



# Autoantibodies

## Diagnostic tools for autoimmune disorders

### What are autoantibodies?

Autoantibodies bind non-foreign structures within us and have been found in most well-defined autoimmune disorders. They also occur in other disorders with an inflammatory component and even in some malignant disorders as paraneoplastic phenomena.

With a few important exceptions, autoantibodies have no direct role in pathogenesis and their main value is as a 'marker' adding weight to a clinical diagnoses.

Circulating forms of autoantibodies may be detected by assays on serum. Tissue-bound antibodies may also be detected by direct immunofluorescence studies of non-fixed biopsy specimens.

### Do autoantibodies ever occur naturally, without clinical associations?

Low-level autoantibodies occur naturally and more commonly in persons who are older, female, have chronic diseases and often a family history of autoimmune abnormalities. These natural autoantibodies occur in low concentrations and have weak binding affinities.

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Thus, low-level autoantibodies have limited utility for the diagnosis of autoimmune diseases.

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### How do I use autoantibodies to diagnose autoimmune disorders?

The clinical utility of autoantibodies as markers for autoimmune diseases comes from large sets of empirical observations on patients rather than a deep understanding of the biology of these diseases. These observations have led to the concepts of clusters of autoimmune disorders, as well as a broader classification of organ-specific and non-organ specific disorders.

Most autoimmune diseases are diagnosed using a combination of clinical and laboratory features. Operational criteria, using a range of clinical and laboratory features, have been developed for the diagnosis of connective tissue disorders. As a general principle, no one clinical or laboratory finding is diagnostic by itself of an autoimmune disease.

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Despite these limitations, autoantibodies are a valuable tool for the diagnosis (when considered with other clinical and laboratory information) and monitoring of many autoimmune disorders.

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### How is tissue injury caused in autoimmune disorders? Are autoantibodies always pathological?

Much tissue damage in autoimmune diseases is probably mediated by T cells and their effector mechanisms, rather than by B cells and their products, autoantibodies. Systemic lupus erythematosus and other connective tissue disorders are characterised by polyclonal self-reactive B cell expansions.

Normal immune system functions include the recognition of, and discrimination between, self and non-self targets and unleashing of effector mechanisms, such as complement proteins, cytotoxic T cells, cytokines and other phagocytic cells onto non-self targets.

Autoantibody production is a consequence of ongoing recognition of self targets by both T and B cells. Recognition of self targets is a necessary part of surveillance by the immune system, but fortunately is seldom coupled with effector mechanisms that lead to tissue damage and autoimmune disease. Breaches of tolerance that may result in autoimmune disease can occur with the expansion of self-reactive T and B cells by infectious agents which have cross-reactive antigens, increased expression of HLA-antigens, often following infection in certain tissues which present antigens to T cells, and a number of other factors.

Tissue injury in autoimmune disease is often the consequence of T cell-mediated tissue damage. In a number of disorders, however, the autoantibodies are directly pathogenic.

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Although many of the individual components of pathological immune responses in autoimmune diseases have been characterised in great detail, autoantibodies remain the markers with greatest clinical utility for the investigation of patients with possible autoimmune diseases.

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## Which serum autoantibodies have significant clinical utility?

- Autoantibodies have been found to add to the diagnosis of many organ-specific diseases.
- For some disorders, however, autoantibodies have little clinical utility and their presence may be inferred by other tests.
- In some disorders, the absence of an autoantibody weighs heavily against the likelihood of the disease being present.

For example, smooth muscle antibodies are a marker of autoimmune chronic active hepatitis, but can occur in low levels without the disease developing. The major clinical value of this test, however, is that the absence of smooth muscle antibodies effectively excludes autoimmune chronic active hepatitis in someone with biochemical features of hepatitis.



Important autoantibody tests, their abbreviations and clinical utility are summarised on the reference sheets at the end of this article.

