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***Approved By**: Prof. C. Feighery, Dr. Katrina Hutchinson, JS Charles.*

Title: Primary Sample Manual - Immunology

***Changes made since previous version:** Plasma as a sample type was removed for all tests.*

***Note**: Please refer to the document record on QPulse / IQM for the revision history of this document.*

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INTRODUCTION

This is a list of the immunology tests performed at Eurofins Biomnis' Dublin Laboratory. For a searchable list of tests performed by Eurofins Biomnis in France, in our laboratories in Lyon and Paris, click here.

If you cannot find details of a test you require, please contact our Client Services department on Free Phone 1800-252-966, or e-mail client.services@eurofins-biomnis.ie.

For sample collection, please contact our Logistics department on Free Phone 1800-252-967, or e-mail logistics@eurofins-biomnis.ie.

LAYOUT OF TEST INFORMATION

TEST INFORMATION TEMPLATE	
Brief information on clinical background, indications for test and interpretation of test results.	
Preparation of Patient: any special preparation required, such as fasting. **Precautions:** any special circumstances, conditions etc. to be aware of.	
Accredited	Whether or not the test is accredited by INAB to ISO 15189. Green indicates accredited, Orange indicates non accredited status.
Method	Test method
Sample Requirements	Type of tube required, transport temperature and other information.
Turnaround Time	The maximum turnaround time from receipt of the sample in the laboratory's pre-analytics department.
Stability	Sample stability under various conditions. **RT = room temperature i.e.: 16 – 25 °C.** Please see **SAMPLE STABILITY** notes below. Stability data indicated by a superscript numeral 1 are taken from the publication referenced below[1].
Units - Reference Ranges and Source	Units and reference range(s) for the test. Source of the reference ranges: 1. Test manufacturer's instructions for use (IFU). 2. National and international guidelines. 3. Ranges established in-house.

NOTES ON SAMPLE STABILITY

The majority of incorrect laboratory test results are due to improper sample collection and transport. For details regarding correct phlebotomy technique and our patient identification requirements, please click here.

In order for you to arrange and properly time phlebotomy and sample collection, we have indicated, for each test, its stability after collection. Stability is indicated for whole blood at various temperatures, and for serum separated from cells, also at various temperatures.

Note: RT = room temperature, i.e. 16 – 25 °C.

Stability data are taken from the manufacturers' instructions for use (IFUs), and from the World Health Organisation publication indicated below[1].

Sample stability data is not available for all tests under all conditions, either in the manufacturers' IFUs or the published literature. If no information is available, in general, unless otherwise specified (such as when the required sample is whole blood), serum should be centrifuged and separated from cells after completion of clotting (20 – 30 minutes), and transported to the laboratory at 2 – 8 °C. Serum may be centrifuged and separated from cells immediately after sampling and gently mixing the sample by inverting the tube 10 times. It should then be transported to the laboratory at 2 – 8 °C. Whole blood should be transported at 2 – 8 °C and reach the laboratory as soon as possible. **However, please check each test for specific stability information.**

If in doubt, please contact our Client Services department on Free Phone 1800-252-966, or e-mail client.services@eurofins-biomnis.ie.

Reference:

1. World Health Organisation: Use of anticoagulants in diagnostic laboratory investigations. WHO/DIL/LAB99.1 Rev.2, 2002.

REASONS FOR REJECTION OF SAMPLES/NON-REPORTING OF TESTS

1. Samples received beyond the stability limits and/or not at the correct temperature indicated below for each test.
2. Samples received in the incorrect tube/with the incorrect anticoagulant or lack of the correct anticoagulant.
3. Samples received without the necessary patient identifiers. For more details, see here.
4. Samples which fail specific criteria for certain tests. See individual tests for details.

Immunology Consultant: Prof. Conleth Feighery
Telephone: 00-353-1-295-8545

ALLERGY TESTING: PHADIA SPECIFIC IGE	
Immunoglobulin E (IgE) specific to individual allergens is measured to determine which allergens are responsible for atopic symptoms and signs in an individual. The concentration of the specific IgE frequently correlates with the severity of symptoms. The following individual allergen specific IgEs are assayed at Eurofins Biomnis: E1, E3, E4, E5, D1, D2, F1, F2, F3, F4, F13, F14, F17, F18, F20, F25, F26, F27, F36, F44, F75, F81, F82, F83, F201, F245, F284, F343. The following panels are assayed: EX1, FX1, FX2, FX5, FX7, FX13, FX15, GX3, MX1, MX2, TX8, WX1, WX2. For details on these tests, see the Phadia Allergy website at http://www.phadia.com/Products/Allergy-testing-products/ImmunoCAP-Allergen-Information/ (Accessed 22/05/20).	
Preparation of Patient: There is no special physical preparation for specific IgE analysis.	
Location	**Eurofins Biomnis Blackthorn Road**
Accredited	Yes
Method	Fluoroenzyme immunoassay on Phadia 250. SOP: CC99
Sample Requirements	Tube Type: Serum (Red and gold cap) Temperature: + 4ºC
Turnaround Time	4 working days
Stability	Whole blood: RT unknown. 7 days @ + 4°C Source: Phadia IFU
Units - Reference Ranges and Source	Panels are reported as Positive or Negative only. Individual allergens are reported in kIU/L, ranging from less than 0.10 to greater than 100. A result of > 0.10 kIU/L is interpreted as positive. Source: Phadia Instructions for Use.

