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1.0 INTRODUCTION

The Immunology section of the Haematology department provides a comprehensive range of clinical and laboratory services for the investigation of Immunology autoimmunity and connective tissue disease.

The laboratory liases closely with other departments within pathology and with the Immunology reference centre at North Bristol.

This handbook provides information about the assays performed, their turnaround times, interpretation of test results and other information about staff and working hours.

Most screening tests are carried out on the same working day or the working day following receipt of the specimen, however many tests are expensive when dealt with in small numbers. Therefore in order to maintain an economic cost and acceptable turnaround time such assays are batched and performed weekly or fortnightly.

If in doubt about the turnaround time for any test or any other aspect of the Immunology service please phone the laboratory for advice.

Address

Immunology Section,
Department of Haematology,
Level 8,
Queens Building,
Bristol Royal Infirmary,
Bristol BS2 8HW

Key Contacts

Dr. Phil Bright
Immunology Consultant
University Hospitals Bristol
Dept. of Immunology
Southmead Hospital
Tel 0117 9595629 or 9595639

Mr Chris Doherty
Principal Biomedical Scientist
(Section Lead for Coagulation & Immunology)
Dept. of Haematology
Bristol Royal Infirmary
Tel 0117 342 2595

Hours of service

The Immunology Laboratory is open:
Monday to Friday 0900 – 17.30 hrs

2.0 URGENT REQUESTS

There are very few tests performed by the Immunology Laboratory that are required clinically on an urgent basis.

Exceptionally, when circumstances justify a more rapid result, the request should be first made to the laboratory Biomedical Scientists.

If the test cannot be performed then the clinician will be referred to the Consultant Immunologist, or his cover, for guidance.

Most requests will be dealt with if received in the laboratory by 14.30 hrs at the latest and you have notified the laboratory that it is urgent. Clinicians must contact the laboratory directly by telephone. There is no contractual on-call for Immunology, however staff familiar with Immunology tests may be available to offer tests outside of routine hours.

3.0 SAMPLE REQUIREMENTS

Patient consent must be confirmed by the requestor prior to taking the samples.

All Immunology tests are performed on a serum (SST) sample or Citrate plasma samples for Antiphospholipid syndrome (anti-cardiolipin / anti-beta2 glycoprotein 1 antibodies).

A 4.5ml sample is preferable to provide adequate serum for testing.

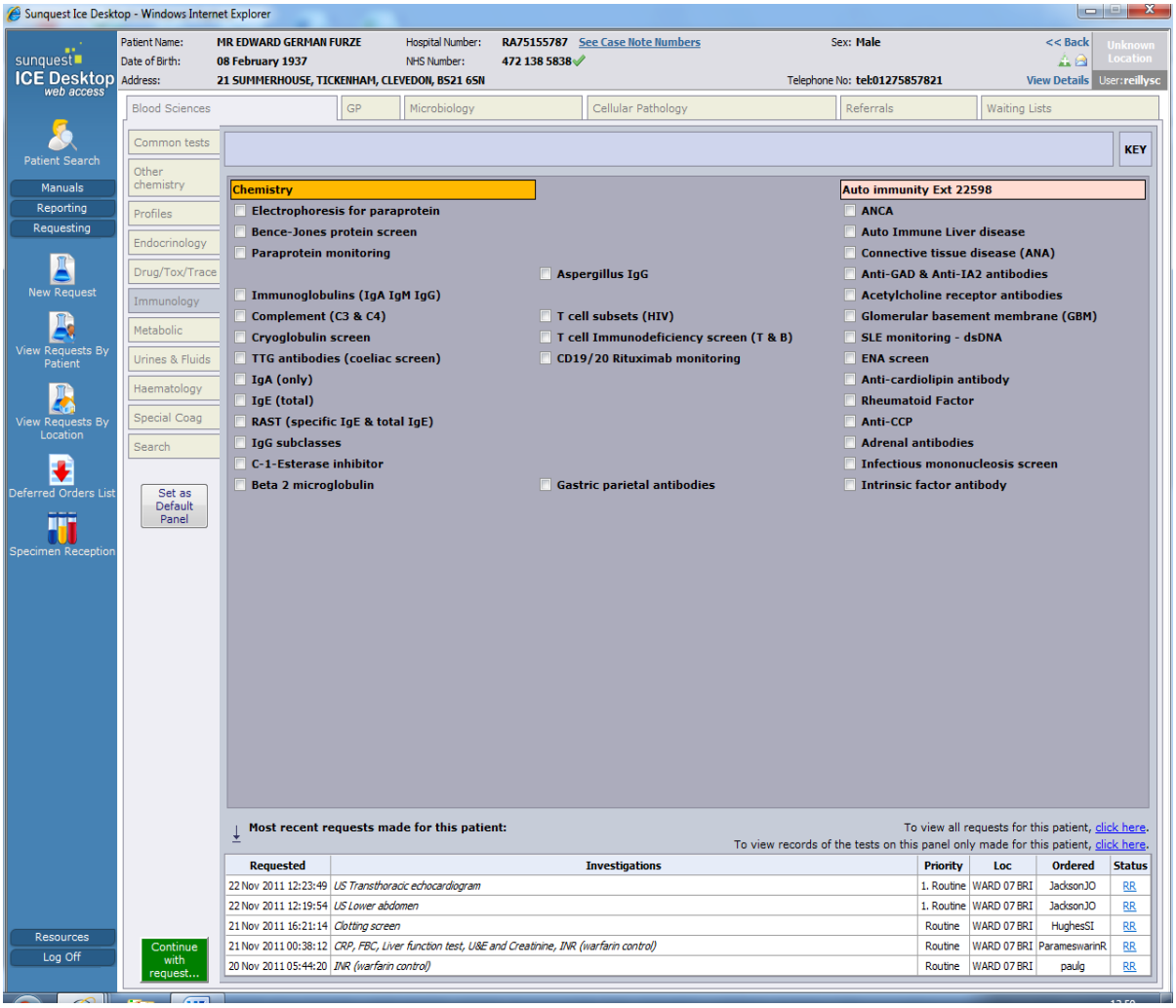
Samples must be sent to, Pathology Central Specimen Reception on level 8 of the Queens Building, Bristol Royal Infirmary.