## Some medications are associated with autoantibodies. What medications should I think of in this regard?

More than 70 medications have been associated with the induction of autoantibodies, particularly ANAs. Many of the first medications implicated are seldom used, but the major ones to remember include hydralazine, procainamide, quinidine, minocycline, carbamazepine, phenytoin, anti-thyroid drugs, anti-tuberculous drugs, slow-acting anti-rheumatic drugs and TNF antagonists.

Drug-induced autoantibodies are more common in slow-acetylators, in women, in Caucasians, with higher doses and with more prolonged therapy.

## What are some other issues I should think of in respect to interpreting autoantibody results?

- Occasionally, clinical features of the disorder may precede the development of significant autoantibody titres or levels. This occurs in some patients with connective tissue disorders. If the clinical features are impressive, repeat testing after a period of time (4-6 weeks) should be considered.
- Some autoantibodies have predictive value for the subsequent development of clinical disease, particularly when persistently present or when present at higher titres. For example, more than low titres of most of the

pathogenic antibodies usually have strong predictive value for the subsequent development of the relevant disease. Similarly, anti-centromere antibodies and anti-mitochondrial antibodies are usually clinically significant, but many years may elapse before the development of clinical features. By contrast, some autoimmune disorders may have been present for a long time, during which the antibody titres may have fallen substantially. This is not uncommon with islet cell antibodies in insulin-dependent diabetes mellitus, adrenal antibodies in Addison's disease and ovarian antibodies in premature ovarian failure.

- Antibody-based tests may be unreliable in immunodeficient patients.

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For example, some patients may be receiving immunosuppressive therapy, have a protein-losing disorder or have specific antibody deficiency disorders. Of the antibody deficiency syndromes, IgA deficiency is the most common, and patients with this disorder usually have no detectable IgA when their total IgA level is measured. This is a potential problem with coeliac serology.

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## What should I do when I have a patient with a positive autoantibody and some clinical features without too much specificity?

Certainly, the finding of positive autoimmune serology with relatively high titre antibodies can add weight to a diagnosis, as well as identify individuals for future scrutiny. Low-level antibodies clearly do not have particular significance, unless the disease process is early or unless the clinical features are very strong. Repeat testing after a period of clinical observation may be useful.

## How is the anti-nuclear antibody (ANA) assay performed?

The assay for ANAs is performed by overlaying diluted patient sera onto cultured human epithelial cells, known as 'HEp2 cells'. After a period of incubation, the sera are washed off and then a fluorescein-labelled antibody to human IgG is overlaid on the cells. This antibody therefore detects antibodies in the patient's serum that have bound to nuclear or cytoplasmic antigens of the HEp2 cell. The results are visualised by immunofluorescence microscopy.

