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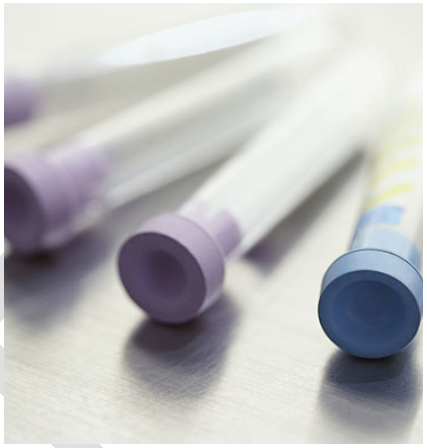

Laboratory Guidelines for Hospitals and Primary Care

5th September 2010

The following document serves as a guideline to appropriate indication and timing of important investigations. It is clear while most investigations are performed for the appropriate reasons there is evidence for the over use and inappropriate use of some tests within hospital and in primary care. The aim of this document is to be helpful and has been put together in a collaborative manner-with local advice and also drawing on international evidence. It is envisaged that this will be updated on an regular basis.

Indications for B12 and Folate testing

Note: B12 and folate should not be requested as repeats within 3 months of assessment unless unusual medical circumstances apply

Unexplained anaemia

Macrocytosis

Suspected malabsorption

Some neurological diseases e.g. peripheral neuropathy

Some psychiatric disorders e.g. unexplained memory loss or dementia

Malnutrition including subjects on restrictive diets e.g. vegetarians

Haematological disease associated with increased cell turnover

Alcohol abuse

Drug therapy e.g. anticonvulsants

Family history of pernicious anaemia

Infertility

Indications for Serum Ferritin

Note: **Ferritin requests for monitoring of haemochromatosis should not be associated with B12/folate requests and follow ups for iron deficiency require ferritin only.**

Unexplained anaemia

Measuring response to iron therapy

Suspected haemochromatosis or unexplained liver disease

Haemochromatosis follow up

Remember a low ferritin ALWAYS means iron deficiency and the cause should be sought if appropriate to do so.

Thrombophilia

Inherited abnormalities which predispose to thrombosis are prevalent in the Irish Population. These can be due to deficiencies or abnormalities of natural inhibitor proteins of the coagulation system. These inhibitors exist to control the rate of formation of a blood clot. A second cause of inherited thrombosis is deficiency of proteins in the fibrinolytic system, these proteins break down blood clots once they are formed. Deficiencies of the fibrinolytic system are very rare and analysis is only carried out following assessment by haematology medical staff.

Protein C.

Protein C is part of the anticoagulant regulatory mechanism. It is converted to activated protein C (APC) by thrombin in the presence of thrombomodulin. APC inactivates activated factors V and VIII. Protein C deficiency has been shown to be a risk factor for thrombosis.

Antithrombin

Antithrombin (AT) is a major inhibitor of blood coagulation and is essential for effective heparin therapy. AT inhibits the coagulation proteases including II a, X a , IX a and XI a. AT deficiency is associated with a high risk of thrombotic disorders.

Free Protein S

Protein S is a vitamin K dependent cofactor for the anticoagulant activity of activated protein C (APC). Two forms of protein S are present in plasma : free protein S (40%) and protein S linked to the C4b-binding protein (60%). Only the free form has functional cofactor activity. Protein S deficiency may be hereditary or acquired – as in normal pregnancy. It has been associated with a high risk of developing venous thromboembolism especially in young people. As only the free form of Protein S has the cofactor activity it is only this form that is measured. Measurement of Protein S in pregnancy is rarely useful.

APC Resistance Assay

Protein C is a naturally occurring inhibitor of blood coagulation, acting on activated factor V and VIII. When patient's plasma does not produce the appropriate anticoagulant response to activated protein C (APC) in the laboratory, this is termed APC resistance. APCR is caused by the VQ506 gene mutation which produces factor V Leiden, a factor V molecule which is resistant to cleavage by activated protein C and therefore prothrombotic.

Factor V Leiden mutation

The identification of Factor V Leiden mutation is carried out using PCR technology. This method is used to identify the genotype of the abnormality. PCR testing is carried out on all samples that have a reduced APCR or have a family history of factor V Leiden.

Prothrombin gene mutation (G-20210-A).

