Derriford Combined Laboratory offers an immunology diagnostic service to South & West Devon and Cornwall. The repertoire offered includes immunochemistry, autoantibodies, cellular immunology and allergy tests. The majority of the tests are performed in-house, but a number are sent to specialist reference centres. A clinical immunology service is also provided for patients with primary immune deficiencies and allergies.

		External	Internal
Dr Claire Bethune	Consultant Immunologist	431002	31002
Dr Andrew Whyte	Consultant Immunologist	431002	31002
Kristen Lilly	Clinical Scientist	430247	30247
Tara Knill	Biomedical Scientist	792293	52293

Opening hours for the laboratory are Monday to Friday 9am-5.30pm

Immunology Tests

Allergy

Total IgE	
Synonyms	None
Indications	There is no role for measurement of total IgE in the routine investigation of allergic disease. There is a role for measurement in suspected allergic bronchopulmonary aspergillosis, as part of the workup for some medications used in specialist care, and in a very rare immunodeficiency.
Requestor	Primary (not recommended) and secondary care
Method	Fluorescent enzyme immunoassay
Clinical Background	Total IgE is elevated in atopic disease (eczema>asthma>allergic rhinitis), as well as in parasitic infections and scabies.
Interpretation	An elevated total IgE level would support a diagnosis of atopy, although this should be a clinical diagnosis and total IgE levels add nothing to the management.
Associated tests	Specific IgE testing, mast cell tryptase
Sample Requirements	Serum

Specific IgE	
Synonyms	Previously referred to as RAST
Indications	These tests should only be performed to confirm a clinically suspected diagnosis of allergy, and should never be used as a “screen” for allergy. Specific IgE testing to drugs is not recommended in primary care. Testing to foods should be requested only if the patient has had onset of symptoms within 1-2 hours of exposure to the food, and not if

	they have subsequently tolerated the allergen. There is some role for measurement of aeroallergens when the allergen can be avoided (eg cat, dog), but pollen-specific IgE need only be measured if the patient is under the care of the clinical immunology service and desensitisation is being considered.
Requestor	Primary and secondary care
Method	Fluorescent enzyme immunoassay
Clinical Background	Specific IgE is produced in susceptible individuals following exposure to an allergen. The IgE is then bound to mast cells and on repeat exposure to the allergen triggers mast cell degranulation and the clinical features of type 1 hyper sensitivity (allergic rhinitis, urticaria/angioedema, anaphylaxis).
Interpretation	A normal specific IgE level to an allergen does not exclude allergy to that substance, and a positive result is not diagnostic. The relevance of the level is dependent on the allergen in question.
Associated tests	Total IgE, mast cell tryptase
Sample Requirements	Serum

Anaphylaxis

Serum tryptase levels are elevated following mast cell degranulation due to anaphylaxis (type I hypersensitivity or pseudoallergic reactions) or mast cell syndromes (rare). Blood should be collected within 3 hours of the event.

Mast cell tryptase	
Synonyms	Tryptase, MCT
Indications	Suspicion of anaphylaxis or systemic mastocytosis
Requestor	Primary or secondary care
Method	Fluorescent enzyme immunoassay
Clinical Background	Tryptase is released from mast cells as a result of degranulation. This may result from type 1 hypersensitivity reactions, due to medications (for example opiates), or spontaneously. Following a suspected episode of anaphylaxis three samples should be taken: at the time of the reaction (within an hour or as soon as possible), no later than 4 hours after onset of symptoms, and a baseline sample at least 24 hours after the reaction.
Interpretation	An elevated mast cell tryptase supports a clinical diagnosis of anaphylaxis. Note that not all cases of anaphylaxis will result in an elevated tryptase level, meaning that a normal tryptase level does not exclude anaphylaxis.
Associated tests	Specific IgE levels, total IgE (not recommended in the routine investigation of allergy)
Sample Requirements	Serum (for timing requirements see above)

Autoimmunity

Requests for autoantibody testing should only be made where there is a clinical suspicion of an autoimmune disease. An autoimmune profile or antinuclear antibody is not a useful screening

test for ‘arthralgia, fatigue or diffuse pain’. Clinical details must be clearly entered on the request form so that appropriate follow-on tests can be added.