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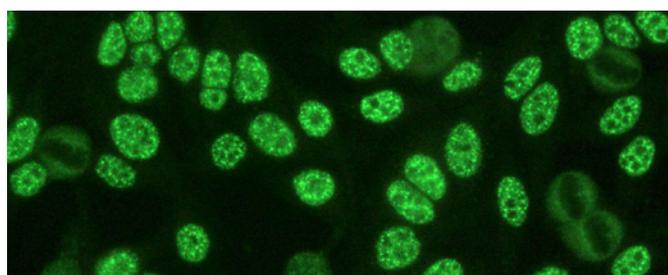
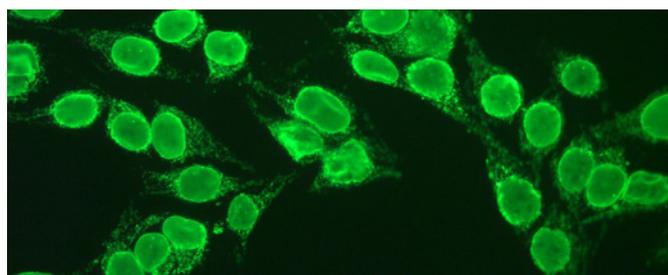
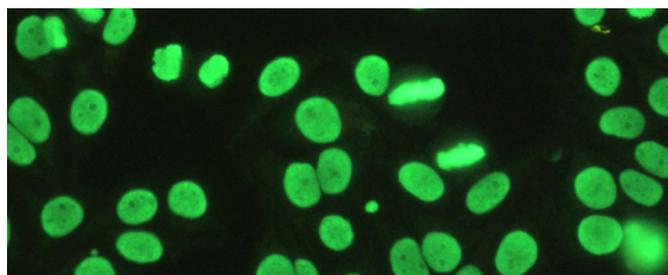
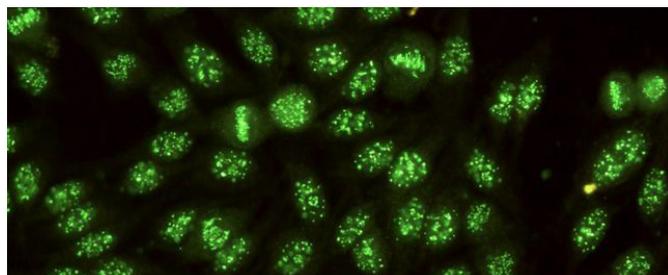
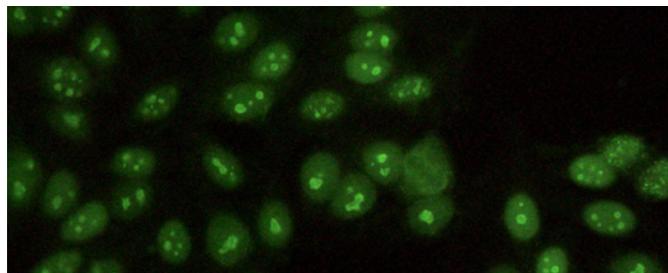
The ANA results are described as 'detected' or 'not detected' and any patterns identified are classified according to defined descriptions. For nuclear (but not cytoplasmic) patterns, the titre (reciprocal of the dilution of serum that the antibody is detected at, a measure of antibody concentration) is determined and reported.

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## What is the significance of the ANA pattern?

Some ANA patterns have clinical associations. For example:

- Nucleolar patterns suggest a greater likelihood of scleroderma.
- Centromere patterns are very strongly suggestive of a more limited form of scleroderma known as CREST syndrome (an acronym for the common characteristics of **C**alcinosis, **R**aynaud's, **O**esophageal involvement, **S**clerodactyly and **T**elangiectasia).
- Homogeneous patterns are common in patients with SLE, drug-induced lupus and autoimmune chronic active hepatitis.
- Nuclear membrane patterns may be associated with cerebral vasculitis and phospholipid antibodies.
- Speckled patterns may occur at low levels without pathological significance, as well as in patients with lupus and Sjögren syndrome.



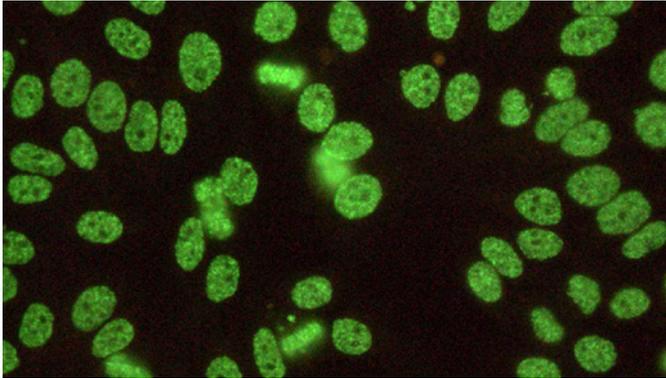
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Other patterns that seldom have clinical significance are not necessarily reported, although different laboratories might have different policies in respect to this. Often, we will also detect antibodies to cytoplasmic antigens (AMA, SMA, GPC) which may have significance for other autoimmune disorders but which must be characterised by specific testing.