The mutation in the factor II gene (G-20210-A) is in the untranslated portion at start of the gene and is probably part of the the regulatory system for the gene. People carrying the mutation have higher levels of factor II than normal and the increased risk of thrombosis is thought to be a function of this.

Lupus anticoagulant (LA)

LA is an acquired abnormality which is associated with an increased risk of venous thrombosis. LA is named for its association with SLE. A lupus anticoagulant is an anti-phospholipid antibody.

Lupus anticoagulant (LA) results in prolongation of coagulation tests such as the APTT, dependent on phospholipid. Dilute Russell Viper Venom TIme (DRVVT) is used to detect the presence of these inhibitors. LA is associated with a range of autoimmune disorders, infections and treatment with some drugs.

The presence of LA may be suggested by:

1. Unexplained prolongation of APTT
2. Recurrent early foetal loss thought to be due to placental infarct
3. Unexplained thrombotic tendency
4. Thrombocytopenia in association with thrombosis

Other tests (second line investigations).

- There are other gene mutations that are occasionally measured such as Methyl tetra hydorfolate reductase (MTHFR) and Thrombomodulin mutations.
- The measurement of homocysteine is also occasionally measured. This is a functional measure of the MTFHR mutation.
- Occasionally Individual clotting factors are measured such as FVIII, FVII and Fibrinogen. As inherited high levels of these factors can lead to a increased risk of thrombosis.

Who Should have Thrombophilia Screening?

Thrombophilia screening is expensive and time consuming and it is therefore important that it is targeted at the correctly. The following guidelines should identify those individuals most at risk. However there is a lack of clear evidence leaving some uncertainty regarding testing.

1. **Patients with a known family history of any of the inherited thrombophilia factors.**
2. **Patients with a family history of proven venous thrombo-embolism. (more than two symptomatic members).**
3. **Patients who have developed a thrombosis with no obvious precipitating cause or at a relatively young age<40**
4. **Women with a history of recurrent miscarriages should be screened for the lupus anticoagulant. In addition women with severe Pre-eclampsia, IUGR and Stillbirth should also be screened.**
5. **Neonates and children with purpura fulminans should be screened urgently**

In all cases the decision to test for thrombophilia should be based on whether or not the results will influence treatment decisions. Thrombophilia testing is very expensive and a full screen may cost in excess of 200 euro – use resources judiciously!

British Journal of Haematology 2010: 149 (2) 209-220 Clinical guidelines for testing for heritable thrombophilia (www.bcshguidelines.com)

When should Thrombophilia Screening be Done?

Thrombophilia Screening should be done when risk factors (above) are identified. If feasible, screen before a patient is exposed to a known precipitating factor such as pregnancy, surgery or the oral contraceptive.

Thrombophila screening should not be done during the acute phase after the patient presents with a clot. Patients should be tested after the acute event and after any anticoagulation therapy (1 month post warfarin therapy). Testing should wait for 4-6 weeks after pregnancy or miscarriage.

Thrombophilia screens will not be processed on patients on heparin, as heparin interferes with all of the coagulation based (non genetic) assays of a thrombophilia screen and reduces Antithrombin. Patients on low molecular weight heparin may be tested depending on the APTT.

If it is not possible to carry out the thrombophilia screen after warfarin therapy or when patients are on lifelong warfarin a limited number of tests may be useful but these should be discussed with haematology medical staff and the laboratory must be informed of the patients therapy. During warfarin therapy it is not possible to interpret low Protein C and S results as these vitamin K dependant proteins are reduced during the treatment.

What tests should be done?

It is recommended that a full thrombophilia screen be done on patients who fall into the “at risk “ categories already defined. This consists of :

- Protein C
- Protein S
- Anti-thrombin
- APCR and Factor V Leiden genetic analysis
- Protein gene mutation (Prothrombin 20210)
- Lupus anticoagulant and B2 glycoprotein 1

Indications for D-dimers

D-Dimers are indicated for decision support in the management of probable acute thrombosis. A negative D- Dimer is rarely found in acute venous thrombosis. D- dimers are usually significantly elevated in Deep Vein Thrombosis (DVT) or Pulmonary Embolism (PE). In the emergency department, a negative D –dimer provides support for a clinical diagnosis other that thrombosis. However if clinical suspicion of a thrombosis is very strong this overrides a negative D-dimer result (see Well's criteria and Emergency Medicine protocol and procedure for further assistance).