ANA	
Synonyms	Anti-nuclear antibodies; connective tissue disease screen,
Indications	An antinuclear antibody should only be requested when a connective tissue disease (e.g. SLE) is suspected clinically. Suspicion may be raised if the following features are present: • Non-erosive arthritis • Late-onset Raynaud’s phenomena • Malar or discoid rash • Photosensitivity • Pleuritis / pericarditis • Renal disease • Neurological disorder • Haemolytic anaemia / leukopaenia, lymphopaenia, thrombocytopaenia ANA testing can also aid diagnosis of autoimmune liver disease in conjunction with the autoimmune liver disease screen. ANA and ENA change very little over time, and have no role in monitoring disease activity. As such, there is usually no role for repeat testing unless the clinical presentation changes.
Requestor	Primary and secondary care
Method	Indirect immunofluorescence on Hep2 cells
Clinical Background	Detects presence of autoantibodies to various nuclear and cytoplasmic proteins. Not all detectable antibodies have disease associations. Further testing is undertaken on clear positive (>1:160) samples to identify disease-associated autoantibodies.
Interpretation	ANA results are reported as a titre (dilution) at which the autoantibodies are detectable; for example 1 part patient serum, 80 parts diluent = 1:80. A negative result virtually excludes connective tissue disease. A result of ANA 1:80 may be non-specific and can be seen in the elderly, pregnancy, inflammatory or infective states. No further testing will be undertaken.
Associated tests	A result of ANA 1:160 (or above) will prompt further testing in the lab. • dsDNA (autoantibodies to double-stranded DNA) • ENA Screen (Extractable nuclear antigens) • ENA Immunotyping
Sample Requirements	Serum
Sensitivity, Specificity, Uncertainty of measurement	Very sensitive. Low specifity at low titres, significance of high titres depends on clinical presentation.

Liver autoantibodies	
Synonyms	Smooth muscle antibodies, anti-mitochondrial antibodies, anti-LKM

	antibodies
Indications	• Unexplained abnormal liver function tests • Suspected autoimmune hepatitis
Requestor	Primary and secondary care
Method	Indirect immunofluorescence on rodent liver/kidney/stomach tissue
Clinical Background	There are two main forms of autoimmune hepatitis. The more common form is type I, which is usually found in young women and may be associated with other autoimmune conditions. Type II is much less common and is seen in younger girls aged 2-14.
Interpretation	Smooth muscle antibodies are present in up to 70% of patients with chronic active autoimmune hepatitis (particularly type I). However, they are relatively non-specific and can be present at lower levels in a variety of infections and other inflammatory conditions. Anti-mitochondrial antibodies (AMA) are sensitive and specific for primary biliary cirrhosis, and may precede clinical disease by years. Cases of positive AMA will be confirmed to be anti-M2 (most frequently associated with PBC) by line immunoassay. Anti-LKM antibodies are found in a subset of patients with type II autoimmune hepatitis.
Associated tests	Liver function tests, ANA
Sample Requirements	Serum

Cardiolipin autoantibodies	
synonyms	ACL antibody
Indications	• Unexplained thrombotic or embolic episode (eg DVT, PE, cerebral venous thrombosis, renal or hepatic artery thrombosis, or AMI/CVA in younger patients). • Recurrent miscarriages, particularly after 10 weeks gestation • Severe pre-eclampsia, eclampsia, or severe placental insufficiency • Prolonged PTT (partial thromboplastin time) • Should be requested in conjunction with lupus anticoagulant and anti-B2GP1 antibodies
Requestor	Primary and secondary care
Method	ELISA
Clinical Background	Antiphospholipid syndrome may present with diverse symptoms due to arterial or venous thrombosis, or recurrent miscarriages. Cardiolipin antibodies may be present in patients with SLE, often in association with lupus anticoagulant. However, antibody levels do not correlate with extent or severity of thrombosis, and as such repeat measurement at least 12 weeks later is necessary.
Interpretation	A diagnosis of antiphospholipid syndrome must not be based solely on anticardiolipin antibodies. Moderate rises in levels can occur transiently following infection (especially IgM ACL), and as such there are defined international clinical and laboratory criteria which should be consulted. These include presence of one or more of the following on two occasions at least 12 weeks apart: (a) lupus anticoagulant in plasma, (b) moderate to high levels of IgG or IgM anticoagulant, or (c) moderate to high levels of anti-B2GP1 antibodies.
Associated tests	Lupus anticoagulant, anti-B2GP1 antibodies, PTT