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## What is a dense fine speckled ANA pattern?

This particular pattern may have been reported as homogeneous and speckled until the last five years. It is distinct from concurrent homogeneous and speckled patterns. A dense fine speckled pattern is negatively associated with connective tissue disease. Although confirmatory tests are becoming available, our approach has been to recommend ENA and dsDNA testing and, if negative, the patient need not generally have follow-up ANA testing.



## What is the significance of the ANA titre?

When ANAs are detected, the titre(s) of the pattern(s) present are determined.

For the initial screening test, the sera have been subjected to doubling dilutions, so that the sera are screened at a dilution of one fortieth of the original specimen. This is a 'titre' of 80. Positive or borderline results are repeated at higher dilutions and the highest dilution at which the antibody is detected is reported as its 'titre'.

Thus, ANA results are reported as 'detected' or 'not detected'. When detected, they are reported at titres of 80, 160, 320, 640, 1280, 2560 or greater than 2560. It is helpful to explain to patients that these results are not on an 'integer scale' and that a result of 1:80 is not necessarily significantly different to one of 1:160. From one assay to another, a change in titre to one lower or greater has no definite clinical significance and could be regarded as within measurement variation. Variation occurs when results from laboratories are compared, and serial results are best done predominantly by the same laboratory.

Low-level ANAs (titres of <320) may be pathological but can also occur in clinically normal persons and do so more commonly with advancing age. Moderate and higher titre ANAs (>320) are more often clinically significant, although not necessarily so.

## When I get an ANA result back as 'detected', are there additional tests I can do?

A not uncommon problem occurs when a patient has been found to have a moderate or even high ANA titre without any definite clinical features of connective tissue disease. While titres of 1:640 are more often significant than not, the presence of some kinds of staining patterns can be strongly suggestive of a particular disorder, as previously mentioned. To further clarify which antigenic specificities may be involved in the positive ANA, testing for antibodies to extractable nuclear antigens (ENA) can be helpful.

Individuals with only one or two clinical features of connective tissue disease and a positive ANA are much more likely to develop problems if they are deficient in the C4 component of complement.

Similarly, the finding of antibodies to ENA and their characterisation, as well as dsDNA antibodies, add to current diagnostic specificity as well as the likelihood of future clinical problems. Higher levels of certain autoantibodies, particularly the ANA, dsDNA, RF and a few others, correlate not only with greater diagnostic likelihood but also disease activity.

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In the presence of mild or non-specific clinical features, repeat estimations of mildly or even moderately abnormal results after several months is often the most useful approach to clarifying clinical questions.

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## Tell me more about the ENA test. What are the associations of the different ENA specificities?

Originally, when different ANA patterns were described, intense efforts were made to try to identify clinical correlations with the different patterns. Subsequently, other methods were developed to identify antibodies to 'extractable nuclear antigens' and a range of clinical associations has been described with antibodies to different ENA specificities. With ENA testing, a screening assay is performed and, if positive, a typing or characterisation assay is then performed.

- Antibodies to ribosomal-P occur in only 15% of patients with SLE, are highly specific and more common in patients with neuropsychiatric manifestations.
- Antibodies to Sm are also highly specific for SLE but occur in only about 10% of patients in Australia, being more prevalent in different patient populations.
- Nearly 30% of patients with SLE may have antibodies to RNP, but otherwise they are quite characteristic as the only antibody in patients with mixed connective tissue disease.
- Antibodies to Scl-70 are highly specific for scleroderma, occurring in about 70% of patients with diffuse scleroderma but also occurring in about 15% of patients with more limited forms of scleroderma, including CREST syndrome. The anti-centromere antibody pattern, which has very high specificity and predictive value for CREST syndrome, is detected by the ANA test, not the ENA test.
- Antibodies to Jo-1 are present in 30% of patients with polymyositis-dermatomyositis and may indicate a greater likelihood of associated pulmonary fibrosis.