Storage of Samples

Sera are stored at 4°C and are retained for a period of 4-6 weeks before disposal.

If further tests are required on the basis of any previous results, then contact should be made with the laboratory staff who will then ensure that further tests are performed on the stored serum. This will prevent the inconvenience of further venesection.

Instructions for completion of request forms



Tests can be requested using the ICE order comms system or via request forms.

Request forms are based on a tick box system for the main requests i.e. autoimmune profiles, ANCA testing, Glandular fever screens and Rheumatoid screens. Other more specialised tests e.g. DNA antibodies, must be added to the `other test` box at the bottom left-hand corner of the form.

Please make sure adequate clinical information is supplied for clinical interpretation.

The following must also be supplied:

- Sufficient information to allow unique identification of the patient
- Time and date of specimen collection
- Degree of urgency
- High risk status
- Contact number of referring clinician
- G.P`s must supply NHS number of patient

4.0 REPORTING OF RESULTS

Printed or computer reports are issued when a group of related assays have been completed.

Further reports may be issued if other, less frequently performed assays, have also been requested.

Unexpected or grossly abnormal results will be telephoned to the clinician whenever possible.

Interpretation of abnormal results will be added to reports, but please contact the laboratory staff if additional information is required.

Clinical Referrals

Please contact Dr. Phil Bright
Department of Immunology
Southmead Hospital
Tel: Contact Southmead switchboard 770 or 0117 9505050

5.0 AVAILABLE TESTS/ASSAYS

		Turnaround Time
1.	Glandular Fever Screening	24 hrs
2.	Anti-CCP antibody tests	10 days
3.	Autoimmune antibody Profile	
	Anti Nuclear antibodies	
	{Hep2000} for Connective tissue disease	72 hrs
	Rodent Tissue for Autoimmune Liver Disease	48 hrs

4. Double Stranded DNA antibodies
5. Extractable Nuclear Antigens (ENA)
   - Screen and typing

   10 days
6. Anti cardiolipin antibodies (ACA) IgG
   IgM
7. ANCA
   - Immunofluorescence Screening test 48 hrs
   - Elisa MPO/PR3 confirmation test 72 hrs

   Please telephone if urgent.
8. Intrinsic Factor antibodies 10 days
9. Send away assays (see Table 1)

Note: These turnaround times apply to routine non-urgent samples. Identified urgent samples will be subject, on discussion, to a shorter turnaround times.

6.0 TESTS SENT AWAY TO OTHER LABORATORIES

Many tests for specific antibodies are sent to other hospitals. This is due to the low volume nature of the requests. These requests will be entered into the UBHT system before dispatch and a report will be issued on return of the results, thereby establishing an audit trail. Return of results will be monitored on a weekly basis.

The Biochemistry send-away department will deal with the despatch of samples to outside hospitals.

A list of assays sent away are listed in Appendix 1 at the end of this document.

7.0 RECOMMENDED INVESTIGATIONS IN AUTOIMMUNE DISEASE

Diagnosis of SLE and other Connective Tissue disorders

SLE

SLE should be considered as a potential cause of symptoms such as small joint arthropathy and rashes or symptoms of serositis (e.g. unexplained pleuritic chest pain, mouth ulcers).

Useful investigations for connective tissue disease screening are ANA, RF, CRP, Immunoglobulins, Anticardiolipin antibodies AND Lupus anticoagulant. Patients with a significantly positive ANA should be screened for dsDNA antibodies and C3 and C4 levels should also be checked, Extractable nuclear antigens such as Ro, La, SM or RNP are useful supportive tests, especially Ro associated complications - cutaneous/neonatal.

In patients with suspected myositis requiring Jo-1 testing an ENA test is required.

Remember patients with SLE often have multiple antibodies.

Autoimmune Hepatitis

Autoimmune Liver screen

- ANA maybe positive. A positive LKM or SM antibody supportive of diagnosis. AMA positive in PBC overlap, this should be confirmed by an M2 Western blot test.

dsDNA

- Seen in Type I autoimmune hepatitis (Lupoid Hepatitis)

Immunoglobulins

- Polyclonal Hypergammaglobulinaemia frequent, IgG predominant.

Rheumatoid arthritis

Rheumatoid Factor

- Positive in approximately 70% of cases and is associated with erosive disease. Remember Rheumatoid factors are not diagnostic of Rheumatoid arthritis.

CCP

- Sensitivity comparable to RF but approximately 95% specific for RA. Patients positive for both RF and CCP tend to have aggressive disease and a worse prognosis.

