Testing for Neuroimmunology Conditions at PathWest Laboratory Medicine

PathWest Laboratory Medicine's QEII Immunology Laboratory provides a comprehensive range of autoantibody tests for range of neurologic conditions. This guide aims to help clinicians choose and request the most appropriate test(s) for their patients. Additional resources include the PathWest Test Directory, available at https://pathwesttd.health.wa.gov.au/testdirectory/.

Assessment of paraneoplastic neurologic syndromes (PNSs)

PNSs are neurologic disorders, occurring in the context of a malignancy, that are associated with anti-neural antibodies. The autoantibodies predominantly target intracellular neural antigens.

Neurological presentations associated with higher likelihood of PNS[1] (so called "high risk phenotypes"):

i) encephalitis, myelitis or encephalomyelitis

ii) limbic encephalitis

iii) rapidly progressive cerebellar syndrome

iv) opsoclonus-myoclonus

v) sensory neuronopathy

vi) enteric neuropathy

vii) Lambert Eaton myasthenic syndrome (LEMS).

Testing in low pre-test probability scenarios increases the chances of false positive results. International consensus guidelines for the diagnosis of PNSs recommend testing serum and CSF for optimal sensitivity[1].

What tests are available at PathWest for assessment of PNSs?

1) **Paraneoplastic anti-neural antibodies, Screening assay**

Sample requirements: Validated for testing SERUM & CSF.

Turnaround time: Test is performed approximately weekly

This is an initial screening test to identify paraneoplastic anti-neural antibodies. The test is performed by indirect immunofluorescence and identifies staining patterns in brain and gastric mucosa that characterise antibodies to: ANNA-1 (Hu), ANNA-2 (Ri), PCA1 (Yo), CV2/CRMP5, amphiphysin, Ma1, Ma2 and Tr (DNER). These are intracellular neural antigens.

If indirect immunofluorescence is negative, results will be reported as "Negative", and this concludes testing for paraneoplastic anti-neural antibodies. If indirect immunofluorescence staining is present,

the results will be reported as positive and the patient sample will be further assessed by a second-line test (see paraneoplastic anti-neural antibodies, confirmatory assay below).

Occasionally, immunofluorescence staining is seen but is not consistent with a well characterised autoantibody. This can either be due to:

a) the presence of a common autoantibody such as anti-nuclear antibody (ANA) which may obscure interpretation of staining patterns. Results will be reported as "NOT EXCLUDED".

b) other staining patterns of uncertain significance. Results will be reported as "UNCHARACTERISED".

In both circumstances, the sample will be further assessed by the Paraneoplastic anti-neural antibodies, confirmatory assay.

2) Paraneoplastic anti-neural antibodies, Confirmatory Assay

Sample requirements: Validated for testing SERUM.

Turnaround time: Test is performed approximately weekly.

Confirmatory testing by line immunoassay is available for ANNA-1 (Hu), ANNA-2 (Ri), PCA1 (Yo), CV2/CRMP5, amphiphysin and Ma2. Results are reported as "Detected" or "Not Detected".

Characteristic disease associations, frequency of underlying cancer & associated tumour types for each paraneoplastic anti-neural antibody are summarised in Appendix A.

3) Voltage gated calcium channel (VGCC) antibodies

Sample requirements: Validated for testing SERUM only.

Turnaround time: Test is performed approximately once per month

PathWest offers testing for antibodies to VGCC for assessment of Lambert Eaton myasthenic syndrome (LEMS). This test is only available for testing on SERUM. The test is a radioimmunoassay and is performed approximately monthly. Results are reported quantitatively.

Note, this test **must** be specifically requested. It is not included in Paraneoplastic anti-neural antibodies, screening or confirmatory tests on account of the distinct clinical features of LEMS.

Testing for anti-neural antibodies associated with Autoimmune Encephalitis (AIE)

A range of anti-neural antibodies are associated with inflammation of the cerebral cortex giving rise to autoimmune encephalitis (AIE).

Antibodies to cell surface neural antigens NMDA-R, LGI1, CASPR2, GABA-R-B1/2 and AMPA-R1/2 are associated with autoimmune limbic encephalitis (limbic-AIE). Limbic-AIE denotes inflammation of limbic structures spanning the border between cerebral cortex and brainstem including the hippocampus, amygdala & hypothalamus. These areas govern emotions, behaviour, memory and autonomic responses. Clinically, limbic-AIE is characterised by subacute-onset personality changes, short-term memory deficits, seizures and psychosis[2]. Furthermore, each antibody subtype has characteristic clinical features in addition to limbic encephalitis, see appendix B.

Testing for antibodies to NMDA-R, LGI1, CASPR2, GABA-R-B1/2 and AMPA-R1/2 is performed using an indirect immunofluorescence cell based assay (IIF-CBA). Results are reported as Detected/Not Detected Tests are available for:

- NMDA-R antibodies
- LGI1 and CASPR2 antibodies (voltage gated potassium channel antibodies)
- GABA-R-B1/2
- AMPA-R1/2

Sample requirements: Validated for testing SERUM & CSF.

