

**ADVICE ON THE USE AND INTERPRETATION OF IMMUNOLOGY LABORATORY TESTS THIS DOCUMENT IS THE PROPERTY OF THE DEPARTMENT OF IMMUNOLOGY.**

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1. Immunoglobulins

The standard assay includes measurement of total serum IgG, IgA and IgM. Serum protein electrophoresis is performed on all samples.

**INVESTIGATION OF POSSIBLE PARAPROTEINAEMIA (MYELOMA, WALDENSTRÖM'S MACROGLOBULINAEMIA)**

Paraproteins are products of expanded monoclonal populations of B-lymphocytes. They can be secreted as intact whole immunoglobulins or free immunoglobulin light chains (it is rare to see free immunoglobulin heavy chains). When the secreted paraprotein is a whole immunoglobulin molecule, this will usually only be detectable in the serum; when the paraprotein is a free immunoglobulin light chain, this will usually only be detectable in the urine (as Bence-Jones protein). If you wish to confidently exclude paraproteinaemia, it is ALWAYS necessary to send both serum and urine sending only serum will miss 85% of cases and sending only urine will miss 15% of cases. Preferably, these should be sent to the laboratory as paired samples 5 ml clotted blood plus 10 ml urine specimen (early morning sample preferred). The potential significance of any reported paraprotein should be considered in the context of the patient's age, clinical condition, and other relevant laboratory findings.

Raised ESR, anaemia, raised urea / creatinine, hypercalcaemia, immune paresis (suppression of the non-paraprotein immunoglobulin classes), bone pain, recurrent infection or hyperviscosity make malignancy more likely. Patients with low-level paraproteins and no immune paresis may remain well for years with MGUS (monoclonal gammopathy of uncertain significance) but should be monitored. All patients with a paraprotein and immune paresis or clinical disease should be referred for specialist haematology assessment.

**INVESTIGATION OF POSSIBLE ANTIBODY DEFICIENCY**

The most common clinical consequence of antibody deficiency is recurrent infection of the respiratory tract (including the ears and sinuses) with polysaccharide-encapsulated organisms (e.g. Strep. pneumoniae, H. influenzae). The majority of patients with antibody deficiency have subnormal total serum IgG levels. However, if there is a high index of

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suspicion (e.g. history of recurrent chest infections or bronchiectasis), specific antibodies should be requested, even if the total IgG is within the normal range. Measurement of IgG subclasses contributes little additional information and is no longer routinely performed. All cases of suspected or proven antibody deficiency should be discussed with a clinical immunologist.

## 2. Complement

### INVESTIGATION OF POSSIBLE COMPLEMENT CONSUMPTION (e.g. active SLE)

Request C3 & C4.

### INVESTIGATION OF POSSIBLE COMPLEMENT DEFICIENCY

If you suspect complement deficiency, this should be discussed with the clinical immunologist, who can advise on the appropriate functional assay.

### INVESTIGATION FOR C1-INHIBITOR DEFICIENCY

Patients with a history of unexplained episodic angioedema and / or abdominal pain, or a family history of these symptoms (or of known hereditary angioedema), may merit investigation for hereditary or acquired C1-inhibitor deficiency. The best screening test is a complement C4 level. In an untreated patient (i.e. not taking danazol or any androgenic steroid) with genuine C1-inhibitor deficiency, the C4 is usually markedly reduced well below the normal range. If the C4 is normal in an untreated patient, C1- inhibitor deficiency is extremely unlikely. However, if there is a particularly high index of suspicion, repeat the C4 during an attack of symptoms if it is still normal, the patient does not have C1-inhibitor deficiency. If a low C4 is found in any of the above settings, it is appropriate to measure C1-inhibitor and possibly functional C1-inhibitor levels (the latter requires a fresh citrate sample and is necessary to exclude type II deficiency, where there are normal levels of a functionally abnormal protein).

**All cases of genuine C1 inhibitor deficiency (hereditary or acquired) should be referred to the immunology clinic.**

Note that: C1-inhibitor deficiency is NOT a cause of urticaria and it is unnecessary and wasteful to investigate patients for C1 inhibitor deficiency because they have urticaria (although C3 and C4 may be useful in suspected hypocomplementaemic urticarial vasculitis). The most common form of angioedema is idiopathic angioedema (which, by definition, evades further explanation). Other causes are allergy (if allergy is the cause, the

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history virtually always gives a clue to a possible or likely cause if it doesn't, allergy is unlikely to be the explanation), and treatment with an ACE inhibitor. No patient with a history of angioedema should be given an ACE inhibitor. Other causes of an isolated low C4 include congenital C4 deficiency (the commonest cause), cryoglobulinaemia and some vasculitides.

### 3. Autoantibodies

#### GENERAL COMMENTS ON AUTOANTIBODY TESTING

##### Connective tissue disease

Although autoantibody testing is a useful adjunct to the diagnosis of autoimmune connective tissue disease, the tests have limited specificity. Also, these diseases affect only a small proportion of the community. As a result, the tests will have a low positive predictive value if they are used indiscriminately that is to say, if the tests are performed on patients who have little or no real clinical evidence of relevant disease, most of the positive results will be found in patients without disease. Therefore, the sensible advice is: If you don't think there are real clinical grounds for suspecting the patient to have an autoimmune connective tissue disease don't ask for autoantibodies. The result is unlikely to tell you anything useful. Similarly, if you receive a positive autoantibody result on a patient, think: Are the clinical findings in keeping with this result? not: The result is positive, so the patient must have disease. For example, it is a very common situation to receive samples from patients whose only clinical feature is non-specific joint discomfort unaccompanied by any signs of an inflammatory process autoantibody testing is unlikely to contribute usefully to the management of such patients. In addition, speckled pattern antinuclear antibodies of undefined specificity (and usually at low titre) occur quite frequently in the normal ageing female population.