Sample Requirements	Serum

Coeliac Screen	
Synonyms	Tissue transglutaminase (TTG) Endomysial antibodies (EMA)
Requestor	Primary or secondary care
Indication	Aid diagnosis of or assess treatment response in coeliac disease
Clinical background	Coaelic disease is relatively common and an often under recognised delayed type IV HS to gluten proteins in wheat, barley, rye & oat. Primary leads to GI symptoms, bloating, variable bowel habit, weight loss and malnutrition. There is significant variability and severity. There is a higher incidence in first degree relatives of those with coeliac disease .
Sample type	Serum
Special considerations	This test detects IgA antibodies to TTG and therefore it is recommended to confirm the patient has normal IgA levels (as per NICE guidelines). TTG may be negative if patient is on a low gluten/gluten free diet (recommend gluten containing food regularly for 6 weeks prior to testing). If clinical suspicion remains high despite negative testing, consider discussion with gastroenterologist.
Associated tests	IgA Endomysial Antibodies
Clinical correlations	Coeliac disease can be associated with thyroid autoimmunity and pernicious anaemia, and in some cases may present with dermatitis herpetiformis.

IgA Endomysial Antibodies	
Synonyms	IgA EMA, ENDOA
Requested by	Not available for direct request.
Indication	Performed by the laboratory to confirm a positive TTG
Background/Clinical context	Positive TTG for confirmation.
Sample type	Serum
Special considerations	
Associated tests	Total IgA, TTG, IgG EMA
Clinical correlations	-
IgG Endomysial Antibodies	
Synonyms	IgG EMA, ENDOG
Requestor	Primary or secondary care
Indication	Screening for coeliac disease in patients with IgA deficiency.
Clinical background	As above.
Sample type	Serum
Special considerations	This test detects IgG antibodies to EMA, which allows screening in patients with IgA deficiency. IgG EMA may be negative if patient is on a low gluten/gluten free diet (recommend gluten containing food regularly for 6 weeks prior to testing).

	If clinical suspicion remains high despite negative testing, consider discussion with gastroenterologist.
Associated tests	Total IgA, TTG, IgA EMA
Clinical correlations	

ANCA	
Synonyms	Anti-neutrophilic cytoplasmic antibodies (ANCA), neutrophil cytoplasmic antibodies, C-ANCA, P-ANCA
Indications	Diagnostic screen in cases of suspected small vessel ANCA-associated vasculitis, which may present with; • Chronic necrotising large airways disease • Cavitating pulmonary nodules • Subglottic stenosis • Pulmonary-renal syndrome • Rapidly progressive glomerulonephritis • Cutaneous vasculitis accompanied by systemic symptoms • Mononeuritis multiplex The main diseases associated with a positive ANCA include; Granulomatous Polyangitis (GPA/Wegener's Granulomatosis), Microscopic Polyarteritis (MPA) and Eosinophilic granulomatous polyangitis (EGPA/Churg-Strauss Syndrome). **Please note that many causes of vasculitis (eg polyarteritis nodosa, giant cell arteritis, cutaneous vasculitis, Behcet's syndrome, Takayasu arteritis) are ANCA negative. As such a negative ANCA does not exclude another cause of vasculitis.**
Requestor	Primary and secondary care
Method	Indirect immunofluorescence on human neutrophils
Clinical Background	Detects presence of autoantibodies to neutrophil proteins. Further testing is undertaken on positive or indeterminate samples to identify disease-associated autoantibodies.
Interpretation	ANCA results are reported as positive, negative or atypical. A negative result will not trigger further testing. A positive C-ANCA or P-ANCA (or atypical) pattern will trigger further confirmatory testing for anti-MPO and anti-R3 antibodies, which increases the specificity for small vessel ANCA associated vasculitis.
Associated tests	Further reflex testing • Myeloperoxidase (MPO) antibodies • Proteinase 3 antibodies Other associated tests; Anti-glomerular basement membrane (GBM) antibodies may be requested in conjunction with ANCA in the context of suspected pulmonary-renal syndrome and/or investigation of anti-GBM (Goodpasture's) disease.
Sample Requirements	Serum
Sensitivity, Specificity, Uncertainty of measurement	Very sensitive, specificity increased with supplementary anti-MPO and anti-PR3 antibodies.
MPO autoantibodies	