ANTI-CARDIOLIPIN IGG ANTIBODIES

Anti-cardiolipin antibodies (ACA) belong to the group of anti-phospholipid antibodies (aPL). Their occurrence was first demonstrated in sera of syphilis patients, but later they have also been described frequently in SLE (systemic lupus erythematosus) patients (prevalence 30-40%) and in patients with other rheumatic diseases. The antiphospholipid syndrome (APS), also known as “Hughes syndrome”, is characterized by typical clinical features such as arterial/venous thrombosis or recurrent miscarriages together with persistently positive tests for aPL. In contrast to “secondary APS” which occurs in association with SLE or other rheumatic disorders, there is no evidence for another relevant underlying disease in primary APS. New criteria for classification of the antiphospholipid syndrome have been defined recently.

Anti-cardiolipin antibodies in infectious diseases and in APS can be distinguished with respect to their dependence on cofactors: whereas ACA from patients with infectious diseases recognize the pure phospholipid as antigen, binding of ACA from patients with APS requires β2-glycoprotein I as a cofactor. For this reason, ACA ELISAs need β2-glycoprotein I to be incorporated into the assay.

The so-called ‘lupus anticoagulant' (LA) describes a phenomenon that is related to the presence of antiphospholipid antibodies. It is defined by the measurement of antibody dependent coagulation inhibition in vitro. ACA/LA are considered to be of significant diagnostic relevance, as a correlation has been found between these antibodies and a tendency towards thromboses. This results in an increased incidence of venous/arterial thromboses, thrombocytopenia, livido reticularis, habitual abortion and neurological manifestations in ACA/LA-positive patients. Elevated levels of ACA/LA may also be found in patients with cerebrovascular insufficiency or myocardial infarction.
aPL are thought to play a direct role in the pathogenesis of APS.

Preparation of Patient: No special preparation.

Location	**Eurofins Biomnis Blackthorn Road**
Accredited	Yes
Method	Fluoroenzyme immunoassay (Phadia 250) SOP CC102
Sample Requirements	Tube Type: Serum (Gold and red cap) Temperature: + 4ºC Source: Phadia IFU
Turnaround Time	4 working days
Stability	Whole blood: unknown. Separated: RT 1 day. 14 days @ + 4ºC Source: Phadia IFU
Units - Reference Ranges and Source	Negative: < 10.0 GPL-U/mL Weak positive: 10.0 - 40.0 GPL-U/mL Positive: > 40.0 GPL-U/mL Source: Phadia IFU

ANTI-CARDIOLIPIN IGM ANTIBODIES

Anti-cardiolipin antibodies (ACA) belong to the group of anti-phospholipid antibodies (aPL). Their occurrence was first demonstrated in sera of syphilis patients, but later they have also been described frequently in SLE (systemic lupus erythematosus) patients (prevalence 30-40%) and in patients with other rheumatic diseases. The antiphospholipid syndrome (APS), also known as “Hughes syndrome”, is characterized by typical clinical features such as arterial/venous thrombosis or recurrent miscarriages together with persistently positive tests for aPL. In contrast to “secondary APS” which occurs in association with SLE or other rheumatic disorders, there is no evidence for another relevant underlying disease in primary APS. New criteria for classification of the antiphospholipid syndrome have been defined recently.

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aPL are thought to play a direct role in the pathogenesis of APS.

Preparation of Patient: No special preparation.

Location	**Eurofins Biomnis Blackthorn Road**
Accredited	Yes
Method	Fluoroenzyme immunoassay (Phadia 250) SOP CC103
Turnaround Time	4 working days
Sample Requirements	Tube Type: Serum (Gold and red cap) Source: Phadia IFU
Stability	14 days @ + 4ºC Source: Phadia IFU
Units - Reference Ranges and Source	Negative: < 10.0 MPL-U/mL Weak positive: 10.0 - 40.0 MPL-U/mL Positive: > 40.0 MPL-U/mL Source: Phadia IFU

ANTI-CYCLIC CITRULLINATED PEPTIDE ANTIBODIES

Rheumatoid Arthritis (RA) is a common, systemic autoimmune disease affecting 0.5-1% of the population. It is characterized by chronic inflammation of the synovium, which commonly leads to progressive joint destruction and in most cases, to disability and reduction of quality of life. Evidence gained over the last few years suggests that aggressive therapy given early in the disease has the greatest therapeutic potential.
The serum of RA patients contains a variety of antibodies directed against self-antigens. The most widely known of these autoantibodies is the rheumatoid factor (RF) antibody directed against the constant domain of IgG molecules. The presence of RF is one of the American College of Rheumatology's criteria for the classification of RA. Although the RF test has good sensitivity for RA, it is not very specific for the disease as it can also be detected in the serum of patients with other rheumatic or inflammatory diseases and even in a substantial percentage of the healthy (elderly) population. For several years it has been recognized that antibodies to anti-perinuclear factor and anti-keratin are highly specific for RA. It was subsequently reported that both of these antibodies reacted with native filaggrin and are now referred to as anti-filaggrin antibodies. More recently it has been shown that all of these antibodies are directed to citrulline-containing epitopes. Citrulline is a non-standard amino acid, as it is not incorporated into proteins during protein synthesis. It can, however, be generated via post-translational modification of arginine residues by the enzyme peptidyl arginine deiminase. In 1998, Schellekens and colleagues reported that linear peptides containing citrulline (CP) were very specific for RA antibodies (96%) in an ELISA based assay. Subsequent work demonstrated that cyclic variants of these peptides, termed cyclic citrullinated peptides (CCP), were equally specific for RA, but with a higher sensitivity than linear peptides. To improve the sensitivity of the CCP test further, several dedicated libraries of citrulline-containing peptides were screened with RA sera and a new set of peptides (CCP2) were discovered which gave superior performance compared to the CCP1 test. Over the last few years, many independent studies have confirmed the diagnostic performance of the CCP2 test. In 2007, the European League against Rheumatism (EULAR) published guidelines for the diagnosis of early RA, and the measurement of antibodies to anti-CCP was included as a serology marker.

