

Autoimmune Antibody Testing

Points of Note:

- The interpretation of all autoantibody tests is highly dependent on the likelihood of disease in the patient.
- The results should always be interpreted with the clinical features of the patient and never in isolation.
- Autoantibodies may be present in healthy individuals and may also occur transiently with intercurrent illness or may be induced by drug therapy. Conversely, autoimmune disease may be present in the absence of detectable autoantibodies.

Do not use these tests as 'screens' for autoimmune disease but rather decide the clinical diagnosis and the likelihood of autoimmune disease and use specific autoantibody tests as diagnostic aids.

Anti-Nuclear Antibodies (ANA). (Incorporating Anti-double stranded DNA (dsDNA) and Anti-Extractable Nuclear Antigen (ENA) Antibodies)

These tests are predominantly used for the investigation and diagnosis of inflammatory connective tissue diseases such as SLE, Sjogren's syndrome, and systemic sclerosis, mixed connective tissue disease, polymyositis and dermatomyositis.

An ANA panel may be positive in healthy individuals (and particularly with increased age) or be induced transiently during acute illness or with infection, and by certain medication. It may also be positive in many other autoimmune diseases including rheumatoid arthritis and autoimmune thyroid disease. The ANA has no particular clinical significance in these situations. **Thus the tests should only be requested where the clinical features are suggestive of inflammatory connective tissue disease.** Occasionally the ANA may be negative where there is underlying CTD.

We currently use an automated machine (Bioplex) to detect antibodies to a panel of antigens relevant to inflammatory connective tissue diseases. These are detailed below. Like any assay, patients with disease may have negative tests (false negatives) and patients without disease may have positive tests (false positives). Low level positive tests may be non-specific in some individuals and test results should always be interpreted with the clinical features. In patients with CTD, tests may be positive before the patient develops the full clinical features of the disease.

The general disease associations with these autoantibodies are as follows:

Anti-dsDNA antibodies	Typically associated with SLE but high levels may be found in autoimmune hepatitis. When positive, the laboratory may check the result by performing a second test (Crithidia assay) to confirm the specificity of the result.
Anti-SSA (Ro) Antibodies. (Ro60 and 52)	Associated with Subacute Cutaneous Lupus Erythematosus, Systemic Lupus Erythematosus and Sjogren's Syndrome and are of particular relevance during pregnancy as their presence may be associated with neonatal lupus and congenital heart block. Antibodies to Ro52 without anti-Ro60 may be associated with polymyositis.
Anti-SSB (La) Antibodies	Generally are present with anti-SSA antibodies and are associated with SLE and Sjogren's Syndrome.
Anti-Sm Antibodies	Pathognomonic of SLE but only occur in a minority of patients with this condition.
Anti-Ribonucleolar protein (RNP) Antibodies (SmRNP, RNP68)	Particularly associated with SLE or Mixed Connective Tissue Disease.
Anti-Scl-70 Antibodies	Particularly associated with diffuse systemic sclerosis (scleroderma). A positive result may be followed by analysing by a second method to confirm specificity.
Anti-Jo 1 Antibodies	Found in a minority of patients with polymyositis, particularly when it is associated with interstitial lung disease.
Anti-Centomere Protein B Antibodies	Typically associated with systemic sclerosis / CREST Syndrome but also found in Primary Biliary Cirrhosis.
Anti-Chromatin antibodies	May be associated with SLE (and other CTDs) but usually in associated with the presence of other autoantibodies. The relevance of low level anti-chromatin antibodies in isolation is unclear.
Anti-Ribosomal P antibodies	Appear to be highly specific for SLE although is also described in some cases of autoimmune hepatitis. May occur in isolation without other autoantibodies.

Rheumatoid Factor

This may be present in Rheumatoid arthritis but also in patients with Sjogren's syndrome, SLE or cryoglobulinaemia.

It is detectable in 15% of the population without RA following chronic inflammation or infection or in the elderly.

RF may be negative in 15-30% of patients with adult RA.

Anti-Cyclic Citrullinated Peptide (CCP) Antibodies

These antibodies appear to be highly specific for Rheumatoid Arthritis and their presence may be associated with erosive joint disease. Anti-CCP is present in about 60-70% of patients with RA. There is no general correlation with presence of RF. Monitoring anti-CCP levels is not useful during treatment of RA.