An elevated D-Dimer result should not be used in isolation in the diagnosis of Thromboembolic events.

The reference range is now:

0 - 0.5 mg/L FEU (Fibrinogen Equivalent Units)

Therefore values of > 0.5 mg/L FEU should be incorporated into the clinical decision pathway of investigation of possible thrombosis. As always, judicious ordering of D-dimer assays should be practiced. Serial D-dimers are not usually requested. CRP is a better marker of infection in an acutely ill patient.

HbA1C

Recent review of the Standards of Medical Care in Diabetes by the American Diabetic Association (ADA) 2010, suggested that laboratory analysis of HbA1C should be carried out as below:

1. Perform the Hb A1C test at least two times a year in patients who are meeting treatment goals (and who have stable glycemic control).

2. Perform the Hb A1C test quarterly in patients whose therapy has changed or who are not meeting glycemic goals.

The haematology laboratory in CUH has adopted the new units of measurement for HbA1C, moving from a % value to a mmol/l standard. This will facilitate comparison of patient values measured in different laboratories and also facilitate research internationally.

	NGSP (%)	IFCC
Normal range	4.0	20
	5.0	31
	6.0	42
Goal	7.0	53
	8.0	64
Action needed	9.0	75
	10.0	86

Table shows the old %

Conversion guide; IFCC mmol/mol = (DCCT% x 11) – 24'

Thyroid Function Testing

Hypothyroidism is an insidious condition with a significant morbidity and the subtle and non-specific symptoms and signs may be mistakenly attributed to other illnesses, particularly in postpartum women and the elderly. The earliest biochemical abnormality in hypothyroidism is an increase in serum TSH concentration associated with normal serum FT4 and FT3 concentrations (subclinical hypothyroidism), followed by a decrease in serum FT4 concentration, at which stage most patients have symptoms and benefit from treatment.

The cause is either chronic autoimmune disease (atrophic autoimmune thyroiditis or goitrous autoimmune thyroiditis (Hashimoto's thyroiditis)) or destructive treatment for hyperthyroidism which may account for up to one-third of cases of hypothyroidism in the community

Hyperthyroidism has a significant short-term morbidity and long-term morbidity and mortality. The prevalence of thyrotoxicosis in women is between 0.5 and 2%, and it is also ten times more common in women than in men. The most common causes of hyperthyroidism are Graves' disease, followed by toxic multinodular goitre, whilst rarer causes include an autonomously functioning thyroid adenoma, or thyroiditis.

Screening-Who?

-**At birth**

-Goitre or nodule

-Atrial fibrillation

-Hyperlipidaemia

-Subfertility/menstrual irregularities

-Clinical features of hyper/hypothyroidism

In general, there is no indication to repeat thyroid function tests within an interval of less than two weeks.

Routine testing of thyroid function in patients admitted acutely to hospital is not warranted, *and may be misleading (sick euthyroid syndrome)*, unless specific clinical indications exist

- Clinical suspicion of hyperthyroidism i.e. conventional symptoms plus:

  (a) Uncontrolled atrial fibrillation
  (b) Unexplained hypercalcaemia
  (c) Unexplained weight loss.

- Clinical suspicion of hypothyroidism i.e. conventional symptoms plus

  (a) Unexplained weight gain
  (b) Unexplained macrocytosis.
  (c) Cerebellar syndrome.
  (d) Apathetic depressed patient not responding to anti- depressant medication.

- Monitoring patients with previously diagnosed hyper or hypothyroidism.

- Patients prior to commencing Amiodarone or Lithium.

- Patients with a history of thyroid surgery.

- Patients with suspected ophthalmic thyroid disease.

C reactive protein

C reactive protein (CRP) is an acute phase protein that is synthesised in the liver in response to inflammation and infection. Mild inflammation and viral infections cause CRP to increase to ~10-50 mg/L whereas active inflammation and bacterial infections generally cause concentrations between 50 and 200 mg/L. Concentrations >200 are seen in more severe infections and trauma.

CRP measurement can be used as a diagnostic tool for infection or inflammation, monitoring the effect of treatment or early detection of relapse.