ANA

- Often present, no need to repeat

ENA

- Ro, La seen in secondary Sjogrens

Complement

- Low complement (particularly C4) seen in rheumatoid vasculitis and cryoglobulinaemia. High levels seen as part of the acute phase reaction

Investigation of Vasculitis

If a diagnosis of vasculitis is suspected then it is advisable to ask for a clinical assessment by a physician experienced in managing this group of disorders. Laboratory investigations are of limited value in arriving at a diagnosis but tests that may be of some use include: ANCA; GBM; CRP; Immunoglobulins; ANA; RF; C3; C4 and cryoglobulins.

ANCA

- c-ANCA/PR3 and p-ANCA/MPO are specifically associated with small vessel vasculitis.
-

Anti GBM

- Associated with pulmonary and renal vasculitis

Rheumatoid Factor

- seen in rheumatoid vasculitis, cryoglobulinaemia

Complement

- Low with immune complex vasculitis

Cryoglobulin

- Low C4, cutaneous vasculitis, neuropathy, nephropathy

AIP/ENA/DNA

- Governed by clinical context.

Always phone the laboratory if clinically urgent.

Raynauds

Anti-Nuclear Abs

- Primary versus Secondary. If ANA is negative then the patient is less likely to develop a connective tissue disorder (CTD)

CTD screen

- Centromere antibodies may be an early finding of an evolving CTD

Complement studies

- Low C4 with cryoglobulinaemia

ENA

- Ro, La, RNP or ScL-70 may be an early finding of an evolving CTD

Scleroderma

ENA

- ScL-70 associated with systemic disease.

CTD screen

- Centromere antibodies associated with limited Scleroderma (CREST)

ANA

- Usually positive and does not need repeating

Complement studies

- Low C4- check for cryoglobulins

Monitoring of Patients with SLE

In some patients with SLE the titre of antibodies dsDNA is an excellent measure of the disease activity. In other patients disease activity is better assessed by using a combination of falling levels

of complement proteins and total white blood cell counts, a rising ESR and increasing microscopic haematuria.

The pattern of changes of laboratory parameters associated with flares of the disease, improvement and remission should be established for each patient.

Ideally a pattern of results will be found that allow prediction of a clinical deterioration in an individual patient which will allow decision on changes in therapy (Ref NEJM (1998) **338** 1359-68).

Women with SLE who are or who are likely to become pregnant should be checked for the presence of Lupus anti-coagulant and for phospholipid and Ro (SSA) and La (SSB) antibodies. A positive ANA does **NOT** need repeating.

Low levels of C3/C4 with active disease are due to consumption, but remember that genetic deficiency of C4 may predispose to SLE. Perform cryoglobulins assay if the C4 is low.

Sjögrens

ANA

- If positive does not need repeating. Mitochondrial also positive if associated with PBC

ENA

- Ro, La – associated with extra glandular disease

Rheumatoid Factor

- found in up to 90% of those cases developing arthritis

Complement studies

- Low C4 seen with cryoglobulinaemia

Immunoglobulins

- Hypergammaglobulinaemia frequent IgG 1 predominance

Antiphospholipid Syndrome (APS)

Cardiolipins and Lupus anticoagulant:

Positive cardiolipins and/ or lupus anticoagulant are supportive of APS. There is a need to check for both as they can also be present in infection.

Positive results should be checked at 12 weeks to confirm.

ANA

- To distinguish primary versus secondary APS. Further add on Testing (ENA/DNA) will be done if the ANA is positive.

All approved requests will receive anti-cardiolipin IgG and Beta2 Glycoprotein 1 IgG antibody testing. If there is connection to pregnancy complications then the anti-cardiolipin IgM and Beta2 Glycoprotein 1 IgM antibody testing will also be performed. If this is required please state in the clinical details or make clear the reason for testing and the lab will add-on the IgM testing.

MCTD

ANA

- Positive, does not need repeat

ENA

- RNP Positive, does not need repeat association

Rheumatoid Factor

- Positive in 40 – 60% of cases

Complement studies

- Low C4 in cryoglobulinaemia

Immunoglobulins

- Polyclonal hypergammaglobulinaemia

Myositis

ANA

- May be positive, especially nucleolar pattern

ENA

- Jo1 associated with pulmonary involvement. Other ENAs seen in overlap syndromes.

Primary Biliary Cirrhosis (PBC)

AIP

- AMA supports PBC, if positive it will be referred for M2 confirmation.

Immunoglobulins

- Polyclonal, increased IgM frequent

Paraneoplastic Syndrome

Anti- neuronal cell

- Anti Yo associated with carcinoma of the breast, ovary antibodies and Hodgkins Lymphoma.

Anti Hu antibodies

- Associated with small cell lung carcinoma

Juvenile arthritis

ANA

- Positive result associated with the development of Uveitis

ENA

- Ro associated with Uveitis

Rheumatoid Factor

- Positive in Polyarticular disease

Complement

- Low levels seen in active disease

Investigation of Renal Failure

Immunological investigations of value in assessing patients with renal failure (where the cause is not apparent) include: ANA; ANCA; GBM antibodies; CRP; C3; C4 Immunoglobulins and urine for BJP. Other tests to include maybe Cryoglobulins and C3 nephritic factor in cases of low C3.

Ankylosing Spondylitis

No diagnostic test

- HLA B27 is not diagnostic.

NB: HLA B27 is a molecular genetic test done on DNA from white blood cells and as such requires an EDTA (purple top) sample.

Thyroid Disease

TPO

- Seen in autoimmune thyroid disease. If positive it does not need repeating.

Myasthenia Gravis

Acetylcholine Receptor Antibodies

- Detected in 90% of patients with systemic features. In 50% of patients with purely ocular myasthenia

Skeletal muscle antibodies

- Marker for underlying Thymoma

Immunoglobulins

- Thymoma associated with antibody deficiency and a riskof pyogenic infections.