➤ The most common antibodies detected by the ENA test are antibodies to SSA and SSB. The finding of antibodies to both SSA and SSB is characteristic of primary Sjögren syndrome. Women with SLE who have both these antibodies are more likely to develop sicca symptoms, cutaneous involvement and, if pregnant, their babies may be more at risk of developing congenital heart block as well as transient cutaneous lupus. Persons who have antibodies to SSA alone may either have SLE or Sjögren syndrome. A small number of patients only have antibodies to SSB and usually have milder disease.

### Are there any new developments in autoantibody testing?

The recognition and reporting of the dense fine speckled pattern will reduce the numbers of persons with positive ANA without disease receiving long-term serologic follow-up. Increasingly, the target autoantigens of autoantibodies or auto-reactive T cells are being characterised, allowing the development of tests that may have greater specificity and sensitivity. Important examples

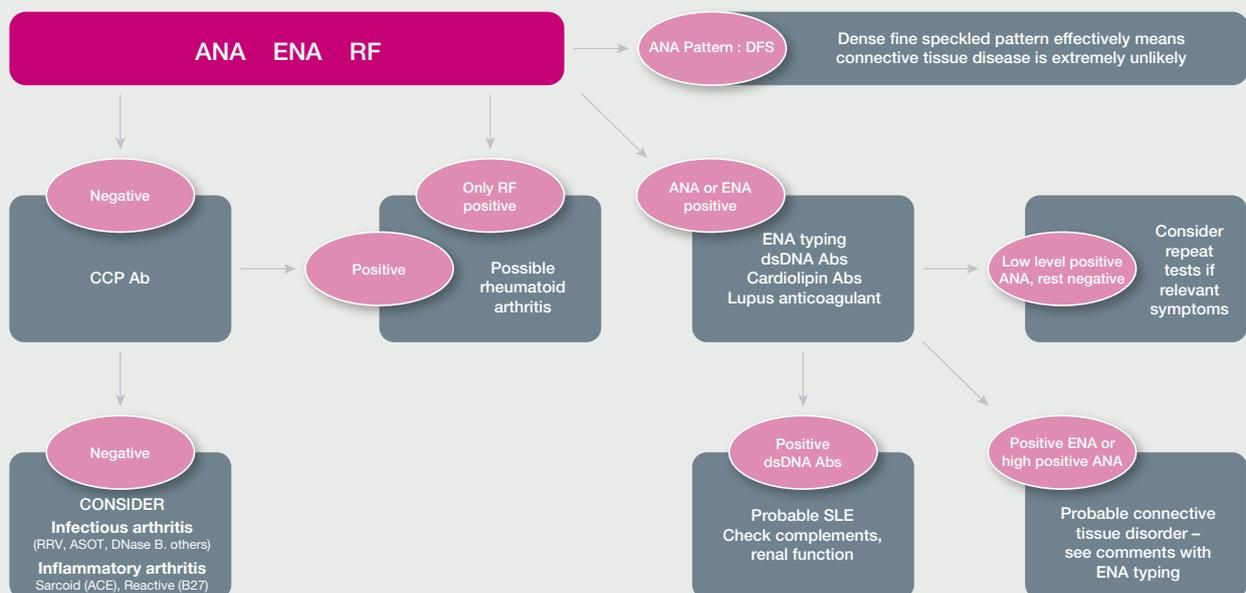
include the development of recombinant or genetically engineered antigens for testing, such as transglutaminase in coeliac disease, as well as extended immunoblot assays for the characterisation of autoantibodies in connective tissue and autoimmune muscle disease. These extended immunoblot assays enhance diagnostic specificity, but are more costly than rebates provided by the Medicare Benefits Schedule. For this reason, we are unable to perform the myositis line immunoassay on a bulk-billed request. In respect to screening tests for connective tissue disease, autoantibodies are often screened by indirect immunofluorescence with a human epithelial cell line (HEp2); this has been enhanced by the use of HEp2000 which hyper-expresses SSA. Other important techniques include RIA, immunoblotting, ELISAs and ALBIA methods; not surprisingly different methods have advantages and disadvantages, and performance characteristics may vary by patient as well as method. The clinical validation of these newer assays, however, has been problematic, since some assays identify similar and overlapping, but not the same, patient populations! Consequently, comparison of assays between laboratories can also be problematic in selected patients.