Measurement of CRP is not indicated in asymptomatic individuals and should not be appended to routine investigations.

Serial measurements every 24-48 hours may be used to monitor effectiveness of treatment.

Note: The CRP assay currently used in CUH is not a high sensitive assay and therefore should not be used to predict risk of future coronary events

Major elevations	
Bacterial infections	pyelonephritis pelvic infections meningitis endocarditis chorio-amnionitis
Hypersensitivity complications of infections	rheumatic fever erythema nodosum
Inflammatory disease	rheumatoid arthritis juvenile chronic arthritis ankylosing spondylitis psoriatic arthritis systemic vasculitis polymyalgia rheumatica Reiter's disease Crohn's disease familial Mediterranean fever
Transplantation	renal transplantation
Cancer	lymphoma sarcoma
Necrosis	myocardial infarction tumour embolisation acute pancreatitis

Trauma	burns fractures
Minor or no elevations	
Inflammatory disease	systemic lupus erythematosus systemic sclerosis dermatomyositis ulcerative colitis Sjogren's syndrome
Transplantation	graft versus host disease
Cancer	leukaemia

Troponin

Troponin T is measured in the biochemistry department to assist in the diagnosis and risk assessment of patients who present with symptoms suggestive of myocardial infarction (MI). It should be used to complement other diagnostic modalities such as clinical examination and ECG testing. Troponin is the preferred biomarker of myocyte necrosis because of its high clinical sensitivity and specificity. Because of their poor specificity, biomarkers such as CK, AST and LD have no role to play in the diagnosis of MI.

To assist in the diagnosis of MI, **blood samples for the measurement of Troponin should be drawn on first assessment and 6-9 hours later.**

In the majority of patients no further Troponin testing is required. In an occasional patient an additional measurement between 12 and 24 hours may be required if the earlier measurements are not elevated and the clinical suspicion of MI is high.

To establish the diagnosis of MI, one elevated value above the decision level is required. The decision level is the 99th percentile of a normal reference population.

The demonstration of a rising/falling level is required to distinguish between background elevated Troponin levels that may occur in non-ischaemic heart disease and some other conditions from elevations that are indicative of MI.

This rising/falling pattern is not absolutely required to make the diagnosis of MI if the patient presents >24 hours after the onset of symptoms.

In patients where recurrent myocardial infarction is suspected from clinical signs and symptoms following the initial infarction, an immediate measurement of Troponin is recommended. A second sample should be obtained 3-6 hours later. Recurrent infarction is diagnosed if there is a ≥20% rise in the value in the second sample. This value should also exceed the 99TH percentile upper reference limit.

Table 1: Elevations of Troponin in the absence of overt IHD

Cardiac contusion, or other trauma including surgery, ablation, pacing etc
Congestive heart failure, acute and chronic
Aortic dissection
Aortic valve disease
Hypertrophic cardiomyopathy
Tachy- or bradyarrhythmias or heart block
Apical ballooning syndrome
Rhabdomyolysis withcardiac injury
Pulmonary embolism, severe pulmonary oedema
Renal failure
Acute neurological disease, including stroke and SAH
Infiltrative diseases, e.g. amyloidosis, haemochromatosis, sarcoidosis and scleroderma
Inflammatory diseases, e.g. myocarditis
Drug toxicity or toxins
Critically ill patients especially with respiratory failure or sepsis
Burns especially if affecting >30% of body surface area
Extreme exertion

Tumour Marker Guidelines

1. Check that the **patient hasn't had recent tumour markers taken already?**
   NB: don't waste resources with an unnecessary test!

2. **Be Specific**! Send tumour markers relevant to the malignancy that's clinically suspected – see table below.
3. In the patient with likely neoplasm (of unknown origin), testing multiple markers is rarely useful, but **use your head** – no point sending PSA in a female!
4. Samples go in a **red-topped bottle.**
5. Samples should not to be sent at night or at the weekend.
6. No tumour marker has **sufficient sensitivity and specificity** to be recommended for mass screening or to be diagnostic – they are "markers".
7. Most tumour markers only **rise when disease is advanced**
8. Tumour markers are most useful in monitoring response to treatment and for recurrence, in patients whose levels were raised initially.
9. A normal level doesn't exclude malignancy, and an abnormal level may occur in a "normal" individual.
10. Changes in level over time are more likely to be clinically useful than absolute levels.