Hereditary Angiodema

C4

- C4 useful screening test. If low and with a history of Angiodema, refer for C1 esterase inhibitor.

Other Diseases

Addison's Disease

Adrenal antibodies

- If positive screen for other endocrinopathies/organ specific autoimmunity as appropriate.

Bullous Skin Disease

Skin ICS antibodies

- seen in Bullous Pemphigus

Skin BM antibodies

- seen in Bullous Pemphigoid

Coeliac Disease

Coeliac screening is carried out by the Clinical Biochemistry Department. Please refer to the **Pathology Online Database** for sample requirements and other test information.

Pernicious Anaemia (PA)

Liver disease screen

- GPC seen in PA but may also occur in other organ specific autoimmune disease and in normal people with increasing age.

IFA

- Intrinsic Factor antibodies seen in early PA.

8.0 ASSAYS

Adrenal Antibodies

Found in 60% of patients with idiopathic **hypoadrenalism** and in 90% of those with **hypoadrenalism** in association with ovarian failure (Autoimmune Poly glandular Syndrome – 1, APS -1) Found in <0.1% of the normal population.

The test is reported as **Positive/Negative** as the titre of the antibody is of no significance. As the adrenal gland atrophies the antibodies may disappear so sequential follow up testing is of no use.

Cyclic Citrullinated Peptide antibodies (CCP)

In 1998, Schellekens et al reported that IgG antibodies reactive with linear synthetic peptides containing the unusual amino acid citrulline were present in 76% of **Rheumatoid Arthritis (RA)** sera with a specificity for RA of 96%.

Citrullinated proteins and peptides are produced naturally in the body during apoptosis. During inflammation citrullinated proteins and peptides can be detected in the body and in some individuals these become antigenic. There appears to be a degree of genetic susceptibility since RA is associated with certain HLA genes, notably the DR B1 *0401 and DR B1 *0404 haplotypes.

Anti CCP antibodies are important markers for diagnosis and prognosis in RA because they:

- Are as sensitive as, and MORE specific than IgM rheumatoid factors in both early and fully established disease.
- May predict eventual development of RA when found in cases of undifferentiated arthritis.
- Are a marker for erosive disease in RA.
- May be detected in healthy individuals years before onset of clinical RA.
- May have a prognostic value, with patients testing positive for BOTH anti-CCP and RF having a more aggressive disease course.

The NICE national clinical guideline for management and treatment of Rheumatoid Arthritis in adults, published in February 2009, recommends that clinicians should consider measuring anti-CCP antibodies in those people with suspected RA who are negative for rheumatoid factor and in whom there is a need to inform decision-making relating to the initiation of combination therapy.

Normal = < 20 U/ml

Anti Nuclear Antibodies (ANA)

These are a heterogenous group of antibodies that bind to nuclear antigens.

ANA negative **Systemic Lupus Erthymatosus (SLE)** is extremely rare. The test is not specific for SLE as Anti Nuclear Antibodies can occur in other conditions

e.g. - RA (Rheumatoid Arthritis)

- Scleroderma
- CAH (Chronic Active Hepatitis)
- Juvenile arthritis
- Sjogrens Syndrome
- MCTD (Mixed Connective Tissue Disease)
- Fibrosing alveolitis
- Systemic Infections
- Drug induced

Patterns of ANA are said to be significant. For example Nucleolar ANA are associated with Scleroderma, Centromere with overlap between various autoimmune conditions. CREST syndrome and speckled patterns with MCTD, Sjögren's syndrome, SLE and Polymyositis. Nuclear rim and Homogeneous ANA have been associated with SLE but overall there is a considerable amount of pattern

Low titre ANA`s can also be found in many healthy elderly people.

All positives), **(>1/100) [measured on Hep2]**, will be further analysed for DNA and ENA antibodies.

Aquaporin 4 or NMO antibodies

These antibodies are associated with **Neuromyelitis Optica (NMO)**, also known as Devic's disease or optic-spinal multiple sclerosis.

This is a severe inflammatory demyelinating disease that affects the optic nerves and spinal cord without affecting the brain.

Aquaporin 4 has been identified as a major NMO antigen. It is used to distinguish NMO from multiple sclerosis

Centromere antibodies

These antibodies are characteristic of the **CREST syndrome**, a variant of systemic sclerosis with limited skin involvement but associated with **C**alcinosis, **R**aynauds phenomenon, o**E**sophageal stricture, **S**clerodactyly, and **T**elangiectasia.

They are also found in 10% of patients with Primary Biliary Cirrhosis (PBC), which often overlaps with systemic sclerosis.

Patients with severe Raynauds should also be tested as a significant percentage evolve into CTD disorders such as CREST/scleroderma.

ds DNA binding antibodies

A positive result for double stranded DNA (dsDNA) antibodies supports the diagnosis of SLE, but it is only one of the features of SLE. However, only 60% of all patients with SLE have these antibodies in their serum i.e. a negative result does not exclude the diagnosis.

DNA antibodies may be detected in the absence of ANA and the level of DNA antibodies can be extremely useful in monitoring the activity of the disease

DNA antibodies may occasionally be found in CAH and weak positives can be found in other CTD and in infections.

Normal range = <27 IU/ml

Borderline = 27-35 IU/ml (suggest repeat sample)

Probable SLE = >35 IU/ml

If an unexpected result is found then repeat the test, in 21 days.