The clinical utility of this test is generally limited to specialist Rheumatology clinics.

Anti-Neutrophil Cytoplasmic Antibodies (ANCA) Antibodies

The ANCA assays are performed first by indirect immunofluorescence providing a negative or positive result. Positive results are reported as a pattern: P(erinuclear), C(ytoplasmic) or Nuclear. *The presence of a positive ANCA result is not disease-defining. A negative test does not exclude vasculitis.*

The disease association (small vessel vasculitis) is with specific antibodies directed to one of two major granule proteins i.e. **Myeloperoxidase (MPO)** or **Proteinase 3 (PR3)**. All positive results are referred on for testing on the Bioplex machine for the presence of IgG to these proteins. Tests for autoantibodies to other neutrophil granule proteins are not available. A negative result will generally be reported without further tests but very occasionally anti-MPO or anti-PR3 antibodies may be present with a negative ANCA.

If the clinical features are strongly suggestive of small vessel vasculitis and the ANCA is negative then these tests may be undertaken on request.

In combination ANCA and anti-MPO and PR3 antibodies are about 90% sensitive in detecting small vessel vasculitis. *Thus a negative test does not exclude vasculitis.*

In general in the context of small vessel vasculitis:

C-ANCA is associated with anti-PR3 antibodies and is found in Wegener's Granulomatosis (Granulomatosis with Polyangiitis), and Churg-Strauss Syndrome (Eosinophilic Granulomatosis with Polyangiitis).

P-ANCA is associated with anti-MPO antibodies and is found in Microscopic Polyangiitis, Crescentic Glomerulonephritis and Churg-Strauss Syndrome. This is variable and occasionally one may have C-ANCA with anti-MPO and P-ANCA with anti-PR3, or combinations of antibodies. A positive ANCA with negative anti-MPO and PR3 may be found in a variety of conditions including autoimmune hepatitis, sclerosing cholangitis, ulcerative colitis, SLE, RA,

malignancy, cystic fibrosis and chronic infections. ANCA testing is generally **not** warranted for these clinical conditions.

Anti-Glomerular Basement Membrane (GBM) Antibodies

Antibodies to GBM are primarily directed towards the non-collagenous domain of the alpha 3 chain of type IV collagen. Since this type of collagen is found predominantly in glomeruli and alveoli, the presence these directly pathogenic antibodies is associated with rapidly progressive glomerulonephritis and alveolitis (Goodpasture's Syndrome).

Anti-GBM antibodies may also be found in some patients with ANCA positive small vessel vasculitis, usually with anti-MPO antibodies.

Repeat tests are useful to determine the effectiveness of plasma exchange.

These tests are performed together as a 'package' on the Bioplex machine and the result is reported as anti-PR3, anti-MPO and anti-GBM antibodies together, even if only one of the autoantibodies is relevant.

Anti-Cardiolipin Antibodies

These tests are utilized to assist in the clinical diagnosis of the Anti-Phospholipid Syndrome (APLS). This condition is characterized by vascular thrombosis and/or recurrent fetal loss. Other features may include livedo reticularis, thrombocytopaenia, heart valve disease, nephropathy and neurological disease. APLS may be Primary, occurring alone, or Secondary, associated with connective tissue disease, especially SLE.

Anti-Cardiolipin (aCL) antibodies of IgG and/or IgM isotype in serum or plasma, present on medium or high titre (> 40 GPL or MPL), on two or more occasions, at least 12 weeks apart, measured by standardized ELISA

Anti- β_2 glycoprotein-I antibody of IgG and/or IgM isotype on serum or plasma (in titre > 99th percentile), present on two or more occasions, at least 12 weeks apart, measured by standardized ELISA, according to recommended protocols

If this diagnosis is strongly suspected then anti-cardiolipin antibodies should be requested. Samples for Anti- β_2 glycoprotein-I antibodies may be sent away, after clinical discussion with the laboratory, if the test is negative and clinical suspicion is high. In addition, aCL antibodies may be weakly positive or transiently abnormal in numerous other conditions, a positive result needs to be confirmed with a repeat sample at least 12 weeks later.

Tissue Specific Autoantibodies

This section refers to **anti-gastric parietal cell**, **anti-mitochondrial**, **anti-smooth muscle**, and **anti-liver kidney microsomal** antibodies.

These tests are performed by indirect immunofluorescence, and all these antibodies can be detected on a single slide.