PSA	**Prostate Cancer** **Also elevated in** Prostatic hypertrophy Prostatitis Urinary retention Post TURP	▪ May be useful for diagnosis and case finding in combination with DRE ▪ Suspicion of Prostatic Cancer / abnormal rectal examination ▪ Male with sclerotic bony change on X-ray ▪ Male with localized bone pain, normal X-ray - ?neoplastic ▪ Male with metastatic carcinomatosis of unknown origin ▪ Obstructive Uropathy due to bilateral ureter obstruction ▪ Patient with previously documented raised PSA ▪ Monitoring effectiveness of Prostatic Cancer treatment ▪ Monitoring for recurrence of Prostatic Cancer
Ca-15.3	**Breast Cancer**	▪ Not useful in Screening ▪ Early diagnosis of Metastatic Breast disease (clinical benefit not established) ▪ Monitoring response to Breast Cancer treatment

Ca-125	**Ovarian Cancer** **Also elevated in** Advanced Breast Cancer Advanced Lung Cancer Gastric Cancer Endometrial Cancer Cervical Cancer Pancreatic Cancer Pancreatitis Cirrhosis Cholangitis	NB: CA125 is not a validated screening test for ovarian cancer CA125 uses: ▪ Females >45 with pelvic masses ▪ suspicious ultrasound ▪ Monitoring response to Ovarian Cancer treatment ▪ Monitoring for recurrence of Ovarian Cancer
CEA	**Colorectal Cancer** Rarely elevated in early disease **Also elevated in** Breast Cancer Gastric Cancer Lung Cancer Oesophageal Cancer Pancreatic Cancer Cirrhosis Inflammatory Bowel Disease Hepatitis	▪ Monitoring relapse in Colorectal Cancer (and also relapse in Breast and Lung Cancer) ▪ Monitoring treatment in Colorectal Cancer

Ca19.9	**Pancreatic Cancer** **Also elevated in** Colorectal Cancer Gastric/oesophageal Biliary Cancer Cirrhosis Pancreatitis Cholangitis	▪ Clinical suspicion of Pancreatic Cancer; painless obstructive jaundice, central abdominal mass, unexplained weight loss with normal or abnormal radiological tests ▪ Monitoring response to Pancreatic Cancer treatment ▪ Monitoring for recurrence of Pancreatic Cancer
AFP	**Hepatocellular Cancer** **Germ Cell Tumours** **Also elevated in** Hepatitis Cirrhosis Alcoholic Liver disease	▪ Screening patients with known liver disease for Hepatocellular Cancer ▪ Dx of Ovarian / Testicular Germ Cell tumours for follow up ▪ Monitoring response to treatment / for recurrence in Hepatocellular and Germ Cell tumours
LDH	**Markedly elevated (>1000)** Burkitts Lymphoma Lymphoblastic Leukaemia **Raised in** Non-Hodgkin's Lymphomas **Also elevated in** Haemolytic anaemia liver disease Severe vitamin B12 deficiency	Note - useful in follow up of lymphoma patients during and after treatment

Haemochromatosis

Molecular Genetic Testing

Hereditatary Haemochromatosis (HH) is a one of the most common, autosomal recessive conditions found in Northern Europeans. It is a disorder of iron metabolism caused by excessive absorption and storage of dietary iron, which leads to progressive iron accumulation and organ damage.

Iron accumulates first in the transferrin pool resulting in raised transferrin saturation, and subsequently in tissue stores resulting in a progressive rise in ferritin levels.

Transferrin saturation is the best marker of iron overload as ferritin (an acute phase protein) may also be raised in inflammatory or infective conditions.

Serum Ferritin >1000ug/L is associated with hepatic fibrosis and cirrhosis in HH patients.

Genetics

HH is associated with two mutations in the HFE gene, C282Y and H63D. Approx. 90% of HH patients are homozygous for C282Y, the remainder are mostly compound heterozygotes for C282Y and H63D mutations.

Clinical expression of haemochromatosis is highly variable due to incomplete penetrance of HFE mutations. In screening studies, less than 20% of men and 40% of women with C282Y homozygosity showed evidence of iron overload.

