gentle pressure to ensure that the plastic tongue has reached the bottom end of the hole.

Notes:

Always start loading into position 1 of the clamp (left side) and do not leave empty spaces between the strips!

After complete loading, check visually the vertical, horizontal and lateral alignment of the strips. Any obvious misalignment should be corrected by unloading the strip(s) from the clamp and loading them again.

Be careful: any plastic bits remaining after breaking apart the individual strip holders may hinder the processing on the instrument and/or the reading with the BlueScan scanner; please remove them with scissors.

5. Replace the clamp in its emplacement on the arm by gently pushing it downwards.
6. Set the number of loaded strips using the up and down arrows on the touch screen.
7. Confirm the number of loaded strips by pressing ✓ on the touch screen → the horizontal arm automatically moves backward to stand over the alignment holes of the cartridge holder → **"Check alignment"** is displayed on the screen.
8. Use the "JOG" function on the screen to check the correct alignment of the strips: maintain a gentle pressure on the down arrow on the touch screen until the bottom of the strips enters into the alignment holes of the cartridge holder. If correctly aligned, the strips will not touch the outlines of the holes.

Note:

In case of misalignment (contact of the strips with the cartridge holder), please refer to the Manual of Use of the BDI).

9. Confirm the correct alignment of the strips by pressing ✓ on the touch screen → the BDI lowers the strips completely into the alignment holes and reads the barcodes of the strips → after complete barcode reading, **"Load reagent"** is displayed on the touch screen.

Note:

In case of failure to read one or more strip barcode(s) (flashing LED at the unread position), please refer to the Manual of Use of the BDI.

10. Unseal the reagent cartridges and insert them under their respective strips in the dedicated notches of the cartridge holder.
11. Confirm complete loading by pressing ✓ on the touch screen → the BDI reads the barcodes of the cartridges and checks the correct association with the strips → after complete barcode reading, the number of strips (validated strips/cartridges associations) is displayed on the screen.

Note:

In case of failure to read one or more cartridge barcode(s), or in case of detection of a wrong strip/cartridge association (flashing LED at the corresponding position), please refer to the Manual of Use of the BDI.

12. Confirm the number of strips by pressing ✓ on the touch screen → the protocol number identified on the barcodes is displayed on the screen (**Protocol ID xx**).
13. Confirm the protocol number by pressing ✓ on the touch screen → **"Please close cover."** is displayed on the screen.
14. Close the cover of the BDI and confirm closing by pressing ✓ on the touch screen → the BDI proceeds to a first washing (pre-treatment) step by incubating the strips into the 2nd well of the cartridges (processing time: 1 minute) → At the end of the wetting step, **"Please open cover."** is displayed on the screen.
15. Open the cover of the BDI and confirm opening by pressing ✓ on the touch screen → the horizontal arm automatically moves forward to the front of the instrument and swings the strips to an oblique position → **"Dry strips"** is displayed on the screen.
16. Dry the strips by gently applying absorbent paper onto the basis of the bottom small cavity (sample loading hole).
17. Confirm drying by pressing ✓ on the touch screen → **"Apply samples"** is displayed on the screen.