Extractable Nuclear Antibodies (ENA)

ENA is a term used to describe antibodies to the soluble components of the nucleus.

Six main antibodies are recognised and are reported as either positive or negative.

SM

Specific for **SLE** but only found in 20 – 30% of **SLE** patients, usually of Afro- Caribbean descent. There is no correlation with the disease activity

RNP

Diagnostic for **MCTD** as found in >95% of patients but also found in 30 – 40% of **SLE** patients.

Ro (SSA)

This is an extractable nuclear antigen which can be present either as a 60KD or 52KD antigen. These are the most common ENA antibodies detected (65% of all positives)

The Ro antigen also occurs in the cell cytoplasm. A patient with **SLE** may be positive for the Ro antibodies even in the absence of ANA. This occurs in 5% of patients and is therefore called ANA negative SLE. This variant is characterised by severe photosensitivity leading to scarring.

These antibodies can cross the placenta and cause congenital heart block. All women of child bearing age suspected of having SLE or Sjögrens syndrome should be screened for Anti Ro especially if they are considering pregnancy.

Ro antibodies are associated with the following:-

- Sjögrens syndrome (75% in primary Sjögrens syndrome)
- Sjögrens syndrome secondary to other autoimmune diseases
- SLE (30-40%)
- Subacute cutaneous SLE (>95%)
- Neonatal Lupus (>95%)
- Systemic Sclerosis (10-20%)
- Rheumatoid arthritis (5-15%)
- SLE resulting from homozygous C2 or C4 deficiency

La (SSB)

Usually found with Anti Ro in both primary and secondary **Sjögrens syndrome** and SLE. Sjögrens syndrome patients with Anti La are likely to have more extra-glandular disease. **SLE** patients with Ro and La are likely to have lower DNA antibody levels and less renal disease.

Jo-1 (antibodies to Aminoacyl- tRNA histidyl synthetase)

Associated with inflammatory muscle disease especially **Polymyositis** (positive in 30% of patients) and **Dermatomyositis**. To test for Jo-1 antibodies please request an ENA screen on the ICE order comms's system or on a paper request form.

ScL-70 (antibodies to Topoisomerase-1 an enzyme catalysing the breakage and reforming of ssDNA)

Found in 20-40% of patients with scleroderma. It is associated with facial skin, kidney and heart involvement, ischaemic fingertip, ulcers and pulmonary fibrosis.

Infectious Mononucleosis (Glandular Fever) Screening

Infectious mononucleosis (Glandular Fever) is an acute infectious disease of viral etiology which is largely, but not exclusively, of young adults. It is characterised by an IgM heterophile antibody. The use of highly purified glandular fever antigen coated on latex particles provides a simple method with improved sensitivity for the specific detection of heterophile antibodies associated with infectious mononucleosis.

Occasionally detectable levels are late in developing and so if symptoms persist it is recommended that the test is repeated several days later. Some patients, especially children, may remain persistently negative. False positive have been found in recent CMV infection, Hepatitis A, Parvovirus and Leptospira.

Detectable levels of heterophile antibodies may persist for many months and more rarely for years in some individuals.

The strength of reaction has little correlation with the severity of infection.

Glomerular basement membrane antibodies (GBM)

These antibodies are found in **Goodpastures Syndrome**, which is a rapidly progressive glomerulonephritis.

Antibody levels can be of value in monitoring response to therapy.

Direct immunfluorecence of a renal biopsy is the suggested method of diagnosis of anti-GBM disease in patients with rapidly progressive glomerulonephritis.

GPC antibodies

Gastric parietal cell antibodies may be found in pernicious anaemia or may be of no significance especially if over 60 years of age.

If positive the more specific assay for antibodies to intrinsic factor (IFA) may be carried out. IFA are present in approximately 40% of patients with Pernicious anaemia.

The presence of IFA`s excludes other causes of vitamin B12 deficiency.

Islet Cell Antibodies

Present in 70% of newly presenting patients with type I Diabetes Mellitis but measurement is rarely clinically useful. Also the antibody levels decrease and eventually disappear over the duration of the disease.

There are no reports of antibodies to pancreatic islet cells in type II diabetes

Liver Kidney Microsomal Antibodies (LKM-abs)

An uncommon but specific marker for a severe condition in a subset of patients with ANA negative auto immune chronic active hepatitis and some drug induced hepatitis conditions.

LKM 1 antibodies are positive in CAH type 2, which is the most common autoimmune liver disease of childhood and has a relatively poor prognosis.

Mitochondrial Antibodies (AMA)

Present in the vast majority of patients with Primary biliary cirrhosis (PBC), usually in high titre and commonly associated with a polyclonal elevation of IgM.

The serologic hallmark of PBC is the presence of antibodies to mitochondria, especially to the antigen M2 component of the pyruvate dehydrogenase complex. The antigen is on the inner mitochondrial membrane.

For this reason all positives are sent to Southmead Immunology for confirmation of M2 Antibodies by Western Blot technique.

Neurological Antibodies

These may be due to autoimmune disease or may occur in patients with certain carcinomas when the tumour cells bear antigens cross reacting with those in the nervous system.

Acetylcholine receptor antibodies (AChR or ACR)

Positive in approximately 95% of **Myasthenia Gravis (MG)** patients.

GAD Antibodies

Anti Glutamic acid Decarboxylase antibodies (**GAD**) are associated with `Stiff man syndrome` (60%) and also with insulin-dependent diabetes (**IDM**) where titres are much lower. The contribution of GAD antibodies to IDM has not been proved.