HH genetic testing is performed in the biochemistry laboratory at CUH. EDTA blood and a special request form indicating patient consent is required.

Request form and further details are available from clinical biochemistry, ext 22531

Testing Criteria

- **Patient has first-degree relative with HH (ie. parent, sibling, offspring)**

or

- **Patient has evidence of iron overload (ie. transferrin saturation >45%)**

Minimum age for HH genetic testing is 16 years of age, in accordance with international guidelines for late onset disorders.

Referrals

Diagnostic testing is performed when biochemical evidence of iron overload exists

Predictive testing is performed for first degree relatives of patients with HH

Indications for Urine Culture

1. Urine culture is **NOT** recommended as part of screening periodic health examinations of asymptomatic infants, children or the elderly.

2. Some guidelines recommend that urine culture is unnecessary if short-term therapy has been prescribed for uncomplicated UTI in female patients.

3. Urine Culture should be ordered once, during the first trimester, for asymptomatic pregnant women

4. Urine culture should be ordered for patients with symptoms and risk factors for urinary tract infection.

5. Clinical judgement should be exercised in ordering urine cultures for asymptomatic individuals with one or more risk factors.

6. The risk factors are listed in the following table.

Previous leukocyte esterase and or nitrates in urine	**Established renal disease**
Pregnancy	**Recurrent urinary tract infections**
Diabetes	**Neurogenic bladder**
Recent urological surgery/cystoscopy	**Renal transplant**
Genitourinary problems	**Treatment failure for cystitis**

Autoimmune Testing

The interpretation of all autoantibody tests is highly dependent on the likelihood of disease in the patient. The results should always be interpreted with the clinical features of the patient and never in isolation. Autoantibodies may be present in healthy individuals and may also occur transiently with intercurrent illness or may be induced by drug therapy. Conversely, autoimmune disease may be present in the absence of detectable autoantibodies. **Do not use these tests as 'screens' for autoimmune disease** but rather decide the clinical diagnosis and the likelihood of autoimmune disease and use specific autoantibody tests as diagnostic aids.

Anti-nuclear (ANA), Anti-double stranded DNA (dsDNA) and Anti-Extractable Nuclear Antigen (ENA) Antibodies

These tests are predominantly used for the investigation and diagnosis of inflammatory connective tissue diseases such as SLE, Sjogren's syndrome, systemic sclerosis, mixed connective tissue disease, polymyositis and dermatomyositis. The initial screening test is the ANA and this is performed using Indirect Immunofluorescence at a screening dilution of 1/80. The autoantibodies detected are IgG and the cells used are a carcinoma cell line, HEp2. If the ANA is positive the result will also be reported as a pattern, as discussed below. If the ANA is positive then the sample will be analysed for anti-dsDNA and anti-ENA antibodies.

Important points about ANA tests:

An ANA may be positive in healthy individuals (and particularly with increased age) or be induced transiently during acute illness or with infection, and by certain medication. It may also be positive in many other autoimmune diseases including rheumatoid arthritis and autoimmune thyroid disease. The ANA has no particular clinical significance in these situations. **Thus the tests should only be requested where the clinical features are suggestive of inflammatory connective tissue disease.** Do not use the tests as 'screens' for autoimmune disease.

Occasionally the ANA may be negative where there is underlying CTD. This is particularly relevant to conditions where anti-Ro (SSA) antibodies may be the only autoantibody present, as in Subacute Cutaneous Lupus, rare cases of SLE, and some patients with Sjogren's syndrome. The ANA may also be negative in polymyositis with anti-Jo 1 antibodies and very occasionally with anti-Scl 70 antibodies in systemic sclerosis. Where these conditions are strongly suspected then the relevant tests (anti-ENA) will be performed even if the ANA is negative. However, this requires that **full clinical details are included on the request forms**. Clinically significant anti-dsDNA antibodies are rarely, if ever, present with a negative ANA.

Antinuclear antibody levels should not be used to monitor disease activity and repeated re-testing is not required.

