

WVT Immunology Handbook

1. Contact Details

General Enquiries:

Immunology telephone number: 01432 364039

Immunology email: immunologylab@wvt.nhs.uk

Clinical Enquiries:

Please email all enquiries to the immunologylab@wvt.nhs.uk. This will allow staff to effectively triage your enquiry.

Urgent requests: Please ensure that the laboratory has had prior warning for any urgent requests so they can be appropriately managed when they arrive.

Sample Volume: Please see *Section 5* for a table of the required volumes for each test.

2. Assay Processing

As a general guide please see the frequency that each assay is run in the table below.

Phadia 250 Tests

Test	Frequency	Method
dsDNA	Daily	ELiA
ENA Screen		
ENA Profile		
MPO & PR3		
Anti-GBM		
Cardiolipin IgG		
Beta-2-glycoprotein IgG		
Intrinsic Factor		
CCP		
Cardiolipin IgM	2x per week	ELiA
Beta-2-glycoprotein IgM		
In house Allergens - Specific IgE	2x per week	ImmunoCAP
Tryptase		

IFT Slides

Test	Frequency
ANA	Daily
ANCA	
Liver	
GPC	
Endomysial IgA	2x per week

Immunoblots

Test	Frequency
Myositis	Once a week
Systemic Sclerosis	

3. In-house test repertoire

Test Additional details Details of reflex testing	Sample requirements Reference range
Anti-Nuclear Antibodies Screening dilution 1:100 dsDNA and/or ENA screen is added onto positive results showing relevant patterns.	Serum – minimum volume 150µl ANA pattern and strength is reported. <i>Negative, Weak Positive, Moderate Positive, Strong Positive.</i>
ANCA Screening dilution 1:10	Serum – minimum volume 150µl ANCA pattern and strength is reported. <i>Pattern: P-ANCA, C-ANCA, atypical</i> <i>Strength: Negative, Weak Positive, Moderate Positive, Strong Positive.</i>
Beta-2-glycoprotein IgG Positive samples will have Cardiolipin antibodies measured.	Serum – minimum volume 200µl Haemolysed and/or lipemic samples unsuitable <i>Negative = <7</i> <i>Equivocal = 7-10</i> <i>Positive = >10 U/ml</i>
Beta-2-glycoprotein IgM Positive samples will have Cardiolipin antibodies measured.	Serum – minimum volume 200µl Haemolysed and/or lipemic samples unsuitable <i>Negative = <7</i> <i>Equivocal = 7-10</i> <i>Positive = >10 U/ml</i>
Cardiolipin IgG Positive samples will have beta-2-glycoprotein antibodies measured.	Serum – minimum volume 200µl Haemolysed and/or lipemic samples unsuitable <i>Negative = <10</i> <i>Equivocal = 10-40</i> <i>Positive = >40 U/ml</i>
Cardiolipin IgM Positive samples will have beta-2-glycoprotein antibodies measured.	Serum – minimum volume 200µl Haemolysed and/or lipemic samples unsuitable <i>Negative = <10</i> <i>Equivocal = 10-40</i> <i>Positive = >40 U/ml</i>
CCP antibodies	Serum – minimum volume 200µl Haemolysed and/or lipemic samples unsuitable <i>Negative = <7</i> <i>Equivocal = 7-10</i> <i>Positive = >10 U/ml</i>

dsDNA dsDNA antibodies are automatically added onto a positive ANA screen with the following patterns: AC-1, AC-2, AC-4/5, AC-19	Serum – minimum volume 200µl Haemolysed and/or lipemic samples unsuitable <i>Negative = <10</i> <i>Equivocal = 10-15</i> <i>Positive = >15 IU/ml</i>
ENA screen An ENA screen is automatically added onto a positive ANA screen with the following patterns: AC-1, AC-2, AC-3, AC-4/5, AC-19, AC-20, AC-29 A positive or equivocal ENA screens will automatically have the ENA profiles added.	Serum – minimum volume 200µl Haemolysed and/or lipemic samples unsuitable <i>Reported as Negative, Equivocal or Positive</i>
ENA Profiles Contains: Centromere, Ro, La, Sm, RNP, Scl70 and Jo-1.	Serum – minimum volume 200µl Haemolysed and/or lipemic samples unsuitable Centromere, Ro, La, Sm, Scl70 and Jo-1: <i>Negative = <7</i> <i>Equivocal = 7-10</i> <i>Positive = >10 U/ml</i> RNP: <i>Negative = <5</i> <i>Equivocal = 5-10</i> <i>Positive = >10 U/ml</i>
Endomysial IgA antibody Screening dilution 1:10	Serum – minimum volume 150µl <i>Reported as: Negative or Positive</i>
GBM	Serum – minimum volume 200µl Haemolysed and/or lipemic samples unsuitable <i>Negative = <7</i> <i>Equivocal = 7-10</i> <i>Positive = >10 U/ml</i>
Gastric parietal cell antibody	Serum – minimum volume 150µl <i>Reported as: Negative, Weak Positive, Moderate Positive, Strong Positive.</i>
Intrinsic Factor	Serum – minimum volume 200µl Haemolysed and/or lipemic samples unsuitable <i>Negative = <7</i> <i>Equivocal = 7-10</i> <i>Positive = >10 U/ml</i>