GAD is an enzyme concentrated in neurones, which control muscle tone and exteroreceptive spinal reflexes

Ganglioside antibodies

- Anti GM1 antibodies (IgM) found in over 50% of acquired motor neuropathies
- Anti GM1 (IgG) found in 5 -15% of patients with **Guillain-Barre Syndrome.**
- Anti GQ1b found in 90% of patients with Miller Fisher syndrome

MAG Antibodies

Myelin associated glycoprotein (MAG) is a glycoprotein component of myelin in the central and peripheral nervous systems. It functions as an adhesion molecule i.e. mediates cell-cell interactions.

MAG IgM anti-MAG antibodies are associated with Paraproteinaemic Polyneuropathy where the paraprotein is IgM.

The levels of antibody do not correlate with the severity of the nerve disease. Sera from patients with neuropathy that are negative for MAG antibodies often exhibit positivity for various other Ganglioside antibodies.

MuSK antibodies

Muscle Specific tyrosine Kinase (MuSK) is a surface membrane enzyme essential in aggregating acetyl choline receptors during the development of the neuromuscular junction. Anti-MuSK antibodies assist in confirming the diagnosis in seronegative Myasthenia Gravis. They are particularly useful when the clinical features are not typical of MG.

Paraneoplastic neuronal antibodies

Found in sub acute sensory neuropathy and cerebral degeneration.

There are three main types:-

I. Anti Hu (ANNA-1) antibodies are associated with Para neoplastic encephalomyelitis. Hu proteins are regulators of mRNA and are needed for neural differentiation, proliferation and maintenance. They are mainly associated with small cell lung carcinomas (SCLC) and gynaecological tumours. Metastases are limited in seropositive patients. Neurological symptoms can precede presentation of malignancy – positive Anti Hu antibodies can precede SCLC in 50% of cases.
II. Anti Yo antibodies. These are antibodies to antigens in the cytoplasm of Purkinje cells and are associated with Paraneoplastic cerebellar degeneration. These tumours are notoriously difficult to find and anti-Yo antibodies may indicate early disease. 99% are found in female patients and 90% in breast cancer patients, antibodies preceding tumour in two out of three patients.
III. Anti-Ri (ANNA-2) antibodies. These antibodies are extremely rare. They are associated with the very rare opsoclonus myoclonus syndrome, breast cancer and SCLC.

Voltage Gated Channel antibodies

I. Anti voltage gated potassium channels (anti **VGKC**) found in 20-30% of acquired neuromyotonia.
II. Anti voltage gated calcium channels (anti **VGCC**) found in 85% of patients with Lambert Eaton syndrome. This is a form of myasthenia often associated with SCLC.

Anti-Neutrophil Cytoplasmic antibodies (ANCA)

ANCAs are antibodies to enzymes within the cytoplasmic granules of neutrophils. They are detected by indirect immunofluorescence using human neutrophils.

There are two main types

i. P-ANCA
ii. C-ANCA

Enzyme Linked ImmunoSorbent Assays (ELISA) to Proteinase 3 (PR3) and

Myeloperoxidase (MPO) are performed for the complete characterisation of ANCA

The presence of ANCA detected by both IF and ELISA (C-ANCA/PR3 and P-ANCA/MPO) is very strongly linked to the presence of small vessel vasculitis.

ELISA assays are also used to monitor activity in known cases.

The following are the main diseases in which ANCA antibodies are found.

1. **Granulomatosis with Polyangitis (GPA)**
   C-ANCA/PR3 is found in up to 85% of patients with active generalised GPA (formerly known as Wegener's granulomatosis). Therefore a negative result does not exclude the diagnosis. Antibody levels may fall with treatment and patients with persistently elevated levels are more likely to relapse. Up to 25% of patients with GPA may be P-ANCA/MPO positive. Five Percent (5%) of patients may give a negative screening test and therefore it is essential to perform both screening and ELISA techniques when GPA is strongly suspected.
2. **Microscopic Polyangitis (MPA)**
   P-ANCA/MPO seen in 50 – 80% of patients. The antibody levels reflect the disease activity. Persistently high levels indicate possible relapse.
   Forty percent (40%) of patients with MPA may be positive for PR3 antibodies.
3. **Churg-Strauss syndrome (CSS)**
   May be positive for either P-ANCA or C-ANCA
4. **Rapidly progressive (crescentic) glomerulonephritis**
   May be positive for either P-ANCA or C-ANCA. In patients with Acute Renal Failure (ARF) and associated pulmonary haemorrhage a rapid battery of test for ANCA, GBM and ANA should be performed.
5. **Drug induced SLE or vasculitis**
   P-ANCA is associated with some forms of drug induced SLE or vasulitis. The levels drop after withdrawal of the drug.
6. **Other diseases**
   Low levels of P-ANCA are occasionally found in RA, SLE, CAH, IBD, UC, Crohns and sclerosing Cholangitis and their significance is uncertain. ANCA should always be requested if there is a possibility of PSC and there is a connection of this diagnosis with UC which is also connected with atypical ANCAs. Atypical ANCA refers to a variety of Immunofluorescence patterns. These antibodies are directed against a range of antigens such as bacterial permeability increasing protein azurocidin, lactoferrin, elastase, cathepsin G and lysozyme.

The clinical significance of atypical ANCA is uncertain.