Anti-double stranded DNA (dsDNA) Antibodies

These tests are performed by ELISA + / - fluorescence methodologies and are reflex tested when the ANA is positive. The ELISA methodology is very sensitive and hence low level positive results may be found when disease is absent. Anti-dsDNA antibodies ca be confirmed by IIF (Crithidia assay) Anti dsDNA antibodies are particularly associated with SLE but they occasionally may be present in other conditions, most notably autoimmune hepatitis. Anti-dsDNA antibodies may correlate with clinical activity in SLE. They are especially useful in long-term monitoring of subsets of patients with more severe manifestations of that disease (renal, cerebral) but should always be used in combination with clinical assessment and complement factors 3 and 4 monitoring. A *fall* rather than a rise in ds DNA antibodies may indicate increasing disease activity. ds DNA antibodies should not be repeated more frequently than once monthly so are not useful in acute monitoring of patients with severe lupus manifestations

Anti-Extractable Nuclear Antigen (ENA) Antibodies

These tests are performed by ELISA and are reflex tested when the ANA is positive. In addition they may be tested, even if the ANA is negative, when the clinical features provided are strongly suggestive of CTD, especially SCLE, SLE, Sjogren's Syndrome or Polymyositis. The sample is first screened for reactivity to all the ENAs in a combined test. If this is negative then no further tests are performed. If this is positive then antibodies to the individual ENAs are tested. The general disease associations with these autoantibodies are as follows:

Anti-SSA (Ro) Antibodies	Associated with Subacute Cutaneous Lupus Erythematosus, Systemic Lupus Erythematosus and Sjogren's Syndrome and are of particular relevance during pregnancy as their presence may be associated with neonatal lupus and congenital heart block.
Anti-SSB (La) Antibodies	Generally are present with anti-SSA antibodies and are associated with SLE and Sjogren's Syndrome.
Anti-Sm Antibodies	Pathognomonic of SLE but only occur in a minority of patients with this condition.
Anti-Ribonucleolar protein (RNP) Antibodies	Particularly associated with SLE, and when in high titre alone, with Mixed Connective Tissue Disease.
Anti-Scl-70 Antibodies	Particularly associated with diffuse systemic sclerosis (scleroderma).
Anti-Jo 1 Antibodies	Found in a minority of patients with polymyositis, particularly when it is associated with interstitial lung disease.

Anti-Neutrophil Cytoplasmic Antibodies (ANCA) Antibodies

The ANCA assays are performed first by indirect immunofluorescence providing a negative or positive result. Positive results are reported as a pattern: P(erinuclear), C(ytoplasmic) or Nuclear. The presence of a positive ANCA result is not disease-defining. The disease association (small vessel vasculitis) is with specific antibodies directed to one of two major granule proteins i.e. **M**yelo**p**er**o**xidase (MPO) or **Pr**oteinase **3** (PR3). Hence all positive results are referred on for ELISA testing for the presence of IgG to these proteins. Tests for autoantibodies to other neutrophil granule proteins are not available. A negative result will generally be reported without further tests but very occasionally anti-MPO or anti-PR3 antibodies may be present with a negative ANCA. If the clinical features are strongly suggestive of small vessel vasculitis and the ANCA is negative then these tests may be undertaken on request. In combination ANCA and anti-MPO and PR3 antibodies are about 90% sensitive in detecting small vessel vasculitis. *Thus a negative test does not exclude vasculitis.*

In general in the context of small vessel vasculitis:

- C-ANCA is associated with anti-PR3 antibodies and is found in Wegener's Granulomatosis, and Churg-Strauss Syndrome.
- P-ANCA is associated with anti-MPO antibodies and is found in Microscopic Polyangiitis,
  Crescentic Glomerulonephritis and Churg-Strauss Syndrome.

But this is variable and one may have C-ANCA with anti-MPO and P-ANCA with anti-PR3, or combinations of antibodies. A positive ANCA with negative anti-MPO and PR3 may be found in a variety of conditions including autoimmune hepatitis, sclerosing cholangitis, ulcerative colitis, SLE, RA, malignancy and chronic infections. ANCA testing is **not** warranted for these clinical conditions.

Anti-Glomerular Basement Membrane (GBM) Antibodies

Antibodies to GBM are primarily directed towards the non-collagenous domain of the alpha 3 chain of type IV collagen. Since this type of collagen is found predominantly in glomeruli and alveoli, the presence these directly pathogenic antibodies is associated with rapidly progressive glomerulonephritis and alveolitis (Goodpasture's Syndrome). Anti-GBM antibodies may also be found in some patients with ANCA positive small vessel vasculitis, usually with anti-MPO antibodies. Repeat test are useful to determine the effectiveness of plasma exchange.