Ideally the specific antibody relevant to the clinical features under investigation should be requested.

As with all autoantibody testing these may be positive in healthy individuals and may be non-specific.

Thus the tests should only be requested with specific reference to the clinical condition.

In general the value of these tests is limited to the investigation of pernicious anaemia and autoimmune liver disease.

Anti-Gastric Parietal Cell Antibodies & Anti-Intrinsic Factor antibodies

These are present in individuals with autoimmune gastritis and pernicious anaemia. However they are not specific for these conditions, as they may be also found in healthy individuals, particularly with increased age, and in those with other autoimmune conditions (thyroiditis, Addison's disease, IDDM).

If Vitamin B12 levels are low then anti-intrinsic factor antibodies should be requested.

Anti-GPC antibodies are more sensitive, but less specific, for pernicious anaemia than anti-IF antibodies.

Anti-Mitochondrial Antibodies

These antibodies are directed to pyruvate dehydrogenase complex and have a close association with Primary Biliary Cirrhosis.

When these are positive for the first time then the sample will be tested for Anti-PDH E2 (M2) antibodies to confirm the antibody specificity.

Anti-Smooth Muscle Antibodies

These are frequently non-specific or transiently detected.

However they may be associated with Type 1 Autoimmune Hepatitis.

These patients may also have a positive ANA.

Anti-Liver Kidney Microsomal Antibodies

These are directed against Cytochrome P450 proteins and are particularly associated with Type 2 Autoimmune Hepatitis.

This form of AIH presents more commonly in children.

Anti-Thyroid peroxidase (TPO) and anti-TSH receptor antibodies.

Anti-TPO antibodies at high titre are found in patients with Hashimoto's thyroiditis (95%), primary myxoedema (90%) and Graves disease (18%).

Low titre anti-TPO antibodies may occur in goitre, thyroid carcinoma and in other organ-specific autoimmune diseases e.g. PA. The main value of this test is in patients with borderline or compensated hypothyroidism. Strong positive anti-TPO antibodies are predictive of progression to permanent hypothyroidism.

Hyperthyroidism in Graves' disease is due to autoantibodies to the TSH receptor (TSHR) and measurement of these autoantibodies can be useful in disease diagnosis and management.

Gluten Sensitive Enteropathy (Coeliac Disease) Testing

Coeliac Disease is an enteropathy that occurs in the presence of gluten found in wheat (gliadins), barley (hordeins) and rye (secalins).

The condition is thought to be far more prevalent than previously realized and may manifest with non-specific and extra-intestinal symptoms (anaemia, osteopaenia, fatigue, abnormal liver function tests).

The enteropathy is essentially an autoimmune condition that occurs in the presence of gluten and resolves with gluten withdrawal. IgA antibodies to gliadin, tissue transglutaminase and to antigens created by the combination of the two are involved in the disease process. GSE predominantly occurs in those individuals with a specific tissue type, HLA DQ2.

Like all autoantibody tests, **the results should be interpreted with the clinical features.**

The tests will not be positive in all patients with GSE, and some patients with positive tests may not have GSE.

For accurate diagnosis the tests should only be undertaken with the patient eating a normal gluten-containing diet for at least 6 weeks. Those on gluten-free diets may have false negative tests.

The gold standard for the diagnosis of Gluten Sensitive Enteropathy is small intestinal biopsy which should also be undertaken on a normal diet containing gluten

In most patients positive tests alone are inadequate to make the diagnosis.

Follow up tests may be useful after the diagnosis has been made to follow compliance with a gluten-free diet.

IgA deficiency, which occurs in about 1 / 400-700, will lead to false negative serological tests for GSE. Using the results of the anti-TTG tests, we are able to determine which samples to test for IgA levels. Where IgA deficiency is present then IgG anti-TTG testing will follow. These are far less specific for GSE and may be present in healthy individuals and those with other intestinal diseases.

IgA Anti-Tissue Transglutaminase (tTG) Antibodies

The antigen within the endomysium to which the IgA antibodies bind in GSE has been identified as tissue transglutaminase (tTG).

This is the main assay for testing for Coeliac Disease.

Anti-Endomysial Antibodies (EMA)

IgA anti-endomysial antibodies are detected by indirect immunofluorescence. The results are reported as positive or negative. This assay will only be performed if the anti-TTG result falls within the borderline range.