Liver autoantibody screen 1:40 screening dilution for adults 1:20 screening dilution for children NOTE: the ANA reported under the liver autoantibodies screen should not be interpreted as an anti-nuclear antibody screen. An ANA should be requested if clinically indicated and ANA clarification is required.	Serum – minimum volume 150µl 4 antibodies reported: Mitochondrial Smooth Muscle LKM Anti-nuclear antibody screen (ANAS) <i>Mitochondrial, smooth muscle and LKM antibodies reported as: Negative, Weak Positive, Moderate Positive or Strong Positive.</i> <i>ANAS reported as Negative or Positive.</i>
MPO and PR3 ANCA IFT will be added to new MPO and PR3 positive samples.	Serum – minimum volume 200µl Haemolysed and/or lipemic samples unsuitable MPO Negative = <3.5 Equivocal = 3.5-5 Positive = >5 IU/ml PR3 Negative = <2 Equivocal = 2-3 Positive = >3 IU/ml
Myositis Immunoblot Contains: anti-OJ, EJ, PL-12, PL-7, SRP, Jo-1, Pm-Scl75, Pm-Scl100, Ku, SAE, NXP-2, MDA5, TIF-1-gamma, Mi-2 and Ro-52.	Serum – minimum volume 50µl <i>Strength: Negative, Weak Positive, Moderate Positive, Strong Positive.</i>
Systemic Sclerosis Immunoblot Contains: anti-Scl-70, CENP A, CENP B, RNAP III, U3-RNP, NOR90, Th/To, Pm-Scl100, Pm-Scl75, Ku, PDGFR and Ro-52.	Serum – minimum volume 50µl <i>Strength: Negative, Weak Positive, Moderate Positive, Strong Positive.</i>
Tryptase	Serum – minimum volume 200µl <i>Reference range: 2-11µg/L</i>
Specific IgE Various tests in house – see section 6.	Serum – minimum volume 150µl + 50µl per allergen <i>Grade 1 = 0.35 - 0.70 kua/L. Grade 2 = 0.70 - 3.5 kua/L. Grade 3 = 3.5 - 17.5 kua/L. Grade 4 = 17.5 - 50 kua/L. Grade 5 = 50 - 100 kua/L. Grade 6 = >100 kua/L</i>

Specific IgE – mixes Individual allergens will be added when food, nut and seafood mixes are positive. Various tests in house – see section 6.	Serum – minimum volume 150µl + 50µl per allergen Grade 1 = P+ Grade 2 = P++ Grade 3 = P+++ Grade 4 = P++++ Grade 5 = P+++++
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4. Reflex Testing & Incidental Findings

Reflex Testing

Anti-Nuclear Antibodies IFT

dsDNA antibodies and/or ENA antibodies are reflexed depending on the pattern seen.

Pattern	Follow on tests
AC-1 homogenous	DSDN, ENAX
AC-2 dense fine speckled	DSDN, ENAX
AC-3 centromere	ENAX
AC-4 nuclear fine speckled	DSDN, ENAX
AC-5 nuclear coarse speckled	DSDN, ENAX
AC-45 nuclear speckled	DSDN, ENAX
AC-19 cytoplasmic dense fine speckled	DSDN, ENAX
AC-20 cytoplasmic fine speckled	ENAX
AC-29 DNA topoisomerase-like	ENAX

Reflex testing for ANAs. DSDN = dsDNA antibodies, ENAX = ENA screen

A systemic sclerosis blot will be added to AC-3 patterns where the following ENA screen and/or centromere B antigen is negative.

dsDNA

DNA by crithidia will be added to any positive or equivocal dsDNA where the ANA is negative or does not have a corresponding pattern (AC-1, AC-2 or AC-4/5).