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

These are present in individuals with autoimmune gastritis and pernicious anaemia. However they are not specific for these conditions, as they may be also found in healthy individuals, particularly with increased age, and in those with other autoimmune conditions (thyroiditis, Addison's disease, IDDM). If Vitamin B12 levels are low then anti-intrinsic factor antibodies should be requested. Anti-GPC antibodies are more sensitive but less specific for pernicious anaemia than anti-IF antibodies.

Gluten Sensitive Enteropathy (Coeliac Disease) Testing

Coeliac Disease is an enteropathy that occurs in the presence of gluten found in wheat (gliadins), barley (hordeins) and rye (secalins). The condition is thought to be far more prevalent than previously realized and may manifest with non-specific and extra-intestinal symptoms (anaemia, osteopaenia, fatigue, abnormal liver function tests). The enteropathy is essentially an autoimmune condition that occurs in the presence of gluten and resolves with gluten withdrawal. IgA antibodies to gliadin, tissue
transglutaminase and to antigens created by the combination of the two are involved in the disease process. GSE predominantly occurs in those individuals with a specific tissue type, HLA DQ2.

Like all autoantibody tests, **the results should be interpreted with the clinical features**. The tests will not be positive in all patients with GSE, and some patients with positive tests may not have GSE. **For accurate diagnosis the tests should only be undertaken with the patient eating a normal gluten-containing diet. Those on gluten free diets may have false negative tests**. The gold standard for the diagnosis of Gluten Sensitive Enteropathy is small intestinal biopsy which should also be undertaken on a gluten containing diet. In most patients positive tests alone are inadequate to make the diagnosis. Follow up tests may be useful after the diagnosis has been made to follow compliance with a gluten-free diet. IgA deficiency, which occurs in about 1/700, will lead to false negative serological tests for GSE. Thus serum IgA is measured in all samples to allow for appropriate interpretation. Where IgA deficiency is present then IgG based tests may then be used but these are far less specific for GSE and may be present in healthy individuals and those with other intestinal diseases.

Anti-Tissue Transglutaminase (tTG) Antibodies

The antigen within the endomysium to which the IgA antibodies bind in GSE has been identified as tissue transglutaminase (tTG). These antibodies are detected by ELISA. this assay, using human recombinant tTG, is more sensitive than anti- EMA testing. The initial screening test is tTG and this is positive the sample is tested for antiendomysial antibodies for confirmation.

Anti-Endomysial Antibodies (EMA)

IgA anti-endomysial antibodies are detected by indirect immunofluorescence. The results are reported as positive or negative.

ALLERGY TEST REQUESTING GUIDELINES

The laboratory can be contacted (021 – 4922535) for discussion and advice as needed.

Laboratory allergy testing is highly useful when requested and interpreted correctly. IgE testing is labour intensive and expensive (each allergen costing 33.19 € and a panel of 5 costing 165.95€)**.** Testing must be used effectively.

All samples received are analysed for total IgE. Elevated levels are a feature of many allergic conditions including asthma, allergic rhinitis and atopic dermatitis. *IgE levels however can be normal in patients with significant allergic or hypersensitivity conditions e*.g. some food and drug allergic presentations. Specialist advice regarding evaluation and management should be sought if required in such cases. Specific IgE testing will not proceed on the basis of the total IgE level, but rather if clinical details are appropriate

An inhalant allergen profile will be performed on samples where clinical information suggests airway allergic disease. Limitation of exposure to inhalant allergens to which the patient has become sensitised may be helpful in clinical management. Serial IgE for aspergillus will be undertaken in specialist circumstances, e.g. monitoring of CF patients andpatients with Allergic Bronchopulmonary Aspergillosis.

Food allergen testing will not be undertaken unless clinical details indicate that it is relevante.g. specific food-associated anaphylaxis and acute urticaria . Food allergen testing is not generally useful in the investigation of patients with chronic urticaria, chronic fatigue, chronic gastrointestinal upset, arthritis etc.

In particular circumstances, such as the investigation of drug allergy and patients experiencing reactions during anaesthesia, it is recommended that specialist advice is sought before requesting tests.