### What tests should I perform to investigate patients with symptoms of arthritis or possible connective tissue disease?

One approach is summarised here.

Formal diagnosis of connective tissue disorders requires a combination of clinical and laboratory features. Negative laboratory tests weigh heavily against, but do not exclude, a particular disorder.

Sometimes, when symptoms are of recent onset, repeat testing after a period of time is helpful.



# Clinical applications of autoantibody tests

ENDOCRINE			
Test	Disease	Clinical features	Significance of laboratory result
Ovarian Ab	Autoimmune ovarian failure	Amenorrhoea/early menopause	Majority have antibodies to theca interna cells and often other endocrine abnormalities.
Sperm Ab	Antisperm Ab	Infertility	A variety of types and targets have been described in females and males as contributing to infertility (pathological).
Adrenal Ab	Autoimmune adrenal disease	Addison's disease	>60% have antibodies to adrenal cortex cells (ACTH receptor); may be pathological.
GAD Ab	Diabetes mellitus type 1	IDDM (type 1)	Almost all have antibodies to glutamic acid decarboxylase and some have features of concomitant scleroderma.
Islet cell Ab	Diabetes mellitus type 1	IDDM (type 1)	Islet cell antibodies may be detected in almost all patients with IDDM prior to the development of disease, and the detection rate falls thereafter.
Thyroid Abs (ATG, TPO)	Hashimoto's thyroiditis	Diverse thyroid disorders	>90% have antibodies to thyroid peroxidase (TPO). >55% have antibodies to thyroglobulin (ATG).
Thyroid receptor Ab (TRAG)	Graves' disease	Diffuse goitre	Almost all have antibodies to TSH receptor. Clinical utility of TSH receptor antibodies is for evaluation of diffuse goitre and prediction of both antenatal and post-treatment risk of disease.

MUSCLE DISEASE			
Test	Disease	Clinical features	Significance of laboratory result
ENA (ANA)	Polymyositis dermatomyositis	Inflammatory muscle	Antibodies of PmScl indicate a high probability of features of concomitant scleroderma.
HMGCR Ab HMG-CoA reductase receptor Ab		Severe statin myopathy	Statins must be discontinued.
GAD Ab	Stiff person syndrome	Progressive rigidity and muscular spasms	Almost all have antibodies to glutamic acid decarboxylase.

## Muscle disease

Additional autoimmune myositis-specific antibodies can be detected by immunoblotting. This assay should be considered for seronegative patients in whom autoimmune myositis is suspected, and can detect additional anti-synthetase antibodies (EJ, OJ, PL7, PL12), Mi-2 antibodies as well as SRP antibodies.

**Patients will be privately billed for these specialised antibody tests.**

NEUROLOGIC DISEASE			
Test	Disease	Clinical features	Significance of laboratory result
ACHR Ab	Myasthenia gravis	Fatiguability of skeletal muscle	>95% have antibodies to acetylcholine receptors. The presence of antibodies to striated muscle is suggestive of an underlying thymoma.
Neuronal Ab	Anti-myelin associated glycoprotein polyneuropathy	Peripheral neuropathy	All have antibodies to nerve sheath of which 35% are to myelin-associated glycoprotein. The majority occur as IgM monoclonal gammopathies.
GM-1 Ab	Guillain-Barré syndrome	Peripheral neuropathy	Limited value as a wide array of antigens have been described of which antibodies to ganglioside GM-1 have been recognised more frequently than others (up to 20%).
Ganglioside Ab	Peripheral neuropathy	Multiple sites of CNS demyelination	Specific autoAbs have limited value. Oligoclonal bands (of IgG) by CSF EPG are useful.