Preparation of Patient: No special preparation.

Location	**Eurofins Biomnis Blackthorn Road**
Accredited	Yes
Method	Abbott Alinity. CMIA. BKICC115
Sample Requirements	Tube Type: Serum (Gold and red cap) Temperature: + 4ºC
Turnaround Time	4 working days
Stability	Serum: RT 22 hours, 2-8ºC 7 days. >7 days @ -20ºC Source: Abbott IFU
Units - Reference Ranges and Source	< 5.0 U/mL: Negative ≥ 5.0 U/mL: Positive Source: Abbott IFU

ANTI-GLIADIN IGA ANTIBODIES

Coeliac disease is a life-long condition in which ingestion of gluten, the water insoluble wheat gliadin and the prolamins in rye and barley, leads to chronic inflammation and damage of the small intestinal mucosa. The disease is multifaceted in nature with clinical presentation ranging from gastrointestinal manifestations to asymptomatic, silent and extraintestinal forms. It is widely accepted that dermatitis herpetiformis, a bullous skin disease, is induced by gluten. The term gluten refers to a whole set of proteins in the so-called endosperm, the nutritive tissue of the grain seed of wheat, rye, oats and barley. The alcohol-soluble polypeptides of gluten, the gliadins, are solely responsible for the toxic effects to the intestinal mucosa. More recent research revealed that gliadin peptides deamidated by tissue transglutaminase represent more specific B-cell epitopes than native peptides.

Preparation of Patient: No special preparation.

Location	**Eurofins Biomnis Blackthorn Road**
Accredited	Yes
Method	Fluoroenzyme immunoassay (Phadia 250) SOP CC104 EliA GliadinDP IgA
Sample Requirements	Tube Type: Serum (Gold and red cap); Temperature: + 4ºC
Turnaround Time	4 working days
Stability	Whole blood: unknown Separated: RT unknown, 14 days @ + 4ºC Source: Phadia IFU
Units - Reference Ranges and Source	Negative: < 7.0 U/mL Borderline: 7.0 - 10.0 U/mL Positive: > 10.0 U/mL Source: Phadia IFU

ANTI-GLIADIN IGG ANTIBODIES

Coeliac disease is a life-long condition in which ingestion of gluten, the water insoluble wheat gliadin and the prolamins in rye and barley, leads to chronic inflammation and damage of the small intestinal mucosa. The disease is multifaceted in nature with clinical presentation ranging from gastrointestinal manifestations to asymptomatic, silent and extraintestinal forms. It is widely accepted that dermatitis herpetiformis, a bullous skin disease, is induced by gluten. The term gluten refers to a whole set of proteins in the so-called endosperm, the nutritive tissue of the grain seed of wheat, rye, oats and barley. The alcohol-soluble polypeptides of gluten, the gliadins, are solely responsible for the toxic effects to the intestinal mucosa. More recent research revealed that gliadin peptides deamidated by tissue transglutaminase represent more specific B-cell epitopes than native peptides.

Preparation of Patient: No special preparation.

Location	**Eurofins Biomnis Blackthorn Road**
Accredited	Yes
Method	Fluoroenzyme immunoassay (Phadia 250) SOP CC105 EliA GliadinDP IgG
Sample Requirements	Tube Type: Serum (Gold and red cap); Temperature: + 4ºC
Turnaround Time	4 working days
Stability	Whole blood: unknown Separated: RT unknown, 14 days @ + 4ºC Source: Phadia IFU
Units - Reference Ranges and Source	Negative: < 7.0 U/mL Weak positive: 7.0 - 10.0 U/mL Positive: > 10.0 U/mL Source: Phadia IFU

ANTI-GLOMERULAR BASEMENT MEMBRANE ANTIBODIES	
GBM antibodies occur in patients suffering from Goodpasture syndrome, anti-GBM-disease and ANCA associated vasculitis. Goodpasture syndrome is defined by the combined occurrence of progressive glomerulonephritis, lung haemorrhage and antibodies to the glomerular basement membrane (GBM). A more limited form only involving the kidney or the lung is referred to as anti-GBM disease. For the diagnosis of both, Goodpasture syndrome and anti-GBM-disease, the presence of GBM antibodies is required. Furthermore up to 10 % of ANCA positive patients show GBM antibodies, which indicate a more severe course of renal damage.	
Preparation of Patient: No special preparation.	
Location	**Eurofins Biomnis Blackthorn Road**
Accredited	Yes
Method	Fluoroenzyme immunoassay (Phadia 250) SOP CC107
Sample Requirements	Tube Type: Serum (Gold and red cap); Temperature: + 4ºC
Turnaround Time	4 working days
Stability	Whole blood: unknown. Separated: RT unknown 14 days @ + 4ºC Source: Phadia IFU
Units - Reference Ranges and Source	Negative: < 7.0 U/mL Weak positive: 7.0 - 10.0 U/mL Positive: > 10.0 U/mL Source: Phadia IFU