Ovarian Antibodies

Found in 15-50% of patients with premature ovarian failure under the age of 40 years. These antibodies react with steroid producing cells and stain the Leydig cells of the testis, placenta and adrenal cortex. They are often seen in autoimmune Polyglandular Syndrome-1 (APS-1) where adrenal and ovarian failure may co-exist. Up to 70% of women may have transient anti ovarian antibodies during IVF therapy.

Pemphigoid and Pemphigus Antibodies

Bullous Pemphigoid is one of the most frequent autoimmune Bullous Pemphigoid Bullous Pemphigoid dermatoses.

Antibodies to epidermal inter-cellular substance (ICS) are found in **pemphigus**.

Antibodies to basement membrane are found in 90% of Pemphigoid patients, **Epidermolysis Bullosa** (EB) and a minority of cases of **herpes gestationis**.

A skin biopsy for the more sensitive test of direct immunofluorescence should also be performed.

Anti-Phospholipid antibodies

These are a family of antibodies (Cardiolipin, ß2-glycoprotein-1 and the Lupus Anti-Coagulant) useful in the investigation of the **'anti-phospholipid syndrome' (APS)**. This may be primary or secondary to SLE and other conditions.

Patients with the APS may be positive for lupus anticoagulant, cardiolipin and ß2-glycoprotein-1 autoantibodies, or they may be positive for some or for only one of these assays.

All approved requests will receive anti-cardiolipin IgG and Beta2 Glycoprotein 1 IgG antibody testing. If there is connection to pregnancy complications then the anti-cardiolipin IgM and Beta2 Glycoprotein 1 IgM antibody testing will also be performed. If this is required please state in the clinical details or make clear the reason for testing and the lab will add-on the IgM testing.

(Please note that the Lupus Anticoagulant assay is performed in the Coagulation Laboratory. Please phone the Coagulation laboratory for further information). The full APS profile is performed on citrated plasma samples.

The diagnosis of the anti phospholipid syndrome is outlined below.

The clinical and laboratory criteria for defining antiphospholipid syndrome are tabulated below. (BSH Guidelines on the investigation and management of antiphospholipid syndrome 2012).

Clinical criteria

Vascular Thrombosis	One or more clinical episodes of arterial, venous or small vessel thrombosis.
Pregnancy morbidity	One or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation.
	One or more pre-term births of a morphogically normal neonate before the 34th week of gestation because of: (i) eclampsia or severe pre-eclampsia or (ii) recognized features of placental insufficiency.
	Three or more unexplained consecutive spontaneous miscarriages before the 10th week of gestation, with maternal anatomic or hormonal abnormalities and parental and maternal chromosomal causes excluded.

Laboratory criteria

Lupus anticoagulant (LA) present in plasma, on two or more occasions at least 12 weeks apart.
Anticardiolipin (aCL) antibody of immunoglobulin (Ig)G and/or IgM isotype in serum or plasma, present in medium or high titre (i.e. >40GPL units or MPL units, or > the 99th centile), on two or more occasions, at least 12 weeks apart.
Anti-β2–glycoprotein I antibody of IgG and/or IgM isotype in serum or plasma (in titre >the 99th centile), present on two or more occasions at least 12 weeks apart.

The cardiolipin antibody assay (particularly IgM) may sometimes give false positive results in patients with infectious diseases (e.g. syphilis) and in some individuals with Anti DNA antibodies.

Normal range IgG = <20 U/ml

IgM = <20 U/ml

Rheumatoid factor

In **Rheumatoid arthritis (RA)** the presence of high RF at onset is of some predictive value. These patients have a worse prognosis and suffer from more systemic manifestations of the disease.

The test is of no value in monitoring RA.

Please also see the section on anti-CCP antibodies and their use in the diagnosis and prognosis of RA

Rheumatoid factor is an IgM immunoglobulin which reacts with IgG and are found in a variety of conditions (viral and bacterial infections, other connective tissue disorders (CTD), lymphoproliferative disorders, some normal elderly people) and by themselves are of low diagnostic value.

The combination of RF, CRP and ANA is useful for screening and differentiating some of the common causes of joint pain and inflammation. SLE is more common in younger women especially of Afro-Carribean and Asian origins and can be virtually excluded by a negative ANA.

	RF	CRP	ANA	CCP
Sero Positive RA	POS	↑	+ / -	+ / -
Sero Negative RA	NEG	↑	+ / -	+ / -
Osteoarthritis	NEG	Normal	NEG	NEG
SLE	NEG	Normal	↑	NEG
Gout	NEG	↑	NEG	NEG

Normal Range <20IU/ML

Smooth Muscle antibodies

Present in up to 75% of cases of autoimmune hepatitis. Only considered of clinical significance if the liver function test results are abnormal. They may also be present in acute viral illness.

Striated Muscle antibodies

Present in some patients with Myasthenia Gravis particularly those with Thymoma. Acetylcholine receptors are a more specific test for Myasthenia Gravis.