18. Apply samples by pipetting 10 µl of patient serum/plasma or positive control into the bottom sample loading holes of the strips.
 Note:
 If preferred the 10µl of the serum/positive control can be directly pipetted into the Diluent Buffer ("Well I") of the cartridge. This operation can be done at any time from opening of the cartridges (see point 8.1.10).
19. **Testing of cerebrospinal fluid CSF:** for the testing of CSF the special CSF cartridge (blue coloured conjugate solution) must be used
 Pipet **50 µl CSF** directly into the Diluent Buffer ("well I") of the CSF cartridge. This operation can be done at any time from opening of the cartridges (see point 8.1.10).
Please note: an intrathecal synthesis of specific autoantibodies can only be detected if a paired serum-CSF sample is tested at equal IgG concentrations.
Performance: recommended IgG concentration **3 mg/Liter**. Pipet the calculated volume of serum and CSF each directly into the Diluent Buffer ("well I") of the CSF cartridge. Do not use more than 150 µl! Usually a high dilution of the serum sample is required. Thus the use of a pre-dilution of 1 : 10 is recommended. Dilute 5 µl serum sample with 45 µl Diluent Buffer taken from the CSF cartridge (see example).
Example: Concentration CSF = 120 mg/Liter → Dilution = 1:40. Serum: 8,4 g/Liter → Dilution = 1:2,800. Volume Diluent Buffer („well I“) = 1,400 µl → CSF: 1,400 µl : 40 = 35 µl, Serum: 1,400 µl : 2,800 = 0,5 µl (1. Dilution 1 : 10. 45 µl Diluent Buffer from the CSF cartridge plus 5 µl Serum). Pipet 5 µl of the pre-diluted serum sample and 35 µl CSF each directly into the Diluent Buffer ("well I") of the CSF cartridge.
20. Confirm samples' loading by pressing ✓ on the touch screen → "**Please close cover**" is displayed on the screen.
21. Close the cover of the BDI and confirm closing by pressing ✓ on the touch screen → the BDI starts the test automatically by proceeding the steps sequence of the protocol.

8.2 Test processing (Protocol 2)

Step	Description	Processing time
01.	The strips are incubated into the 1 st well of the cartridge (<i>Diluent Buffer</i>). Upon contact with the liquid in the wells and agitation, the pre-loaded patients' samples (see 8.1.18) are released from the small cavity at the bottom of the strips and are diluted in the buffer.	30 min
02.	The clamp moves forwards and the strips are incubated into the 2 nd well of the cartridge (<i>Wash Buffer</i>)	2 min
03.	The clamp moves forwards and the strips are incubated into the 3 rd well of the cartridge (<i>Wash Buffer</i>)	2 min
04.	The clamp moves forwards and the strips are incubated into the 6 th well of the cartridge (<i>Wash Buffer</i>)	2 min
05.	The clamp moves backwards and the strips are incubated into the 5 th well of the cartridge (<i>Conjugate</i>)	10 min
06.	The clamp moves backwards and the strips are incubated into the 4 th well of the cartridge (<i>Wash Buffer</i>)	2 min
07.	The clamp moves backwards and the strips are incubated into the 3 rd well of the cartridge (<i>Wash Buffer</i>)	2 min
08.	The clamp moves backwards and the strips are incubated into the 2 nd well of the cartridge (<i>Wash Buffer</i>)	2 min
09.	The clamp moves forwards and the strips are incubated into the 7 th well of the cartridge (<i>Substrate</i>)	10 min
10.	The clamp moves backwards and the strips are incubated into the 6 th well of the cartridge (<i>Wash Buffer</i>)	2 min

After completion of the process, the clamp moves to a central (stand-by) position in the BDI to allow easy manipulation of the clamp. The instrument beeps and "**Finished test**" is displayed on the screen.

Gently apply absorbent paper onto the basis of the strips to remove liquid from the bottom small cavity (sample loading hole) and allow the strips to dry for at least 120 minutes before interpretation of the results. The interpretation has to be done within 24 hours following the test processing.

In case of use of the BlueScan for help of results interpretation, please leave the processed strips attached to the clamp.

TEST DATA REGISTRATION

The test protocol can be downloaded by pressing the USB stick symbol and following the indications on the screen (Insert USB → Writing USB → Remove USB). This step is not obligatory but is highly recommended for traceability and regulatory matters.

9 RESULTS INTERPRETATION

A visual evaluation of the results can be performed, however the use of the Dr DOT Software and Scanning system is generally recommended for more precise and semi-quantitative interpretation.

More information on Dr DOT is available via your distributor or via our website.