ANTI-B2-GLYCOPROTEIN 1 IGG ANTIBODIES	
The antiphospholipid syndrome (APS), also known as "Hughes syndrome", is characterized by typical clinical features such as arterial/venous thromboses or recurrent miscarriages together with persistently positive tests for antiphospholipid antibodies. The criteria for classification of the APS have been revised in 2004 in Sydney. Besides the clinical criteria, three different laboratory tests are listed: lupus anticoagulant, anticardiolipin antibodies (IgG and IgM) and anti-β2-Glycoprotein I antibodies (IgG and IgM). The latter was not included in the former Sapporo criteria. However, by majority, the Sydney committee agreed that they are an independent risk factor for thrombosis and pregnancy complications. For APS diagnosis, β2-Glycoprotein I antibody tests show higher specificity than anticardiolipin assays. In 3-10% of APS patients, β2-Glycoprotein I antibodies may be the only positive test. The association of β2-Glycoprotein I antibodies with pre-eclampsia and/or eclampsia in unselected pregnant women who tested negative for anticardiolipin antibodies implies that the inclusion of β2-Glycoprotein I antibodies may also help clarify this type of pregnancy morbidity. Outside the context of clinical studies, testing for β2-Glycoprotein I antibodies can be helpful for APS diagnosis, particularly when anticardiolipin antibodies and lupus anticoagulant are negative and APS is strongly suspected.	
Preparation of Patient: No special preparation.	
Location	**Eurofins Biomnis Blackthorn Road**
Accredited	Yes
Method	Fluoroenzyme immunoassay (Phadia 250) SOP CC113
Sample Requirements	Tube Type: Serum (Gold and red cap); Temperature: + 4ºC
Turnaround Time	4 working days
Stability	Whole blood: unknown. Separated: RT unknown; 14 days @ + 4ºC Source: Phadia IFU
Units - Reference Ranges and Source	Negative: < 7.0 U/mL Weak positive: 7.0 U/mL to 10.0 U/mL Positive: > 10.0 U/mL Source: Phadia IFU

ANTI-B2-GLYCOPROTEIN 1 IGM ANTIBODIES

The antiphospholipid syndrome (APS), also known as “Hughes syndrome”, is characterized by typical clinical features such as arterial/venous thromboses or recurrent miscarriages together with persistently positive tests for antiphospholipid antibodies. The criteria for classification of the APS have been revised in 2004 in Sydney. Besides the clinical criteria, three different laboratory tests are listed: lupus anticoagulant, anticardiolipin antibodies (IgG and IgM) and anti-β2-Glycoprotein I antibodies (IgG and IgM). The latter was not included in the former Sapporo criteria. However, by majority, the Sydney committee agreed that they are an independent risk factor for thrombosis and pregnancy complications. For APS diagnosis, β2-Glycoprotein I antibody tests show higher specificity than anticardiolipin assays. In 3-10% of APS patients, β2-Glycoprotein I antibodies may be the only positive test. The association of β2-Glycoprotein I antibodies with pre-eclampsia and/or eclampsia in unselected pregnant women who tested negative for anticardiolipin antibodies implies that the inclusion of β2-Glycoprotein I antibodies may also help clarify this type of pregnancy morbidity. Outside the context of clinical studies, testing for β2-Glycoprotein I antibodies can be helpful for APS diagnosis, particularly when anticardiolipin antibodies and lupus anticoagulant are negative and APS is strongly suspected.

Preparation of Patient: No special preparation.

Location	**Eurofins Biomnis Blackthorn Road**
Accredited	Yes
Method	Fluoroenzyme immunoassay (Phadia 250) SOP CC108
Sample Requirements	Tube Type: Serum (Gold and red cap); Temperature: + 4ºC
Turnaround Time	4 working days
Stability	Whole blood: unknown. Separated: RT unknown; 14 days @ + 4ºC Source: Phadia IFU
Units - Reference Ranges and Source	Negative: < 7.0 U/mL Weak Positive: 7.0 U/mL to 10.0 U/mL Positive: > 10.0 U/mL Source: Phadia IFU

ANTI-INTRINSIC FACTOR ANTIBODIES	
Pernicious anaemia (also known as Biermer's disease) is an autoimmune atrophic gastritis, predominantly of the fundus, and is responsible for a deficiency in vitamin B12 (cobalamin) due to its malabsorption. Its prevalence is 0.1% in the general population and 1.9% in subjects over the age of 60 years. Pernicious anemia represents 20%–50% of the causes of vitamin B12 deficiency in adults. Anti intrinsic factor antibodies do not appear to have a clearly defi ned pathogenic role in the development of gastritis. By contrast, they have a well-documented role in the onset of pernicious anemia, via the vitamin B12 deficiency they induce. The finding of a low total serum cobalamin level may be further evaluated by testing for anti-intrinsic factor antibodies. If positive, the antibodies have a high positive predictive value (95%) for the presence of pernicious anaemia with a concurrent low false positive rate (1–2%) i.e. a high specificity. It identifies those patients with a need for lifelong cobalamin replacement therapy.2 With regard to diagnostic performance sensitivity is low for anti-intrinsic factor antibodies, in the most recent studies while specificity is 100%. In combination with anti-parietal cell antibodies they yield 73% sensitivity and 100% specificity.	
Preparation of Patient: No special preparation.	
Location	**Eurofins Biomnis Blackthorn Road**
Accredited	Yes
Method	Fluoroenzyme immunoassay (Phadia 250) SOP CC159
Sample Requirements	Tube Type: Serum (Gold and red cap); Temperature: + 4ºC
Turnaround Time	4 working days
Stability	Whole blood: unknown. Separated: RT 8 hours; 14 Days @ + 4ºC Source: Phadia IFU
Units - Reference Ranges and Source	U/mL Negative: less than 7 Equivocal: 7 - 10 Positive: greater than 10 Source: Phadia IFU