Special Autoantibody Tests

Anti-*Saccharomyces cerevisiae* Antibodies

Anti *Saccharomyces* antibodies (ASCA) are associated with Crohn's disease (CD) and are found in 50-60% of patients with CD and 10-15% of patients with Ulcerative colitis (UC).

Combined IgG and IgA ASCA can give better discrimination between CD and UC.

ASCA testing can be used to distinguish between CD and UC especially in a Gastro-enterology clinic setting.

Anti-Striated Muscle Antibodies

These are detected by indirect immunofluorescence using skeletal muscle.

Testing for these antibodies is of most relevance in patients with myasthenia gravis where their presence is associated with underlying thymoma.

Anti-Skin Antibodies

Skin antibodies are assayed by indirect immunofluorescence.

This test is used to aid in the diagnosis of autoimmune bullous skin disease (Bullous Pemphigoid, Pemphigus vulgaris), particularly when direct immunofluorescence on skin biopsy is unavailable.

Anti-Adrenal Cortex Antibodies

These are detected by indirect immunofluorescence using adrenal tissue.

This test is used to aid the diagnosis of autoimmune adrenal failure (Addison's disease).

Anti-Islet Cell Antibodies

These antibodies are used to assist in the diagnosis of type 1 (autoimmune) diabetes.

The tests are usually positive early in the clinical course.

Additional tests (anti-IA2 and anti-GAD65) may be sent away to an outside reference laboratory if necessary.

NICE Guidance (2015) states that "With autoantibody testing, carrying out tests for 2 different diabetes-specific autoantibodies, with at least 1 being positive, reduces the false negative rate".

Neurological Disease Antibodies

These are expensive tests and are referred to specialist laboratories

Acetylcholine Receptor (anti-AChR) <i>IgG, detected by RIA</i>	Myasthenia Gravis: Generalised Ocular	85% 50%
MuSK (anti-MuSK) <i>IgG, detected by RIA</i>	Generalised AChR antibody negative Myasthenia Gravis (15% of all MG patients approx)	up to 50% of AChR neg MG (variable)
Voltage gated Ca²⁺ channel (anti-VGCC) <i>IgG, detected by RIA</i>	Lambert-Eaton Syndrome (with or without SCLC) Cerebellar ataxia ass with SCLC	>85% Around 30%
Voltage gated K⁺ channel (anti-VGKC) <i>IgG, detected by RIA</i>	Acquired neuromyotonia Limbic encephalitis-like syndromes (both sometimes associated with thymoma or SCLC)	40% Not known
Ganglioside (GM1) (anti-GM1) <i>IgG and IgM (combined), detected by ELISA</i>	Guillain Barre Syndrome (IgG) Multifocal motor neuropathy (IgM)	~40% ~60%
Ganglioside (GQ1b) (anti-GQ1b) <i>IgG and IgM (combined), detected by ELISA</i>	Miller-Fisher syndrome (IgG) Chronic sensory neuropathy (IgM)	>90% Some
Glutamic acid decarboxylase (GAD) (anti GAD) <i>IgG, detected by RIA</i>	High levels >300 U/ml in Stiff-man syndrome Cerebellar ataxia (usually with other autoimmune disorders) Low levels <100 U/ml in Diabetes	~60% Not known
Myelin associated glycoprotein (MAG) (anti-MAG) <i>IgM, detected by ELISA</i>	Chronic sensory neuropathies	Some
Markers for Paraneoplastic neurological syndromes <i>Anti-neuronal antibodies detected by immunohistochemistry (Hu, Yo, Ri, Ma, Tr and potentially other autoantibodies); if positive, followed by confirmation using immunoblotting on RAVO kit</i> RAVO kit Immunoblotting detects Hu, Yo, Ri, Ma, Amphiphysin, CRMP/CV2 but not Tr or other antibodies)	Antigen: most common presentation (most frequent associated tumour) Hu, ANNA: Subacute sensory neuropathy/encephalitis (SCLC) Yo, APCA1: Cerebellar degeneration (breast, ovary) Ri, ANNA2: Opsoclonus/Myoclonus and other (breast) Ma2: Limbic encephalitis and other syndromes (testicular and other cancers) Amphiphysin: Opsoclonus, ataxia (breast, SCLC) CRMP/CV2: Various (various) Tr: Cerebellar ataxia (lymphomas)	Variable