Synonyms	Myeloperoxidase antibodies, Anti-neutrophilic cytoplasmic antibodies (ANCA), Neutrophil cytoplasmic antibodies, P-ANCA
Indications	Undertaken on positive ANCA samples or requested in isolation for disease monitoring.
Requestor	Reflex testing from positive ANCA. Primary and secondary care for disease monitoring in known positive cases.
Method	Chemiluminescence
Clinical Background	MPO testing can be requested directly or reflexed from a positive ANCA pattern by immunofluorescence. Detects presence of autoantibodies to myeloperoxidase; a protein found in the neutrophil cytoplasm.
Interpretation	MPO provides a quantitative result to indicate presence of absence of the disease-associated autoantibody.
Associated tests	Often performed in parallel with proteinase 3 (PR3) antibodies Other associated tests; Anti-glomerular basement membrane (GBM) antibodies may be requested in conjunction with ANCA in the context of pulmonary-renal syndrome and/or investigation of anti-GBM (Goodpasture's) disease.
Sample Requirements	Serum
Turn Around Time (TAT)	This is not routinely processed as an urgent test. Urgent processing can be requested Monday – Friday by contacting the immunology laboratory.
Sensitivity, Specificity, Uncertainty of measurement	Less sensitive but more specific than ANCA immunoflorescence.
PR3 autoantibodies	
Synonyms	Proteinase 3 antibodies, Anti-neutrophilic cytoplasmic antibodies (ANCA), Neutrophil cytoplasmic antibodies, C-ANCA
Indications	Undertaken on positive ANCA samples or requested in isolation for disease monitoring.
Requestor	Reflex testing from positive ANCA. Primary and secondary care for disease monitoring in known positive cases.
Method	Chemiluminescence
Clinical Background	PR3 testing can be requested directly or reflexed from a positive ANCA pattern by immunofluorescence. Detects presence of autoantibodies to myeloperoxidase; a protein found in the neutrophil cytoplasm.
Interpretation	PR3 provides a quantitative result to indicate presence of absence of the disease-associated auto-antibody.
Associated tests	Often performed in parallel with myeloperoxidase (MPO) antibodies Other associated tests; Anti-glomerular basement membrane (GBM) antibodies may be requested in conjunction with ANCA in the context of pulmonary-renal syndrome and/or investigation of anti-GBM (Goodpasture's) disease.
Sample Requirements	Serum

Turn Around Time (TAT)	This is not routinely processed as an urgent test. Urgent processing can be requested Monday – Friday by contacting the immunology laboratory.
Sensitivity, Specificity, Uncertainty of measurement	Less sensitive but more specific than ANCA immunoflorescence.

Anti-GBM antibodies	
Synonyms	Glomerular basement membrane antibodies
Indications	Anti-glomerular basement membrane (GBM) antibodies are found in anti-GBM disease (Goodpasture's syndrome). The autoantibody is directed against the NC1 domain of the α- chain of type IV collagen. Approximately 25% of patients presenting with anti GBM antibodies also present with anti-neutrophil cytoplasmic antibodies, so parallel testing is recommended.
Requestor	Primary and secondary care for screening, and disease monitoring in known positive cases.
Method	Fluorescent enzyme immunoassay
Interpretation	GBM provides a quantitative result to indicate presence of absence of the disease-associated auto-antibody, and the level in monitoring disease.
Associated tests	Often performed in parallel with ANCA, myeloperoxidase (MPO) and PR3 antibodies
Sample Requirements	Serum
Turn Around Time (TAT)	This is not routinely processed as an urgent test. Urgent processing can be requested Monday – Friday by contacting the immunology laboratory.
Sensitivity, Specificity, Uncertainty of measurement	Very sensitive and specific

Skin autoimmunity

Skin autoantibodies	
Synonyms	Pemphigus antibodies, pemphigoid antibodies
Indications	Investigation of blistering autoimmune skin disease (i.e. pemphigus vulgaris and bullous pemphigoid).
Requestor	Primary and secondary care
Method	Indirect Immunofluorescence on monkey oesophagus
Clinical Background	Detects presence of autoantibodies to antigens found in the skin that are associated with blistering skin diseases (i.e. pemphigus vulgaris and bullous pemphigoid).
Interpretation	Two patterns are detected by immunofluorescence for skin antibodies and are reported as POS or NEG; - Skin intracellular cement (SICS) - Skin basement membrane (SBM)