9.1 Visual interpretation:

1. Remove the clamp from the BDI and unload the strips from the clamp.
2. Place the strips with reactive face up onto the marked fields of the Diver Interpretation Template provided with the kit. This will indicate the respective positions of the different controls and antigens on the membrane.
3. The first upper line (Reaction Control) must be positive for all patients.
Only a clearly coloured Reaction Control line ensures your results are valid and operation was correct and/or kit components were not degraded. If the first upper line is not coloured, the test has failed and cannot be interpreted further.
4. Compare the specific antigen lines to the Negative Control line (which always is the last bottom line). The colour intensity of the Antigen lines is directly proportional to the titer of the specific antibody in the patient sample.
The colour intensity of the Negative Control line may vary depending on the sample characteristics. If the sample is free of interfering substances the Negative Control line may be even close to uncoloured. In contrast, a highly coloured Negative Control line indicates a high rate of unspecific binding in the sample.

POSITIVE RESULT:

A sample is positive for a specific antibody if the colour intensity of the corresponding Antigen line is higher than the intensity of the Negative Control line.

NEGATIVE RESULT:

A sample is negative for a specific antibody if the colour intensity of corresponding Antigen line is lower than or equal to the intensity of the Negative Control line.

NB: A weak coloration of an antigen line, when close to the colour intensity of the Negative Control line may be difficult to differentiate by visual inspection only. In such cases, it is recommended to use Dr DOT software and scanning system (see 9.2) and refer to the corresponding instructions for more accurate interpretation.

Usually strong bands are observed in serum samples from patients with clinically defined paraneoplastic symptoms.

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 proves a paraneoplastic etiology of a neurological disease but it does not hint at a special type of cancer.

In contrast to the well-known paraneoplastic autoantibodies low antibodies titers to SOX1 are sometimes detected. The meaning of these weak reactions is unknown at the moment.

Antibodies to GAD (glutamat-decarboxylase) are considered as serological markers for Stiff-Person-Syndrome. The majority of patients have high titers of antibodies to both isoforms, GAD65 and GAD67.
 High titer GAD antibodies have recently been described to define a form of non-paraneoplastic Limbic Encephalitis.

Testing of cerebrospinal fluid CSF

An intrathecal synthesis of specific autoantibodies can only be detected if a paired serum-CSF sample is tested at equal IgG concentrations.

Visual Interpretation:

A more intensive band for the CSF-sample hints at an intrathecal synthesis of specific autoantibodies.

Use of Dr DOT and scanning system:

Divide the Dr DOT arbitrary units of the CSF (= A) by the arbitrary units of the serum sample (=B) → A/B

Negative result: A/B < 1,5

Equivocal result: 1,5 < A/B < 2,0

Positive result: A/B > 2,0

Example:

3 mg/L	Conc.IgG	Dilution	Calculated µl per 1400 µl Diluent Buffer ("well I")	Dr Dot arbitrary units	A/B
CSF	120 mg/L	40	35 µl	A = 40	
Serum	8,4 g/L	2,800	5 µl of 1 : 10 pre-dilution	B = 10	
					4

9.2 Use of Dr DOT Software and Scanning system

1. Remove the clamp from the BDI. Leave the processed strips attached to the clamp.
2. Insert the clamp, the reactive side of the strips facing down, into the dedicated emplacement in the cover of the BlueScan scanner.
3. Start scanning the strips using the Dr DOT software.
 For detailed information about the BlueScan and Dr DOT software please refer to the Manual of Use of your Dr DOT software

Dr DOT arbitrary unit (AU)	Interpretation
< 5	Negative
5 - 10	Equivocal (*)
>10	Positive

** Low titers of auto-antibodies may also occur in healthy patients. For this reason low positive results (between 5 to 10 AU), although valid, should be considered equivocal. Retesting of the patient after 8-12 weeks, preferably by using a new sample, is therefore recommended.*

Coloured strips : some samples give a more or less purple background due to serum components of unknown origin and the interpretation of the results is only partly feasible. In these cases the results should be interpreted visually.

10 TEST PERFORMANCES

Sensitivity and Specificity

Characterized samples (confirmed positive or negative for specific antibodies by reference laboratories and/or methodologies) are assayed following the test instructions.