Myositis/Systemic Sclerosis Immunoblots

An ANA slide will be added to any positive immunoblots which don't already have an ANA or haven't had an ANA results within 3 months.

Incidental Findings

GPC antibodies and Liver autoantibodies

The same substrate is used for determination of GPC antibodies and liver autoantibodies, therefore it can be common to find incidental findings on these requests.

Where incidental strong GPC antibodies are seen on the liver autoantibodies request, the GPC test will be added to the sample to allow this result to be reported. A comment will also be added to the liver autoantibodies screen to clarify that GPC antibodies were seen.

Where incidental liver antibodies are seen on a GPC antibodies request, the liver autoantibodies test will be added to the sample to allow this result to be reported. A comment will also be added to the GPC screen to clarify that liver antibodies were seen.

5. Sample Volumes – in-house and referral tests

Allergens & IgE

Test	Test Code	Minimum Volume required
Specific IgE	Various	150µl + 50µl per allergen
Total IgE	IGE	150µl

IF Sprinter – Slides

Test	Test Code	Minimum Volume required
ANA	ANA	150µl
Liver autoantibodies	LIVAA	150µl
GPC antibodies	PAAAX	150µl
Endomysial IgA	ENDOM	150µl

Phadia

Dead volume is 150µl and then an additional 50µl per Phadia test as a minimum.

Test	Test Code	Minimum Volume required
dsDNA	DSDN	200µl
ENA screen	ENAX	200µl
ENA profile	ENAX1	200µl
MPO & PR3	MPOPR3	200µl
Cardiolipin IgG	CARD	200µl
Cardiolipin IgM	CARDM	200µl
β-Glycoprotein IgG	B2GG	200µl
β-Glycoprotein IgM	B2GM	200µl
Tryptase	TRYPX	200µl
Intrinsic Factor	IFAX	200µl
GBM	GBM	200µl
CCP	CCPA1	200µl

EuroBlot Master – Immunoblots

Test	Test Code	Minimum Volume required
Systemic Sclerosis Profile	SSIMMX	100µl
Myositis Immunoblot	MYOIMM	100µl

Referral Laboratory Tests

Test	Test Code	Location	TAT	Minimum Volume required
ACR antibody	ACRAX	Oxford Immunology	4 weeks	500µl
Adrenal abs	ADREX	Birmingham Med Immunology	4 weeks	500µl
AMPA1/AMPA2/GABA abs	GMREC	Oxford Immunology	4 weeks	1ml
CSF AMPA1/AMPA2/GABA Abs	CFGMRX	Oxford Immunology	4 weeks	1ml
Aquaporin 4 abs	AQU4X	Oxford Immunology	4 weeks	500µl
Basal Ganglia Abs	ABAGX	Queen's Square Immunology	4 weeks	500µl
C1-esterase inhibitor	C1ESTX	Synnovis	4 weeks	500µl
Voltage Gated Potassium Abs <i>VGKC only completed if LGI1 and CASPR2 positive</i>	VGKC	Oxford Immunology	4 weeks	500µl
CSF Voltage Gated Potassium Abs <i>VGKC only completed if LGI1 and CASPR2 positive</i>	CFVGKX	Oxford Immunology	4 weeks	500µl
NMDA receptor antibody	NMDARX	Oxford Immunology	4 weeks	500µl
CSF NMDA Abs	CFNMDX	Oxford Immunology	4 weeks	500µl
Paraneoplastic Antibodies <i>(Yo, Hu, Ri, CV-2, Ma2, Amphyphisin, GAD, SOX1, Zic4, TrDNA)</i>	BRAINX	Birmingham Med Immunology	4 weeks	500µl
CSF Paraneoplastic Abs <i>(Yo, Hu, Ri, CV-2, Ma2, Amphyphisin, GAD, SOX1, Zic4, TrDNA)</i>	CFBRNX	Birmingham Med Immunology	4 weeks	500µl
Voltage gated Calcium channels	VGCX	Oxford Immunology	4 weeks	500µl
CSF Voltage Gated Calcium Abs	CFVGCX	Oxford Immunology	4 weeks	500µl
dsDNA by Crithidia	DNAAX	Birmingham Med Immunology	3 weeks	500µl