9.0 Appendix 1: Available Send away test list

Test Name	Referral Laboratory	Laboratory Website
ADALIMUMAB LEVELS AND ANTIBODIES	Blood Sciences (Exeter)	www.exeterlaboratory.com
ANTI MULLERIAN HORMONE	Automation, Clinical Chemistry (NBT)	https://www.nbt.nhs.uk/severn-pathology
CARDIOLIPIN	UCLH London	https://www.uclh.nhs.uk/our-services/find-service/queen-square-laboratories/neurometabolic-unit
GANGLIOSIDE SCREEN	Queen Elizabeth University Hospital	
IOHEXOL - REFERRAL	Immunology Department (NBT)	https://www.nbt.nhs.uk/severn-pathology
INHIBIN	Immunology and Protein Reference Unit (Sheffield)	
ACETYL CHOLINE RECEPTOR ANTIBODIES	Immunology Department (Oxford)	http://www.ouh.nhs.uk/immunology
ANAPHYLAXIS STUDIES	Immunology Department (NBT)	https://www.nbt.nhs.uk/severn-pathology
ANTI ADRENAL ANTIBODIES	Immunology Department (NBT)	https://www.nbt.nhs.uk/severn-pathology
ANTI PLA2R	Immunology Department (NBT)	https://www.nbt.nhs.uk/severn-pathology
AP50	Immunology Department (NBT)	https://www.nbt.nhs.uk/severn-pathology
CH50	Immunology Department (NBT)	https://www.nbt.nhs.uk/severn-pathology
AQUAPORIN 4 ANTIBODIES	Immunology Department (Oxford)	http://www.ouh.nhs.uk/immunology
ASPERGILLUS (IgG)	Immunology Department (NBT)	https://www.nbt.nhs.uk/severn-pathology
C 1 ESTERASE INHIBITOR	Immunology Department (NBT)	https://www.nbt.nhs.uk/severn-pathology

CALCIUM GATED CHANNEL	Immunology Department (Oxford)	http://www.ouh.nhs.uk/immunology
CRYOGLOBULIN	Immunology Department (NBT)	https://www.nbt.nhs.uk/severn-pathology
ANTI GAD ANTIBODIES repeated	Synnovis, London	www.viapath.co.uk
ANTI GLUTAMIC ACID DECARBOXYLASE ANTIBODY repeated	Immunology Department (Oxford)	http://www.ouh.nhs.uk/immunology
GLOMERULAR BASEMENT MEMBRANE ANTIBODIES	Immunology Department (NBT)	https://www.nbt.nhs.uk/severn-pathology
HLA-A29	Tissue Typing (NBT)	https://www.nbt.nhs.uk/severn-pathology
HLA-B27	Tissue Typing (NBT)	https://www.nbt.nhs.uk/severn-pathology
HLA DQ2/DQ8	Immunology Department (NBT)	https://www.nbt.nhs.uk/severn-pathology
IGG SUBCLASSES	Immunology Department (NBT)	https://www.nbt.nhs.uk/severn-pathology
IGA ANTI ENDOMYSIAL ANTIBODIES	Immunology Department (NBT)	https://www.nbt.nhs.uk/severn-pathology
LIVER LINE BLOT	Immunology Department (NBT)	https://www.nbt.nhs.uk/severn-pathology
MYELIN ASSOCIATED GLYCOPROTEIN	Immunology Department (Oxford)	http://www.ouh.nhs.uk/immunology
MANNOSE BINDING LECTIN	Immunology, UHW, Cardiff	
MAST CELL TRYPTASE	Immunology Department (NBT)	https://www.nbt.nhs.uk/severn-pathology
ANTI MOG ANTIBODY (SERUM)	Immunology Department (Oxford)	http://www.ouh.nhs.uk/immunology
ANTI MOG ANTIBODY (CSF)	Immunology Department (Oxford)	http://www.ouh.nhs.uk/immunology
ANTI MUSK ANTIBODIES	Immunology Department (Oxford)	http://www.ouh.nhs.uk/immunology
MYOSITIS LINE IMMUNOBLOT	Immunology Department (NBT)	https://www.nbt.nhs.uk/severn-pathology
HLA- ASSOCIATION WITH NARCOLEPSY (DQB1*06:02)	Immunology Department (NBT)	https://www.nbt.nhs.uk/severn-pathology

ANTI NEURONAL ANTIBODIES	Immunology and Protein Reference Unit (Sheffield)	
NMDA RECEPTOR ANTIBODIES (SERUM)	Immunology Department (Oxford)	http://www.ouh.nhs.uk/immunology
OLIGOCLONAL STUDIES	Immunology Department (NBT)	https://www.nbt.nhs.uk/severn-pathology
ANTI OVARY ANTIBODIES	Immunology and Protein Reference Unit (Sheffield)	
PEMPHIGOID / PEMPHIGUS	Immunology Department (NBT)	https://www.nbt.nhs.uk/severn-pathology
POTASSIUM GATED CHANNEL (BLOOD)	Immunology Department (Oxford)	http://www.ouh.nhs.uk/immunology
POTASSIUM GATED CHANNEL (CSF)	Immunology Department (Oxford)	http://www.ouh.nhs.uk/immunology
ANTI SKELETAL MUSCLE ANTIBODIES	Immunology and Protein Reference Unit (Sheffield)	
SYSTEMIC SCLEROSIS IMMUNOBLOT	Immunology Department (NBT)	https://www.nbt.nhs.uk/severn-pathology
T CELL SUBSETS	Immunology Department (NBT)	https://www.nbt.nhs.uk/severn-pathology
IMMUNODEFICIENCY	Immunology Department (NBT)	https://www.nbt.nhs.uk/severn-pathology
RITUXIMAB MONIORING	Immunology Department (NBT)	https://www.nbt.nhs.uk/severn-pathology
ANTI TESTIS ANTIBODIES	Immunology and Protein Reference Unit (Sheffield)	
TSH RECEPTOR ANTIBODIES	Immunology and Protein Reference Unit (Sheffield)	
Allergens (NBT)	Immunology Department (NBT)	https://www.nbt.nhs.uk/severn-pathology
Allergens (PRU)	Immunology and Protein Reference Unit (Sheffield)	