ANTI-M2 ANTI-MITOCHONDRIAL ANTIBODIES

Primary biliary cirrhosis (PBC) is a chronic, cholestatic liver disease which affects mainly middle-aged women. It starts with an inflammatory process of the small and middle-sized interlobular bile ducts leading first to a proliferation and then to a loss of bile ducts, to portal inflammation and in late stages to liver cirrhosis.
PBC occurs all over the world but with varying incidence, ranging from 0.7-49 per million per year. In most recent studies the point prevalence was estimated to range from 6.7 to 402 per million.
Typical clinical features of PBC are fatigue, pruritus and Sicca-syndrome. However, nowadays at diagnosis, the majority of patients are asymptomatic and present for other reasons, e.g. for workup of elevated serum levels of AP or cholesterol. A diagnosis of PBC is made “with confidence” when biochemical markers of cholestasis, particularly alkaline phosphatase, are elevated persistently for more than 6 months in the presence of antibodies against mitochondria and in the absence of an alternative explanation.
PBC-related antibodies against mitochondria react with subunits of the 2-oxoacid-dehydrogenase complex (2-OADC) and, in most cases, recognize the E2-subunit of pyruvate dehydrogenase (PDH-E2). Individuals, who are positive for these so-called anti-M2 antibodies, even if they have no signs of cholestasis and/or liver inflammation, are very likely to develop PBC.
Anti-M2 antibodies are present in about 95% of PBC-patients.

Preparation of Patient: No special preparation.

Location	**Eurofins Biomnis Blackthorn Road**
Accredited	Yes
Method	Fluoroenzyme immunoassay (Phadia 250) SOP CC149
Sample Requirements	Tube Type: Serum (Gold and red cap); Temperature: + 4ºC
Turnaround Time	4 working days
Stability	Whole blood: unknown. Separated: RT 8 hours; 14 Days @ + 4ºC; months at - 20 ºC Source: Phadia IFU
Units - Reference Ranges and Source	Negative: < 4.0 IU/mL Equivocal: 4.0 IU/mL to 6.0 IU/mL Positive: > 6.0 IU/mL Source: Phadia IFU

ANTI-NUCLEAR ANTIBODIES, DS-DNA AND ENA ANTIBODIES

The determination of antinuclear antibodies (ANA) is of central importance for the clinical diagnosis of connective tissue diseases, which are systemic inflammatory diseases with a chronic course of disease. Connective tissue diseases exhibit overlapping symptomatic features that render an accurate diagnosis difficult.

The Phadia EliA ANA CTD screen wells are coated with human recombinant U1RNP (RNP70, A, C),SS-A/Ro (60 kDa, 52 kDa), SS-B/La, Centromere B, Scl-70, Jo-1, Fibrillarin, RNA Pol III, Rib-P, PM-Scl, PCNA, Mi-2 proteins, Sm proteins and native purified DNA. This ANA screen is more specific than traditional IF ANA methods, which show a high false-positive rate.

A positive ANA CTD screen requires the specific quantification of the antibodies listed above: ds-DNA and the individual anti-extractable nuclear antigen (ENA) antibodies.

For the diagnosis of systemic lupus erythematosus (SLE), dsDNA antibodies are considered to be a highly specific marker representing one of the diagnostic criteria for SLE (ACR criteria). More than 90 % of sera from patients with active SLE contain dsDNA antibodies. Additionally, the determination of dsDNA antibodies is a tool to monitor the clinical course of a defined SLE patient, because a clear-cut relationship exists between anti-dsDNA titre and disease activity, in particular renal involvement. Sm antibodies offer a highly specific, but comparatively insensitive, clinical marker for SLE. Indeed, their presence constitutes one of the revised ACR criteria for diagnosis, even though their overall prevalence ranges from 20 % to 30 % in SLE. U1-snRNP antibodies typically appear in both SLE and mixed connective tissue disease (MCTD, Sharp Syndrome).

In MCTD, the presence of U1-snRNP antibodies is required for diagnosis, whereas they occur in only 30 to 40 % of SLE patients. Detection of SS-A/Ro antibodies is of interest and significance for the clinical diagnosis of SLE (prevalence 40-50%) and Sjögren's syndrome (prevalence 60-75% for primary Sjögren's syndrome). They have been reported to occur in tight association with certain disease subsets, such as subacute cutaneous LE, neonatal lupus erythematosus or vasculitis in Sjögren's syndrome. As anti-SS-A/Ro may be the only antibody present in many patients with SLE or Sjögren's syndrome, failure to measure anti-SS-A/Ro leaves a diagnostic void which cannot be filled by other tests. SS-B/La antibodies are the serological hallmark of Sjögren's syndrome but a small proportion of patients remains anti-SS-B/La negative. La antibodies are found in 6-15 % of sera from SLE patients. Here, they are associated with a lower prevalence of dsDNA antibodies and renal disease.

Although a strong association of neonatal lupus erythematosus (NLE) with Ro antibodies was recognized first, the majority of mothers with babies with NLE are now known to have La antibodies as well.

CENP antibodies are found in 70-90 % of patients with CREST Syndrome, a limited form of scleroderma with a comparatively favourable prognosis. However, they may also occur in Raynaud's phenomenon and primary biliary cirrhosis (about 10-20 %).