Turnaround time: Tests for antibodies to NMDA-R, LGI1, CASPR2, GABA-R-B1/2 and AMPA-R1/2 are performed approximately once per week.

Clinicians can decide whether to select individual antibody tests (eg for patients with characteristic clinical features of a specific syndrome associated with a particular antibody) or to request a panel of antibodies (eg if the clinical presentation of AIE has fewer specific clinical features). For the latter, requesting "Autoimmune Encephalitis antibody panel" will prompt testing for:

a) paraneoplastic anti-neural antibodies, screening assay (see above): autoimmune encephalitis is seen in some patients with antibodies to intracellular neural antigens.

b) NMDA-R, LGI1, CASPR2, GABA-R-B1/2 and AMPA-R1/2 tests using IIF-CBA.

** **For severely unwell patients, requests to expedite testing must be discussed directly with an Immunopathologist. Monday to Friday, please call 63834306. ****

****For urgent tests, it is recommended to test <u>serum and CSF</u> for optimal sensitivity and specificity.**

Testing for GAD-antibody (GAD-AB) associated neurologic syndromes

The most well recognised neurologic disorders associated with GAD-AB are[3]:

- Stiff Person Syndrome (SPS)
- Cerebellar ataxia
- Refractory epilepsy, most typically affecting the temporal lobes.

GAD-AB are tested at PathWest by ELISA method and a quantitative level of GAD-AB is reported. Patients may present with overlapping features. Typically, patients with GAD-AB neurologic syndromes have much higher antibody levels than patients with diabetes mellitus without neurologic disease[4].

Sample requirements: Quantitative ELISA for GAD antibodies is validated for SERUM samples only Evaluation of CSF samples for GAD antibodies cannot be performed by GAD ELISA. CSF samples can be tested for "Paraneoplastic anti-neural antibodies, Screening assay" as GAD antibodies are associated with a recognisable pattern of immunofluorescence staining.

Turnaround time: Test performed approximately once per fortnight

Testing for Neuromyelitis Optica Spectrum Disorders (NMOSD)

NMOSD is a relapsing autoimmune condition involving demyelination of the optic nerves and / spinal cord causing visual loss and weakness. It is associated with antibodies to aquaporin-4 (AQP4) and myelin oligodendrocyte glycoprotein (MOG)[5].

1) AQP4 antibodies

Sample requirements (AQP4): Validated for SERUM only

Turnaround time (AQP4): Approximately once per fortnight.

Testing for AQP4 antibodies is performed at PathWest using an indirect immunofluorescence cell based assay. Results are reported as "Detected" or "Not detected".

2) MOG antibodies

Sample requirements (MOG): SERUM (& CSF*)

Turn-around time (MOG): Variable turnaround time, sendaway test.

Testing for MOG antibodies is not performed at PathWest. Samples can be sent to the Brain Autoimmunity Laboratory, The Children's Hospital at Westmead where testing is performed using a live, cell based assay. Note, currently this test is not accredited by the National Association of Testing Authorities (NATA) and is considered research use only.

* Note, serum testing is the preferred sample type for routine evaluation for MOG-antibody associated disease (MOGAD). On rare occasions when CSF testing is required, The Brain Autoimmunity Laboratory requests that all CSF samples to be tested for MOG antibodies are accompanied by a paired / matching serum sample.

Testing for neuroimmunology conditions affecting the neuromuscular junction

1) Acetylcholine receptor (AChR) antibodies

Sample requirements: Validated for SERUM only

Turn-around time: Testing is performed approximately once per fortnight

Test is performed by radioimmunoassay (RIA) and results are reported quantitatively.

2) Muscle specific kinase (MuSK) antibodies

Sample requirements: Validated for SERUM only

Turn-around time: Testing is performed approximately once per month

Test is performed by radioimmunoassay (RIA) and results are reported quantitatively.

3) Voltage gated calcium channel (VGCC) antibodies

Refer to section 3 of Assessment of Paraneoplastic Neurologic Syndromes above.

Testing for neuroimmunology conditions affecting peripheral nerves

1) Ganglioside antibodies

Sample requirements: Validated for SERUM only

Turn-around time: Testing is performed once per fortnight

Test is performed using an immunoblot method and assesses for IgM and IgG antibodies to GM1, GM2, GM3, GM4, GD1a, GD1a, GD1b, GD2, GD3, GT1a, GT1b and GQ1b. Results for each specificity are reported as not detected or detected.

2) Myelin associated glycoprotein (MAG) antibodies

Sample requirements: Validated for SERUM only

Turn-around time: Testing is performed once per fortnight

Test is performed by ELISA and results are reported quantitatively.

Clinical information accompanying requests for neuroimmunology tests

Please provide details of the patient's clinical presentation to assist in processing and interpretation of the patient's sample. This helps the laboratory to ensure relevant tests are performed & also assists in the interpretation and communication of results. Requests for urgent testing must be discussed directly with an Immunopathology Advanced Trainee or Consultant.