## Neurologic disease

Neuronal antibodies detected by the immunoblot assay include amphiphysin, CV2.1, PNMA2 (Ma2/Ta), Ri, Yo and Hu.

**An extended neuronal antibody panel, including another six antigens, is available for which patients will be privately billed.**

Ganglioside antibodies detected by the immunoblot assay include IgG and IgM antibodies to GM1, GM2, GD1a, GD1b and Gq1b.

**Patients will be privately billed for these specialised ganglioside antibody tests.**

# Tests for connective tissue diseases

## RHEUMATOLOGICAL DISORDERS

Test	Disease	Clinical features	Significance of laboratory result
<b>RF</b>	Rheumatoid arthritis Other disorders	Inflammatory polyarthritis	The level of RF is accurately measured by a number of methods with the presence of, and higher titres of, RF having prognostic significance.
<b>CCP Ab</b> (Cyclic citrullinated polypeptide Ab)	Rheumatoid arthritis	Inflammatory polyarthritis	May precede RF. Predicts more aggressive erosive disease. Level reflects inflammatory activity.
<b>ANA</b> (Anti-nuclear AB)	Connective tissue disorders	SLE, scleroderma, CREST syndrome, other connective tissue disorders	The majority of persons with connective tissue disorders develop antibodies to nuclear antigens. Many patients may have low-level antibodies in the initial phase of their illness. In some patients, the titre of ANAs may vary with the clinical activity of their disease.
<b>dsDNA</b> (Double-stranded DNA Ab)	SLE	SLE	Present in patients with renal or cerebral lupus, and when present, are a useful marker of disease activity.
<b>Chromatin Ab</b> (Chromatin or nucleosome Ab)	SLE Drug-induced lupus		Identifies antibodies to histone as well as dsDNA. Inferior disease activity marker to dsDNA.
<b>ENA</b> (Antibodies to extractable nuclear antigens)	Connective tissue disorders		It is possible to classify connective tissue disorders on the basis of clinical and laboratory features into a number of different subsets with different outcomes and management. The detection and typing or characterisation of antibodies to extractable nuclear antigens is useful in this context.
<b>ACL Ab (LAC)</b> Anti-phospholipid Ab (Lupus anticoagulant)	Excessive clotting	Venous clotting, miscarriages	Abs to cardiolipin of IgG (and less commonly IgM) isotopes have been found in increasing frequency in individuals with excess fetal loss as well as venous and arterial thrombosis. Higher levels, particularly IgG isotype with beta2-glycoprotein I antibodies, are more frequently correlated with disease. Many, but not necessarily the majority, of such persons have other connective tissue disorders.

### Extended systemic sclerosis antibodies

Detection of antibodies to CENPA, RP11, RP155, fibrillarin, NOR90, Th/To, PM-Scl75, Ku, PDGFR by immunoblotting is possible although **patients will be privately billed for this specialised test.**

## SKIN

Test	Disease	Clinical features	Significance of laboratory result
<b>Skin BM Ab</b>	Bullous pemphigoid	Subepidermal blisters	>70% have Abs to dermal-epidermal basement membrane (pathological).
<b>Skin ICCS Ab</b>	Pemphigus vulgaris	Intraepidermal blisters	>90% have Abs to intercellular cement substance (pathological).
<b>Gliadin. tTG, EMA Ab</b> (Deamidated gliadin IgG, IgA; tissue transglutaminase IgG, IgA endomysial IgA Ab)	Dermatitis herpetiformis	Pruritic papules & blisters	These Abs are usually positive in dermatitis herpetiformis. Skin biopsy is recommended. Small bowel biopsy is useful.