Sensitivity: 99 %

Specificity: 98 %

11 TEST LIMITATIONS

1. According to the German (14) and European guidelines (15) a confirmation of positive results by a second method, e.g. Line Assay versus IFA and vice versa is recommended. Interpretation of laboratory test results must be made within the context of the clinical symptoms.
2. *ravo* and its authorised distributors **shall not be liable for any damages resulting from a change or modification in the procedure indicated.** The kit should be performed by trained technical staff only.
3. In any case, GLP should be applied with all general and individual regulations to the use of this kit.
4. *ravo's* liability shall in any event be limited to the replacement of the kit.



12 References:

1. O,Stich, S.Rauer. Paraneoplastische Neurologische Syndrome. Der Nervenarzt. 2013; 4
2. S. Rauer. Paraneoplastische Neurologische Syndrome. Fortschr Neurol Psychiatr. 2011;79(1):51-8; quiz 59-61. Review.
3. Oliver Stich, Sven Jarius, Christiane Rasiah, Raymond Volz und Sebastian Rauer. Recombinant immunoblot for assessment of intrathecally synthesized paraneoplastic antineuronal antibodies in cerebrospinal fluid from patients with paraneoplastic neurological syndromes. Clin Chem Lab Med 2008;46(12):1793-1795.
4. Lisa M. DeAngelis and Jerome B. Posner. Neurologic Complications of Cancer. Oxford University Press 2008 Second Edition
5. F. Graus, J.Y. Delattre, J. C. Antoine, J. Dalmau, B. Giometto, W. Grishold, J. Honnorat, P. Sillevs Smitt, Ch. Vedeler, J. J. G. M. Verschuuren, A. Vincent, R. Voltz, for the Paraneoplastic Neurological Syndrome Euronetwork. Recommended diagnostic criteria for paraneoplastic neurological syndromes. J Neurol Neurosurg Psychiatry 2004;75:1135-1140.
6. Voltz R. Paraneoplastische neurologische Autoimmunerkrankungen. Nervenarzt 2002;73:909 – 929.
7. Honnorat J., Antoine J.C., Derrington E., Aguira M., Belin M.F. Antibodies to a subpopulation of glial cells and a 66 kDa developmental protein in patients with paraneoplastic neurological syndromes. J.Neurol.Neurosurg.Psychiatry 1996;61(3): 270-8.
8. Sillevs-Smitt P., Manley G., Dalmau J., Posner J.B.Pitfalls in the diagnosis of autoantibodies associated with paraneoplastic neurologic disease. Neurology 1996;46:1739-1741.
9. Moll J.W., Antoine J.C., Brashear H.R. et al. Guidelines on the detection of paraneoplastic anti-neuronal-specific antibodies: report from the Workshop to the fourth Meeting of the International Society of Neuro-Immunology on paraneoplastic neurological disease. Neurology 1995;45:1937-1941.
10. Moll J.W., Vecht C.J. Immune diagnosis of paraneoplastic neurological diseases. Clin. Neurol. Neurosurg.1995;97: 711-81.
11. Dalmau J., Posner J.B. Neurologic paraneoplastic antibodies (anti-Yo; anti-Hu; anti-Ri): the case for a nomenclature based on antibody and antigen specificity.Neurology 1994;44:2241-2246.
12. L. Sabater, M.Titulaer, A, Saiz, J.Verschuuren, A. O. Güre and F. Graus. SOX1 antibodies are markers of paraneoplastic Lambert-Eaton myasthenic syndrome. Neurology, 2008; 70: 924-928.
13. Michael P. Malter, Christoph Helmstaedter, Horst Urbach, Angela Vincent, and Christian G.Bien. Antibodies to Glutamic Decarboxylase Define a Form of Limbic Encephalitis. Ann Neurol 2010;67:470-478.
14. DGN Leitlinie: Paraneoplastische neurologische Syndrome. www.dgn.org
15. M.J. Titulaer, R. Soffiatti, J. Dalmau, N.E. Gilhus, B. Giometto, F. Graus, W. Grisold, J. Honnorat, P.A.E. Sillevs-Smitt, R. Tanasescu, C.A.Vedeler, R. Voltz and J.J.G.M. Verschuuren. Eur J Neurol 2011; 18(1): 19-e3