Antibodies against Scl-70 are characteristic and specific for scleroderma (particularly the diffuse form; frequency up to 70%). Jo-1 antibodies can be found as markers in dermatomyositis/polymyositis (prevalence of about 25%), but also in patients with polymyositis overlap syndrome. They are associated with interstitial pneumonitis (in the context of myositis) and occur in a far smaller proportion of children with myositis than of adults. Patients with Jo-1 antibodies tend to have a severe form of the disease with a tendency to relapse and a poorer prognosis. Fibrillarin antibodies produce a nucleolar pattern in immunofluorescence. They occur in less than 15% of patients with scleroderma and seem to be associated with internal organ involvement including pulmonary hypertension, myositis, and renal disease. The presence of fibrillarin antibodies in diseases other than scleroderma and their clinical relevance requires further investigation. RNA polymerase III antibodies are highly specific for scleroderma and are here more frequent in patients with diffuse cutaneous scleroderma than in those with limited cutaneous

scleroderma. Among patients with diffuse cutaneous involvement, RNA polymerase III antibody was the most common antibody detected (35-45%).

Antibodies against Ribosomal P proteins react with the specific ribosomal proteins (P0, P1 and P2). These autoantibodies occur in SLE during active disease and are associated with neuropsychiatric, renal and hepatic involvement. They are found in 23% of SLE patients.
Autoantibodies to the polymyositis/scleroderma (PM-Scl) complex were the first antinucleolar antibodies identified in systemic sclerosis. Anti-PM-Scl are associated with a specific form of scleroderma; indeed, only 2% of the patient population with scleroderma, but 24% of the patients with myositis-scleroderma overlap syndrome produce these antibodies. They correlate with a benign course of disease and a positive response to steroid therapy. Antibodies against PCNA (proliferating cell nuclear antigen) occur in 2 to 10% of SLE patients but seem to be not very specific for SLE as it was found in 12.3% of hepatitis B and 18.7% of hepatitis C patients, respectively.
Autoantibodies targeting the Mi-2 nuclear antigen represent one of the serologic hallmarks of polymyositis/dermatomyositis, with a diagnostic sensitivity and specificity of approximately 4-18% and 98-100%, respectively. They are strongly associated with dermatomyositis with a frequency of up to 31%.

Preparation of Patient: No special preparation.

Location	**Eurofins Biomnis Blackthorn Road**
Accredited	Yes
Reflex testing	The Phadia EliA ANA CTD wells are coated with human recombinant U1RNP (RNP70, A, C),SS-A/Ro (60 kDa, 52 kDa), SS-B/La, Centromere B, Scl-70, Jo-1, Fibrillarin, RNA Pol III, Rib-P, PM-Scl, PCNA, Mi-2 proteins, Sm proteins and native purified DNA. A positive ANA CTD screen will lead to analysis of specific ds-DNA antibodies and ENA antibody typing.
Method	Fluoroenzyme immunoassay (Phadia 250) SOPs CC117, CC118 and CC119 – CC125
Sample Requirements	Tube Type: Serum (Gold and red cap); Temperature: + 4ºC
Turnaround Time	4 working days
Stability	Whole blood: unknown Separated: RT unknown; 14 days @ + 4ºC Source: Phadia IFU
Units - Reference Ranges and Source	**ANA:** ratio less than 0.7 **ds-DNA:** less than 10 IU/mL **Anti-Sm, anti-SSA/RO, anti-SSB/La, anti-Scl-70, anti-Jo-1 and anti-centromere Antibodies:** each less than 7 EliA U/mL. **Anti-U1 RNP:** less than 5 EliA U/mL. Source: Phadia IFUs.

ANTI-MPO AND ANTI-PR3 ANTIBODIES	
First described in patients with necrotizing crescentic glomerulonephritis (NCGN) without immune deposits (pauci-immune), the clinical spectrum associated with anti-MPO includes also NCGN associated with systemic vasculitis, either Granulomatosis with polyangiitis (GPA) or a microscopic polyangiitis (MPA). Indeed, anti-MPO are detectable in 65% of patients with idiopathic NCGN, 45% of patients with MPA and 20% to 30% of patients with GPA. Additionally, anti-MPO are present in some 60% of patients with the eosinophilic granulomatosis with polyangiitis (EGPA). Antibodies to PR3 are highly sensitive (81%) and specific (97%) for GPA. The sensitivity is dependent on the phase and on the activity of the disease. Despite the strong association between PR3 antibodies and GPA, there is a small percentage of patients with microscopic polyangiitis and about 30% of EGPA patients who are PR3 antibodies positive. PR3 antibodies may also occur in 20% to 30% of patients with necrotizing glomerulonephritis with no obvious extrarenal manifestations of small vessel vasculitis.	
Preparation of Patient: There is no special physical preparation required.	
Location	**Eurofins Biomnis Blackthorn Road**
Accredited	Yes
Method	Chemistry – Fluoroenzyme immunoassay. SOP: CC127 (MPO) and CC126 (PR3).
Sample Requirements	Tube Type: Serum (Red and gold cap); Lipemic and haemolysed samples are unsuitable. Temperature: + 4ºC
Turnaround Time	4 working days
Stability	Whole blood: unknown Separated: RT unknown; 14 days @ + 4ºC Source: Phadia IFU
Units - Units - Reference Ranges and Source	Anti-MPO: less than 3.5 IU/mL (<3.5 = negative; 3.5-5.0 = equivocal; >5.0 = positive. Anti-PR3: less than 2.0 IU/mL (<2.0 = negative; 2.0-3.0 = equivocal; >3.0 = positive). Source: Phadia IFUs