## HAEMATOLOGICAL

Test	Disease	Clinical features	Significance of laboratory result
<b>Platelet Ab</b>	Immune thrombocytopenia	Various forms (acute, drug and chronic)	Limited value. Anti-platelet Ab (pathological). Often inferred after bone marrow biopsy. Associated Ab tests (ANA) are important.
<b>Neutrophil Ab</b>	Immune neutropenia	Various forms	Limited value (pathological). Often inferred after bone marrow biopsy.
<b>Coombs test</b>	Immune haemolytic anaemia	Warm & cold forms	Coombs test with modification. Classification relevant to treatment, prognosis through associated diseases (pathological).

## RENAL & VASCULITIS

Test	Disease	Clinical features	Significance of laboratory result
<b>GBM Ab</b>	Goodpasture's syndrome	Haemoptysis and haematuria	Ab to glomerular basement membrane (pathological).
<b>ANCA</b> (PR3, MPO)	Granulomatosis with polyangiitis	Systemic vasculitis	The c-ANCA (classic cytoplasmic) pattern of anti-neutrophil cytoplasmic Ab (target antigen, being neutrophil proteinase 3), has strong specificity and sensitivity for active vasculitis.

## GASTROINTESTINAL

Test	Disease	Clinical features	Significance of laboratory result
<b>ANCA</b> (Anti-neutrophil cytoplasmic Ab)	Ulcerative colitis	Inflammatory bowel disease	c-ANCA and PR3 antibodies may occur with ulcerative colitis and predict concurrent sclerosing cholangitis. p-ANCA, the perinuclear staining cytoplasmic staining pattern, may occur with Crohn's disease.
<b>ASCA</b> (Anti- <i>saccharomyces cerevisiae</i> IgG IgA Ab)	Crohn's disease	Inflammatory bowel disease	Only likely significant if both IgA and IgG ASCA antibodies positive.
<b>Gliadin, tTG Ab</b> (Deamidated gliadin IgA IgG Ab; tissue transglutaminase IgA, IgG Ab)	Coeliac disease	Gluten intolerance	Deamidated gliadin IgA and IgG Ab; TTG IgA and IgG; All four are tested concurrently in our coeliac serology assay. IgA deficiency may confound IgA antibody assays. Elevated levels suggest coeliac disease and normalise with gluten restriction over some months.
<b>GPC, IF Ab</b> (Gastric parietal cell Ab, intrinsic factor Ab)	Pernicious anaemia	Impaired B12 absorption	90% have parietal cell Abs or intrinsic factor antibodies. Intrinsic factor Abs (block binding of B12 or block absorption of B12 bound to intrinsic factor) are more specific, but may disappear with established disease. (Schilling's test is no longer available.)

## LIVER

Test	Disease	Clinical features	Significance of laboratory result
<b>ANCA</b> (Anti-neutrophil cytoplasmic Ab)	Sclerosing cholangitis (primary)	Chronic fibrosis of hepatobiliary tree	90% have c-ANCA Abs and often PR3 antibodies.
<b>SMA</b> (Smooth muscle Ab)	Autoimmune chronic active hepatitis	Chronic hepatitis without evidence of viral infection	80% have smooth muscle Abs. High titre. IgG Abs more specific.
<b>LKM Ab</b> (Liver kidney microsomal Ab)	LKM - Ab associated chronic active hepatitis	Chronic hepatitis	Other rare variants have liver kidney microsomal antibodies, and antibodies to asialoglycoprotein receptors have been described.
<b>AMA (M2 Ab)</b> (Anti-mitochondrial Ab and also M-2 subtype)	Primary biliary cirrhosis	Progressive portal fibrosis	>95% have anti-mitochondrial antibodies. Many (>9) subtypes of mitochondrial antibodies have been described. Rarely AMA negative patients have M2 antibodies which can be detected by a specific immunoblot.

**Additional liver disease autoantibodies** can be detected by immunoblotting. These are AMA-M2, AMA M2-3E, Sp100, PML, gp210, LC-1, SLA/LP.

**Patients will be privately billed for these specialised antibody tests.**



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