	The skin intracellular cement pattern is associated with autoantibodies specific for the cell surface of epidermal keratinocytes. These are found in 90% of patients with pemphigus and correlates with disease activity. The basement membrane pattern is associated with autoantibodies directed against the skin basement membrane. These autoantibodies are present in 70% of patients with bullous pemphigoid but do not reflect disease activity and tends to persist following successful therapy. Presence of basement membrane antibodies is uncommon in normal individuals or in other skin diseases.
Sample Requirements	Serum

Thyroid autoimmunity

TSH-receptor autoantibodies (TRAb)	
Synonyms	TSH antibodies, thyroid stimulating antibodies
Indications	• Hyperthyroidism, or suspected Grave's disease • Monitoring of anti-thyroid therapy • Pregnancy in the context of known autoimmune thyroid disease, to determine risk of neonatal thyroid dysfunction
Requestor	Primary and secondary care
Method	ELISA
Clinical Background	TRAbs are closely associated with autoimmune hyperthyroidism. They may be detected before hyperthyroidism becomes biochemically or clinically apparent, and decrease after anti-thyroid therapy. TRAbs in pregnant women may cross the placenta and result in neonatal hyperthyroidism.
Interpretation	An elevated TRAb level is relatively specific for Grave's disease, and a negative result virtually excludes it. Regardless, presence of hyperthyroidism should prompt referral to endocrinology for investigation/treatment.
Associated tests	TSH, free T3, free T4, thyroglobulin

Immunochemistry

Total immunoglobulins	
Synonyms	IgG, IgA, IgM
Indications	Recurrent infections raising the possibility of immune deficiency. Symptoms or signs raising the possibility of myeloma. Failure to thrive in infants.
Requestor	Primary and secondary care
Method	Immunoturbidimetry
Clinical Background	Total immunoglobulin levels (IgG, IgA, and IgM) should be requested in the context of failure to thrive in infants, recurrent bacterial sinopulmonary infections, or suspicion of myeloma.
Interpretation	IgA deficiency occurs in approximately 1 in 700 of the healthy population, and is associated with a slightly increased risk of

	autoimmune disease including coeliac disease. IgG deficiency may be due to protein loss (renal or GI), reduced production (myeloma, primary immune deficiency), or immunosuppressive medication. In the context of recurrent bacterial sinopulmonary infections consider discussion with the clinical immunology service. IgM deficiency is generally of little clinical significance.
Associated tests	Serum and urine protein electrophoresis
Sample Requirements	Serum

Serum Electrophoresis	
Synonyms	SPEP, EP, PE
Indications	The main indications for serum electrophoresis are in the diagnosis, exclusion or monitoring of patients with B cell malignancies or plasma cell malignancies (MGUS or myeloma), or in the investigation of patients with increased frequency or severity of infections. Requests should be accompanied by samples for urine protein electrophoresis or serum free light chains.
Requestor	Primary and secondary care
Method	Capillary zone electrophoresis, and agarose gel immunofixation
Clinical Background	Serum immunoglobulins are normally polyclonal, but in some B cell malignancies and myeloma a single immunoglobulin clone develops in higher quantities and may suggest MGUS or multiple myeloma. Occasionally this can result in suppression of other immunoglobulin types leading to an increase in frequency and severity of infections.
Interpretation	A normal serum electrophoresis does not exclude a B cell or plasma cell malignancy; a paired urine sample for Bence Jones protein or serum sample for serum free light chains is essential. The presence of a paraprotein raises the possibility of a B cell or plasma cell malignancy, such as MGUS, multiple myeloma, or a lymphoproliferative disorder. If there is associated lymphadenopathy, organomegaly, or unexplained bone pain, or abnormalities of calcium, renal function, and haemoglobin suggest discussion with or referral to haematology.
Associated tests	Immunoglobulins, urine electrophoresis, immunofixation, serum free light chains
Sample Requirements	Serum

Urine protein electrophoresis	
Synonyms	BJP, UPE
Indications	Requests for urine protein electrophoresis are recommended as part of the investigation of suspected B cell or plasma cell malignancies (including MGUS and multiple myeloma), immune deficiencies, and amyloidosis.
Requestor	Primary and secondary care
Method	Agarose gel electrophoresis
Clinical Background	Immunglobulin free light chains are usually present in a polyclonal pattern. In some conditions there is an increase in monoclonal light chains, which can be excreted in the urine. Detection of this in urine (Bence Jones protein), would suggest a B cell or plasma cell

	abnormality.
Interpretation	The absence of a BJP does not exclude a B cell or plasma cell abnormality. A paired serum sample for serum electrophoresis is recommended, with or without serum free light chains. Detection of a BJP warrants discussion with or referral to haematology.
Associated tests	Immunoglobulins, serum electrophoresis, serum free light chains.
Sample Requirements	Urine (ideally early morning sample) or 24-hour collection (no preservative). A Universal container will be accepted.