Sendaway tests

Developments in the field of neuroimmunology research has led to a growing number of reported autoantibody specificities relevant to patients presenting with neurologic syndromes. Tests for such autoantibodies may not yet have commercially available tests or are not viable to offer routinely as part of PathWest's diagnostic service. For clinicians considering rarer, autoantibody associated neuroimmunology conditions, please contact the Immunology Laboratory to discuss with the Immunopathology Advanced Trainee or Consultant what testing might be relevant and feasible. In such cases, relevant first-line tests available at PathWest will be completed first, prior to arranging sendaway for further testing at other centres.

Appendix A: Characteristic clinical features, frequency of cancer association and tumour types for paraneoplastic anti-neural antibodies.

Antibody	Neurologic syndrome and characteristic clinical features	Frequency of underlying cancer	Cancer type(s)
ANNA-1 (Hu)[6]	Sensory neuronopathy, limbic encephalitis, encephalomyelitis (which may include bulbar dysfunction and central hypoventilation) and gastrointestinal pseudo-obstruction.	Varies according to age of patient: 80% for patients >45 yo 25% for adults <45 yo 45% for children	SCLC++ NSCLC Neuroendocrine tumours Neuroblastoma, particularly in paediatric cases
ANNA-2 (Ri)[7]	Multisystem neurologic disease with prominent cerebellum and brainstem involvement; opsoclonus-myoclonus and other oculomotor presentations; movement disorders including spasticity, dystonia and parkinsonism.	>75% of patients	Breast Lung Bladder Testicular
PCA-1 (Yo)[8]	Rapidly progressive cerebellar syndrome comprising vertigo (which is the most frequent presenting symptom); limb, trunk and gait ataxia; dysarthria; diplopia. Extracerebellar findings include peripheral neuropathy, pyramidal and extrapyramidal features, cognitive dysfunction and psychiatric symptoms. Rare cases of patients presenting with cranial neuropathies, opsoclonus-myoclonus and myopathy are also reported. Most patients with PCA1 antibodies and neurologic syndromes are women (>90%).	80%	Gynaecologic cancers For men, gastrointestinal tumours are most common
CV2/ CRMP5[9]	Limbic encephalitis; chorea; ocular syndromes (including optic neuritis, retinitis, uveitis, central nystagmus, diplopia, opsoclonus); cerebellar ataxia and peripheral neuropathy. Patients often present with more than one of these syndromes. However, isolated, longitudinally extensive symmetric myelopathy is also reported.	>80%	SCLC Thymoma
Amphiphysin[10]	Limbic encephalitis and stiff-person syndrome (SPS) are the most common neurologic syndromes associated with anti-amphiphysin antibodies. Cases of brainstem encephalitis, myelopathy (which may be an isolated syndrome), peripheral neuropathy, polyradiculoneuropathy and cerebellar syndrome are also reported.	80%	Breast SCLC
Ma1 and Ma2[11]	Anti-Ma antibodies associate with limbic encephalitis, brainstem encephalitis and cerebellar syndrome. It is rare to find Ma1 antibodies in isolation, usually they are found alongside Ma2 antibodies. Conversely, Ma2 antibodies may be identified alone.	>75%	Testicular (younger patients) NSCLC (older patients)
Tr (DNER)[12]	Rapidly progressive cerebellar syndrome. Extracerebellar features are present in less than 10% of cases.	>90%	Hodgkin lymphoma
Voltage gated calcium channel (VGCC) antibodies[13]	Lambert Eaton Myasthenic syndrome (LEMS) and cerebellar degeneration.	50%	SCLC (more frequent among males, smokers and over 50 yo)

SCLC = small cell lung cancer NSCLC = non small cell lung cancer

Appendix B: Characteristic clinical features, frequency of cancer association and tumour type for cell surface anti-neural antibodies

Antibody	Neurologic syndrome and characteristic clinical features	Frequency of underlying cancer	Cancer type(s)
NMDA-R[14]	Prodrome of headache, fever, nausea and vomiting; followed within 2 weeks by psychiatric and behavioural symptoms (including psychosis, agitation, hyperactivity, delusions, hallucinations and memory impairment). Neurologic manifestations include seizures, speech changes, involuntary movements and impaired alertness / reduced level of consciousness. These changes may be accompanied by autonomic instability.	15-40%	Ovarian teratoma (mainly among females <45yo). Patients >45yo are more likely to have carcinoma.
LGI1[15]	Faciobrachial dystonic seizures; limbic encephalitis; hyponatraemia in two-thirds of patients. Rarely can present with neuromyotonia and Morvan's syndrome.	<10%	Thymoma Neuroendocrine
CASPR2[15]	Limbic encephalitis, neuromyotonia, Morvan's syndrome.	20-50% (especially in those with Morvan's syndrome)	Thymoma
AMPAR1/R2[16]	Limbic encephalitis +/- more diffuse encephalitis, particularly affecting the orbito-frontal cortex.	Up to 66%	SCLC Thymoma Breast Ovarian
GABARB1/B2[17]	A rare form of autoimmune encephalitis which can present quite acutely, often with a prodrome including fever. Neurologic manifestations typically include acute onset frequent seizures, limbic encephalitis & cerebellar ataxia.	Approximately 50%	SCLC (especially in older, male smokers).

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