Skin antibodies BM and ICS	SKINX	Bristol	3 weeks	1ml
Endomysial (IgG) Abs	ENDOGX	Synnovis	2 weeks	500µl
Diabetes antibodies <i>anti-GAD, anti-IA2 and anti-zinc transporter 8 antibodies</i>	GADAX	Synnovis	4 weeks	500µl
Glycolipid antibodies <i>(GD1a IgG and IgM GD1b IgG and IgM GM1 IgG and IgM GM2 IgG and IgM GQ1b IgG and IgM)</i>	AGAX	Glasgow Immunology	4 weeks	1ml
Glycolipid extended panel <i>(GM3 IgG and IgM GD3 IgG and IgM GA1 IgG and IgM GT1b IgG and IgM Sulfatide antibodies IgG and IgM)</i>	AGAX1	Queen's Square Immunology	4 weeks	1ml
Histone antibodies	HISTX	Sheffield	3 weeks	500µl
HMG-CoA Abs	HMGCOX	Oxford Immunology	3 weeks	500µl
IgG Subclasses	IGGSC	Bristol	2 weeks	500µl
Insulin Antibodies	INSAAX	Sheffield	2 weeks	500µl
Islet cell Abs	ISLEX	Birmingham Med Immunology	3 weeks	500µl
Liver Line Blot <i>(M2, LKM-1, LC-1, SLA/LP)</i>	EXLLBX	Bristol	2 weeks	1ml
Extended Liver Line Blot <i>(M2, LKM-1, LC-1, GP210, SP100, F-actin, SLA/LP)</i>	EXLLBX	Bristol	3 weeks	1ml
MAG Ab (Screen)	MAGIX	Bristol	3 weeks	500µl
Myelin Oligodendrocyte GP Abs	AMOGX	Oxford Immunology	4 weeks	1ml
Anti-muscle specific kinase antibodies	MSKAX	Oxford Immunology	4 weeks	500µl
Phospholipase A2 Receptor Ab	PARAB	Birmingham Med Immunology	3 weeks	500µl
Specific IgE allergens – <i>various</i>	Various	Sheffield or New Cross	2 weeks	150µl + 50µl per allergen
TTG-IgG abs (Quant)	TTGIGX	Sheffield	2 weeks	500µl

6. In-House Allergens

Allergen			
Almond	Egg white	Peach	Tree mix (late)
Aspergillus	Egg yolk	Peanut	Tuna
Bee venom	Feather (poultry) mix	Pecan	Walnut
Birch tree	Food mix	Penicilloyl G	Wasp Venom
Blue mussel	Grain mix	Penicilloyl V	Weed mix
Brazil nut	Grass (timothy)	Pine nut	Wheat
Cashew	Hazelnut	Pistachio	
Cat dander	Horse dander	Rabbit epithelium	
Cereal mix	House dust mite	Salmon	
Cheese	Kiwi	Sea food mix	
Chestnut	Latex	Sesame seed	
Chicken meat	Lobster	Shrimp	
Chickpea	Macadamia nut	Soya	
Coconut	Milk	Tomato	
Crab	Mould mix	Tree mix	
Dog dander	Nut mix	Tree mix (early)	

Allergen Components			
Ana o3 cashew nut component	Ara h1 peanut component	Ara h8 peanut component	Cor a14 hazelnut component
Gal d 2 egg component	Ara h2 peanut component	Ara h9 peanut component	Cor a1 hazelnut component
Ovomucoid egg component	Ara h3 peanut component	Bet v1 PR-10 birch component	Cor a8 hazelnut component
Peach Pru p3 LTP	Ara h6 peanut component	Bet v2 birch component	Cor a9 hazelnut component

All other specific IgEs are sent to either Sheffield or New Cross Hospital for analysis.

Reflex testing on allergens:

When a food mix is positive, the individual allergen contained in that mix will be tested.

Allergen Mix	Individual allergens added
Sea food mix	Fish (Cod), Shrimp, Blue mussel, Tuna, and Salmon
Food mix	Egg white, Milk, Fish, Wheat, Peanut, Soybean
Nut mix	Peanut, Hazelnut, Brazil nut, Almond, Coconut