Serum free light chains	
Synonyms	SFLC
Indications	Investigation of suspected multiple myeloma, light chain diseases, and amyloidosis.
Requestor	Primary and secondary care.
Method	Nephelometry
Clinical Background	Polyclonal serum free light chains are present in normal individuals. In patients with B cell or plasma cell malignancies or amyloidosis the level of one type (kappa or lambda) may be disproportionately increased. Increases in both levels suggests inflammation, but may mask an underlying abnormality.
Interpretation	An abnormal kappa/lambda ratio in itself is not diagnostic; given the levels represent total (polyclonal and monoclonal) light chains. Therefore, abnormalities may represent a B cell or plasma cell malignancy, or infection/inflammation. A serum free light chain result alone is uninterpretable without serum protein electrophoresis and urine Bence Jones protein.
Associated tests	Serum electrophoresis, BJP, immunoglobulins
Sample Requirements	Serum

IgG subclass levels	
Synonyms	IgG1, IgG2, IgG3, IgG4
Indications	Suspicion of IgG4-related disease. There is no role for measurement of IgG subclasses in the routine investigation of suspected immune deficiency. If immunodeficiency is suspected please contact the clinical immunology service on 01752 431672.
Requestor	Primary and secondary care
Method	Nephelometry
Clinical Background	A reduced total IgG may cause recurrent sinopulmonary infections. A total IgG level is a combination of four subclasses: IgG1, IgG2, IgG3, and IgG4. A reduction in one or more of these classes with a normal total IgG represents a subclass deficiency. In the past this was considered an immune deficiency, although it can be seen in otherwise healthy individuals and has little clinical relevance. IgG4-related disease is a chronic inflammatory, relapsing-remitting condition characterised by mass-producing fibrosing masses in one or more organs, and is the common cause of autoimmune pancreatitis, as well as some cases of retroperitoneal fibrosis, Mikulicz's disease, fibrosing thyroiditis, and others.
Interpretation	A reduction in one or more of these classes with a normal total IgG represents a subclass deficiency. In the past this was considered an

	immune deficiency, although it can be seen in otherwise healthy individuals and has little clinical relevance. A raised IgG4 level may be due to IgG4-related disease, but also some other conditions including atopic dermatitis and some other autoimmune conditions.
Associated tests	Total immunoglobulins (IgG, IgA, IgM), total IgE, serum electrophoresis
Sample Requirements	Serum

Cryoglobulin	
Synonyms	
Indications	Reduced C4 with features of vasculitis (skin, peripheral neuropathy, renal), hepatitis C infection, paraproteins with organ dysfunction, cold-induced symptoms.
Requestor	Secondary care (difficult to ensure meticulous collection requirements in primary care).
Method	Incubation at 4*C, capillary zone electrophoresis and agarose gel immunofixation.
Clinical Background	Cryoglobulins are serum proteins (often immunoglobulins) which precipitate in cold temperatures and redissolve at 37*C. Precipitation in vivo can lead to vasculitis and potentially significant organ dysfunction in multiple organs. Cryoglobulins can be associated with some infections, autoimmune diseases, and liver disease (hepatitis C in particular). Cryoglobulins are divided into type 1 (paraprotein-associated), type 2 (immune complex-associated with increased rheumatoid factor and low C4), and type 3 (increased RF with normal C4).
Interpretation	Interpretation depends very much on the clinical presentation. If the sample is not collected correctly the cryoglobulin may be lost during collection, causing a false negative result. The level of the cryoprotein does not necessarily correlate with symptom severity.
Associated tests	C3 and C4, rheumatoid factor, hepatitis C serology.
Sample Requirements	Samples must be collected into a red top tube warmed to 37oC, and the sample must be transported to the laboratory maintaining the temperature at 37oC. Any drop in temperature may cause the cryoglobulin to precipitate and be removed during centrifugation. Please contact the laboratory prior to collection.