##### Organ-specific autoimmunity

The detection of some autoantibodies is of relevance to the diagnosis of organ-specific autoimmune conditions e.g. thyroid autoantibodies in autoimmune thyroid disease. These are less likely to be requested when there is no evidence of relevant disease. However, it still needs to be borne in mind that treatment should be based on evidence of organ dysfunction (e.g. deranged hormone levels), not positive autoantibody results.

#### INTERPRETATION

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Problems of interpretation are most frequently encountered with antinuclear antibodies (ANA) and antibodies to extractable nuclear antigens (ENA), so these are discussed further below.

There are two main features of the ANA report the pattern and the strength of the antibody.

Patterns that are routinely reported:

1. homogeneous due to autoantibodies binding to DNA, or proteins which are closely associated with it, and therefore widely distributed throughout the nucleus. When we detect a homogeneous ANA, we automatically perform further testing for double-stranded DNA (dsDNA) antibodies, which are classically associated with SLE. However, even when these aren't found, SLE is still the main disease association of a homogeneous ANA.

2. speckled due to autoantibodies binding to antigens which are less widely distributed through the nucleus. When we detect a speckled ANA, we automatically perform further testing for antibodies to extractable nuclear antigens (ENAs). The ENA antibodies routinely tested for are: Ro (SS-A) found in SLE (increased risk of vasculitis, nephritis, lymphadenopathy, leucopenia), and primary Sjögren's syndrome. Ro is also associated with subacute cutaneous lupus (when it is usually the only autoantibody present) and neonatal lupus (sometimes complicated by heart block) La (SS-B) usually found in association with Ro. Sm (an abbreviation of Smith, not smooth muscle) found in SLE (increased risk of renal involvement) RNP (or u1-RNP) found in SLE, mixed connective tissue disease (when it is usually the only autoantibody present), sometimes in rheumatoid arthritis Scl-70 found in the disseminated form of scleroderma (increased risk of visceral involvement) Jo-1 found in polymyositis/dermatomyositis When ENA antibodies aren't found, the significance of the speckled ANA is unclear.

3. nucleolar due to autoantibodies binding to antigens only present in the nucleolus. Nucleolar antibodies are associated with scleroderma and scleroderma-polymyositis overlap syndromes.

4. centromere found in limited cutaneous scleroderma (also known as CREST syndrome); likely to be relevant if the patient has Raynaud's phenomenon.

5. nuclear dots - not often seen, but associated with primary biliary cholangitis.

6. cytoplasmic patterns – seen in a variety of connective tissue diseases and primary biliary cholangitis.

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The strength of the antibody is no longer reported as a titre. Instead results are reported as negative, weak positive, positive and strong positive. A weakly positive ANA result is approximately a 1/80. A positive result is between 1/160 and 1/640. A strong ANA can be interpreted as a titre of >1/640.

Other autoantibodies.

Two other antibodies need specific mention because of their importance and / or ability to confuse:

1. Liver / kidney microsomal antibodies (LKM) the major association of LKM antibodies is autoimmune hepatitis, but they have been found in viral and drug-induced hepatitis and cryptogenic cirrhosis.

2. Antineutrophil cytoplasmic antibodies (ANCA) there are two diagnostically significant ANCAs: antibodies to neutrophil myeloperoxidase (anti-MPO), which typically give a perinuclear pattern on immunofluorescence (pANCA). They are predominantly associated with microscopic polyangiitis and necrotising crescentic glomerulonephritis (or rapidly progressing glomerulonephritis RPGN), but are also present in about 60 % of cases of Churg-Strauss vasculitis. antibodies to proteinase 3 (anti-PR3), which typically give a cytoplasmic pattern on immunofluorescence (cANCA). They are predominantly associated with Wegener's granulomatosis, but also found in some patients with microscopic polyangiitis and Churg- Strauss vasculitis. Sometimes a cANCA pattern will be reported with negative antibodies (to PR3 and MPO) this is of uncertain significance. However, a pANCA with negative antibodies is not unusual in inflammatory bowel disease (especially ulcerative colitis) or primary sclerosing cholangitis. Finally, it should be remembered that a negative ANCA does not exclude a diagnosis of vasculitis some well characterised vasculitides are typically ANCA-negative, e.g. polyarteritis nodosa and rheumatoid vasculitis

#### 4. Allergy tests

Total serum IgE and a variety of antigen-specific IgE tests are available. Think critically when requesting IgE tests there is little point in random, "blind" screening for specific IgE against antigens which the clinical history has not implicated as potential causes of symptoms. If there is a suspected antigen, request an IgE test specific to that antigen, not to other irrelevant ones. Remember that IgE tests are only useful in cases of suspected type I (immediate) hypersensitivity and are essentially useless for the investigation of non-specific symptoms or delayed hypersensitivity (such as contact eczema). Finally, interpret

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IgE test results with caution a positive specific IgE does not always mean that the patient is clinically allergic to the antigen (especially in strongly atopic patients with eczema and high total IgE levels); a negative specific IgE does not guarantee that the patient is not allergic (especially if the total IgE is low)