ANTI-PARIETAL CELL ANTIBODIES	
Pernicious anaemia (also known as Biermer's disease) is an autoimmune atrophic gastritis, predominantly of the fundus, and is responsible for a deficiency in vitamin B12 (cobalamin) due to its malabsorption. Its prevalence is 0.1% in the general population and 1.9% in subjects over the age of 60 years. Pernicious anaemia represents 20%–50% of the causes of vitamin B12 deficiency in adults. Parietal cell antibodies are found in about 90% of Caucasian patients with pernicious anaemia. In the later stages of the disease, the incidence of these antibodies decreases due to the progression of autoimmune gastritis and a loss of parietal cell mass, as a result of the decrease in antigenic rate. In recent studies, an average incidence of 55% of anti-parietal cell antibodies was documented in patients with advanced pernicious anaemia. Parietal cell antibodies are present in 7.8–19.5% of the general healthy adult population. A not fully explained question is whether parietal cell antibodies presence is related to Helicobacter pylori infection. Anti-parietal cell antibodies are found in up to 20.7% of these patients.	
Preparation of Patient: No special preparation.	
Location	**Eurofins Biomnis Blackthorn Road**
Accredited	Yes
Method	Fluoroenzyme immunoassay (Phadia 250) SOP CC156
Sample Requirements	Tube Type: Serum (Gold and red cap); Temperature: + 4ºC
Turnaround Time	4 working days
Stability	Whole blood: unknown. Separated: RT 8 hours; 14 Days @ + 4ºC Source: Phadia IFU
Units - Reference Ranges and Source	U/mL Negative: less than 7 Equivocal: 7 - 10 Positive: greater than 10 Source: Phadia IFU

CALPROTECTIN (FAECES)

Calprotectin is a calcium- and zinc-binding protein which is predominantly present in the cytoplasm of cells involved in pathogen defence, such as neutrophil granulocytes, monocytes and macrophages. In neutrophil granulocytes it accounts for as much as 60% of the cytosolic protein. In intestinal inflammation neutrophil granulocytes migrate through the intestinal wall into the intestinal lumen, which leads to an elevated calprotectin level in the stool. The level of faecal calprotectin correlates directly with the number of neutrophil granulocytes in the intestinal lumen and is thus specifically elevated in inflammatory bowel diseases (IBD), such as Crohn's disease and ulcerative colitis, and to a much smaller extent in other entities such as neoplasia and polyps. Faecal calprotectin measurement is an easy, non-invasive first line test which clearly differentiates IBD from IBS (irritable bowel syndrome) and other functional disorders. It has been shown to be the most sensitive and most specific test for this discrimination; clearly outperforming blood tests such as CRP or ESR. Faecal calprotectin correlates with disease activity and is able to predict relapses in IBD. This makes faecal calprotectin useful for both diagnosis and monitoring of IBD patients.

Preparation of Patient: There is no special physical preparation for calprotectin analysis.

Location	**Eurofins Biomnis Blackthorn Road**
Accredited	Yes
Method	Fluoroenzyme immunoassay on Phadia 250 - Phadia EliA 2nd Generation CC139
Sample Requirements	At least 20g stool sample. Temperature: + 4ºC or room temp or frozen
Turnaround Time	2 weeks maximum (batched).
Stability	Stool: 8 days RT or at 2°C to 8°C. The stool extract can be stored at room temperature for a max 3 days, at +4°C for 7 days and at <=20°C for 3 months. Source: Phadia IFU
Units - Reference Ranges and Source	<50 ug/g: Negative. Calprotectin level not suggestive of organic pathology. 50-100 ug/g: Gray Zone. Organic pathology cannot be excluded. A repeat sample in 4 to 6 weeks is suggested. >100 ug/g: Positive. Calprotectin level is consistent with organic pathology. Sources: 1. Phadia Calprotectin 2 IFU 2. "EliA Calprotectin Assay Background Technical & Clinical 15.11.2012", presentation from ThermoFisher. 3. NICE guideline DG11.

ANTI-TRANSGLUTAMINASE IGA ANTIBODIES	
Celiac disease is a life-long condition in which ingestion of gluten, the water insoluble wheat gliadin and the prolamins in rye and barley, leads to chronic inflammation and damage of the small intestinal mucosa. Tissue transglutaminase has been identified as the major autoantigen in celiac disease. IgA antibodies against tTG are highly disease specific serological markers for celiac disease and dermatitis herpetiformis. tTG IgG antibodies are less specific for these diseases but are helpful markers in patients with IgA deficiency.	
Preparation of Patient: No special preparation.	
Location	**Eurofins Biomnis Blackthorn Road**
Accredited	Yes
Method	Fluoroenzyme immunoassay (Phadia 250) SOP CC106
Sample Requirements	Tube Type: Serum (Gold and red cap); Temperature: + 4ºC
Turnaround Time	4 working days
Stability	Whole blood: unknown. Separated: RT unknown. 14 days @ + 4ºC Source: Phadia IFU
Units - Reference Ranges and Source	Negative: less than 7 U/mL Weak Positive: 7 - 10 U/mL Positive: greater than 10 U/mL Source: Phadia IFU