Hereditary angioedema

This condition is very rare, is associated with recurrent angioedema *without* urticaria and there is usually a positive family history. Complement C4 levels are invariably low and C3 levels normal in patients with this condition, and a normal C4 level virtually excludes the condition and should be the first line screening test. If suspicion remains please discuss with the clinical immunology service on 01752 431675.

C1 esterase inhibitor levels may be low or normal, and are not recommended as a first line screening test.

Haptoglobin	
Synonyms	
Indications	Diagnosis and monitoring of haemolysis.
Requestor	Primary and secondary care.
Method	Immunoturbidimetry
Clinical Background	Haptoglobin conserves iron by binding haemoglobin released during haemolysis. This complex is cleared rapidly, leading to reduced haptoglobin levels in haemolysis.
Interpretation	Decreased levels are seen in intravascular haemolysis. Increased levels may be seen in inflammatory conditions, trauma, and malignancies.
Associated tests	LDH
Sample Requirements	Serum

Isoelectric focusing	
Synonyms	Oligoclonal banding, OCB
Indications	Suspicion of central nervous system inflammation, particularly demyelinating conditions such as multiple sclerosis.
Requestor	Secondary care
Method	Agarose gel-based serum and/or CNS isoelectric focusing
Clinical Background	In normal individuals immunoglobulins are predominantly serum-based (for IgG), and to some extent cross the blood-brain barrier. In cases of systemic inflammation these immunoglobulins cross the blood brain barrier, creating a similar pattern in CSF as in serum. In cases of CNS inflammation or demyelination immunoglobulins are produced locally in the CNS as well, leading to a mismatch of appearances between serum and CSF.
Interpretation	Up to 95% of patients with multiple sclerosis have intrathecal synthesis (oligoclonal banding in CSF) detectable. The more bands that are present in CSF but not serum, the higher the predictive value of demyelinating disease.
Associated tests	Serum immunoglobulins, CSF protein levels
Sample Requirements	Paired serum and CSF (within 10-14 days or each other, but preferably at the same time) are essential to identify matched or mismatched bands.

Immunodeficiency and immunity testing

Pneumococcal antibodies	
Synonyms	Total pneumococcal antibodies, pneumococcal serotype specific antibodies
Indications	Measurement of antibodies against specific antigens is useful in the assessment of suspected immune deficiency, and occasionally to demonstrate immunity for other reasons (eg after splenectomy). Pneumococcal serotype specific antibodies are preferred and

	provide more information than total pneumococcal antibody levels.
Requestor	Primary and secondary care
Method	
Clinical Background	Antibodies are produced in response to antigen exposure. Measurement of antibodies prior to and after vaccination contributes to the assessment of immunity and immune deficiency.
Interpretation	Levels considered protective are quoted, although the significance depends on the clinical situation. The assessment of a dynamic response should be undertaken only in conjunction with the clinical immunology service.
Associated tests	Hib antibodies, tetanus antibodies
Sample Requirements	Serum

Tetanus antibodies	
Synonyms	
Indications	Measurement of antibodies against specific antigens is useful in the assessment of suspected immune deficiency, and occasionally to demonstrate immunity for other reasons (eg occupational, pregnancy).
Requestor	Primary and secondary care
Method	
Clinical Background	Antibodies are produced in response to antigen exposure. Measurement of antibodies prior to and after vaccination contributes to the assessment of immunity. The levels can also be used to assess protection following vaccination for other reasons.
Interpretation	The protective level against tetanus is >0.1 IU/mL. The assessment of a dynamic response depends on the clinical situation and should be undertaken only in conjunction with the clinical immunology service.
Associated tests	Hib antibodies, pneumococcal antibodies (total and serotype-specific)
Sample Requirements	Serum

Hib antibodies	
Synonyms	Haemophilus influenzae type b (Hib) antibodies
Indications	Hib antibodies may be used to assess protection in some populations (eg post-splenectomy), although this is not routine. Hib antibodies are not recommended in the first line investigation of suspected immune deficiency in view of its being a protein-conjugated vaccine. Measurement of Hib-specific antibodies may be useful in certain circumstances, although this should be done following discussion with the clinical immunology service.
Requestor	Primary and secondary care
Method	
Clinical Background	The levels can also be used to assess protection following vaccination in some circumstances. Antibodies are produced in response to antigen exposure and therefore measurement of antibodies prior to and after vaccination contributes to the assessment of immunity, although this should only be done in consultation with the clinical immunology service.
Interpretation	Levels considered protective are quoted, although this depends on the clinical situation. The assessment of a dynamic response should be

	undertaken only in conjunction with the clinical immunology service. Note Hib antibodies are not related to infections with non-typable Haemophilus influenzae strains that commonly cause respiratory tract infections.
Associated tests	Tetanus antibodies, pneumococcal antibodies (total and serotype-specific)
Sample Requirements	Serum

Flow cytometry

Lymphocyte subsets	
Synonyms	LSM, T cell count, CD4 count, B cell count, CD19 count, CD20
Indications	CD4 count monitoring in HIV, monitoring of B cell numbers after some biologics, monitoring of immune suppression, suspected immunodeficiency, evaluation of lymphopenia
Requestor	Primary and secondary care
Method	Flow cytometry
Clinical Background	The total lymphocyte count comprises T cell (CD4 and CD8), B cell, and NK cell numbers. The CD4 count is part of the routine monitoring of HIV infection, and is also relevant in the risk assessment of opportunistic infections.
Interpretation	A CD4 count of less than 200/uL should prompt investigation for HIV or other immunodeficiency, and consideration of prophylaxis against opportunistic infections. Discussion with clinical immunology is recommended for new cases of HIV-negative individuals with significantly reduced CD4 or B cell numbers. NK cell numbers commonly decrease with age and unless there are signs to suggest immune deficiency this is of limited significance.
Associated tests	Full blood count
Sample Requirements	EDTA

Neutrophil function test	
synonyms	Neutrophil burst test, NBT, DHR
Indications	Suspicion of neutrophil dysfunction as part of the workup for chronic granulomatous disease (CGD).
Requestor	Immunology only
Method	Flow cytometry
Clinical Background	Neutrophil dysfunction can lead to severe recurrent bacterial and fungal infections affecting a variety of organs. Presentation is usually very early in life, although very occasionally the diagnosis is made in adults.
Interpretation	A normal neutrophil oxidative burst in response to antigen stimulation makes CGD unlikely. A reduced burst must be interpreted in the context of the clinical history, as abnormal results may also be due to collection or transport issues. This would warrant repeat testing.
Sample Requirements	Lithium Heparin (green top) within 4 hours of collection

B cell Immunophenotyping

synonyms	B cell subtyping
Indications	Evaluation of immunodeficiency
Requestor	Immunology only
Method	Flow cytometry
Clinical Background	Evaluation of B cell subtypes can be useful in the evaluation of certain conditions.
Interpretation	Interpretation depends on the clinical context.
Associated tests	Lymphocyte subset analysis, full blood count.
Sample Requirements	EDTA

Lymphoma/leukaemia flow cytometry	
Synonyms	Flow cytometry
Indications	Suspected lymphoproliferative disorder, myeloma, acute leukaemia, and some other rare conditions.
Requestor	Haematology only (or other requestors as suggested by haematology)
Method	Flow cytometry
Clinical Background	Immunophenotyping is important in the diagnosis and characterisation of haematological disorders including lymphoproliferative disorders, acute leukaemia, and myeloma.
Interpretation	Interpretation very much depends on the clinical context, and this test should only be requested after discussion with haematology.
Associated tests	Bone marrow aspirate and trephine, morphology, molecular testing
Sample Requirements	CSF (within 3 hours of collection), bone marrow, peripheral blood, and fine needle aspirate/ascitic/pleural fluid after discussion with the laboratory.

PNH screen	
Synonyms	Paroxysmal nocturnal haemoglobinuria screen
Indications	Unexplained cytopenias in the context of abdominal pain or thrombosis, thrombosis in unusual sites, haemoglobinuria
Requestor	Secondary care, only after discussion with haematology.
Method	Flow cytometry
Clinical Background	PNH is a rare, acquired haematological disease characterised by red cell destruction due to reduced inhibition of complement activation. The symptoms are variable, although include haemoglobinuria, anaemia, abdominal pain, and particularly thrombosis (in both common and unusual sites).
Interpretation	On the basis of the flow cytometric measurement of levels of proteins on the surface of red and white blood cells a percentage of cells is derived.
Associated tests	Full blood count, urinanalysis, imaging of suspected thrombosis
Sample Requirements	EDTA