INFLIXIMAB DRUG LEVEL

Tumor Necrosis Factor alpha (TNFα) belongs to the proinflammatory cytokines which promote and sustain inflammatory reactions. It is produced by macrophages and T cells and plays a central role in both acute and chronic inflammations. Consequently, chronic inflammatory diseases like Crohn's disease, ulcerative colitis, rheumatoid arthritis, or psoriasis are increasingly being treated with antibodies against TNFα, which target directly the underlying inflammatory process.
During recent years, reports of an association between anti-drug antibodies (ADAs) and adverse effects of treatments both in CD and UC have surfaced. Development of ADAs is usually considered to be associated to immunogenicity of monoclonal antibodies. The clinical efficacy of an anti-TNFα therapy usually correlates with the trough level of the therapeutic antibody, or the drug level just before the next application of the anti-TNFα antibody. Several factors influence the trough level, among them dosage and frequency of anti-TNFα blocker infusion, disease activity, individual pharmacokinetics and immune reaction (formation of anti-drug antibodies, ADA)
The IDKmonitor® infliximab drug level ELISA for the determination of the drug level of infliximab (eg REMICADE®) measures quantitatively free infliximab in serum. In combination with the detection of ADA against infliximab, the IDKmonitor®infliximab drug level ELISA is an opportunity for the treating physician to monitor and optimize treatment early on.

Preparation of Patient: No special preparation.

Location	**Eurofins Biomnis Three Rock Road**
Accredited	No
Method	ELISA SOP CC179
Sample Requirements	Tube Type: Serum (Gold and red cap); Temperature: + 4ºC or spun, seperated and frozen at -20°C
Turnaround Time	10 working days
Stability	Whole blood: unknown. Separated: RT 7 days. Longer @ -20ºC
Units - Reference Ranges and Source	ug/mL A detectable anti-TNF trough level (immediately pre-dose) is associated with a higher rate of clinical and endoscopic remission. IDK Infliximab drug level IFU

INFLIXIMAB TOTAL ANTI-DRUG ANTIBODY

Tumor Necrosis Factor alpha (TNFα) belongs to the proinflammatory cytokines which promote and sustain inflammatory reactions. It is produced by macrophages and T cells and plays a central role in both acute and chronic inflammations. Consequently, chronic inflammatory diseases like Crohn's disease, ulcerative colitis, rheumatoid arthritis, or psoriasis are increasingly being treated with antibodies against TNFα, which target directly the underlying inflammatory process.
During recent years, reports of an association between anti-drug antibodies (ADAs) and adverse effects of treatments both in CD and UC have surfaced. Development of ADAs is usually considered to be associated to immunogenicity of monoclonal antibodies. The clinical efficacy of an anti-TNFα therapy usually correlates with the trough level of the therapeutic antibody, or the drug level just before the next application of the anti-TNFα antibody. Several factors influence the trough level, among them dosage and frequency of anti-TNFα blocker infusion, disease activity, individual pharmacokinetics and immune reaction (formation of anti-drug antibodies, ADA). It is thought that ADA functionally neutralize the therapeutic antibodies or induce their rapid elimination. Consequences of ADA formation can be therapy failure and allergic reactions during anti-TNFα antibody application.
The IDKmonitor® Infliximab total ADA ELISA for the detection of total antibodies against infliximab measures free and bound antibodies against infliximab. This assay allows a reliable determination of ADA even in the presence of infliximab; therefore, it is ideal for therapy monitoring when a measureable infliximab concentration is expected, for example shortly after last infusion. In combination with the drug level determination, the IDKmonitor® Infliximab total ADA ELISA is an opportunity for the treating physician to monitor and optimize treatment early on.

Preparation of Patient: No special preparation.

Location	**Eurofins Biomnis Three Rock Road**
Accredited	No
Method	ELISA SOP 181
Sample Requirements	Tube Type: Serum (Gold and red cap); Temperature: + 4ºC or spun, seperated and frozen at -20°C
Turnaround Time	10 working days
Stability	Whole blood: unknown. Separated: RT 7 days. Longer @ -20ºC
Units - Reference Ranges and Source	AU/mL A total Infliximab Antibody concentration >/= 10 AU/mL is positive. This assay measures TOTAL infliximab antibody concentration, and is not sensitive to the presence of drug. IDK total Infliximab Antibody level IFU

THYROID-STIMULATING HORMONE RECEPTOR ANTIBODIES (TRAB)	
TRAb is a third generation assay to detect anti-TSH receptor antibody using the human thyroid-stimulating monoclonal antibody M22. The M22 antibody acridinium-labeled conjugate competes with TRAb in the specimen for the bound human TSH receptor on the microparticle. Elevations of these thyrotropin receptor antibodies demonstrated high clinical sensitivity and specificity for Graves' disease when used as an aid in the differential diagnosis and etiology of hyperthyroidism. These antibodies can be classified as stimulating, inhibitory, or neutral; and the stimulating type of antibody is not subject to the typical negative feedback of elevated thyroid hormone levels. This results in continuous stimulation of the thyroid leading to the typical thyrotoxic symptoms of Graves' hyperthyroidism.	
Preparation of Patient: No special preparation.	
Location	**Eurofins Biomnis Blackthorn Road**
Accredited	Yes
Method	Abbott Alinity. CMIA. BKICC223
Sample Requirements	Tube Type: Serum (Gold and red cap) Temperature: + 4ºC
Turnaround Time	4 working days
Stability	Serum: RT 24 hours, 2-8ºC 3 days. 30 days @ -20ºC Source: Abbott IFU
Units - Reference Ranges and Source	< 3.1 IU/L: Negative ≥ 3.1 IU/L: Positive Source: Abbott IFU