

# Ampath Desk Reference: Guide to Laboratory Tests

PATHOLOGISTS · PATHOLOGISTES

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Your consulting pathologists



# **Ampath Desk Reference:**

## Guide to laboratory tests

Edition 2

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# CONTENTS

<b>Abbreviations and Symbols key .....</b>	xii
<b>General notes .....</b>	xviii
<b>BIOCHEMISTRY .....</b>	1
<b>Electrolytes and renal function .....</b>	1
<b>Diagnosis of chronic kidney disease (CKD) .....</b>	3
Classification of CKD using the CGA system.....	3
Assignment of the cause of CKD .....	3
Recommendations regarding GFR estimation .....	4
Albuminuria as a marker of kidney damage .....	7
Areas of controversy .....	8
<b>Calcium, magnesium, phosphate, vitamin D and PTH.....</b>	9
<b>Liver function tests .....</b>	10
Interpretation of unconjugated hyperbilirubinaemia .....	13
Interpretation of liver profiles .....	13
Evaluation of isolated mild chronic elevation of serum ALT and AST .....	14
<b>Pancreas.....</b>	15
<b>Inflammatory markers .....</b>	16
C-reactive protein (CRP) .....	16
Procalcitonin .....	16
<b>Cardiac and skeletal muscle markers.....</b>	17
Homocysteine interpretation.....	19
NT-ProBNP interpretation .....	19
Diagnosis and classification of Acute Coronary Syndrome in the emergency department setting .....	20
List of contemporary sensitive troponin assays with relevant cut-off values (ng/l).....	22
Proposed algorithm for work-up of suspected acute coronary syndrome (ACS) for hsTroponin T.....	23
Proposed algorithm for work-up of suspected acute coronary syndrome (ACS) for Troponin I.....	24
Causes of cardiac troponin elevation (other than acute coronary syndrome).....	25

<b>Carbohydrate metabolism .....</b>	<b>26</b>
Criteria for diagnosing diabetes and categories of intermediate hyperglycaemia.....	27
WHO recommendations (2013 update) for classification of hyperglycaemia during pregnancy .....	28
Factors influencing HbA1c measurement .....	29
HbA1C targets for glycaemic control .....	29
Diagnostic criteria for insulin resistance syndrome / metabolic syndrome .....	30
<b>Lipid metabolism .....</b>	<b>31</b>
CVD Risk Stratification and Cholesterol Targets.....	31
Framingham 10 year risk assessment score.....	33
Laboratory tests for dyslipidaemia .....	37
Interpretation of abnormal lipid profiles .....	39
Effects of secondary causes of dyslipidaemia on the lipogram.....	43
<b>Iron studies.....</b>	<b>45</b>
Interpretation of Iron profile .....	45
<b>Folate and vitamin B12.....</b>	<b>46</b>
<b>ENDOCRINOLOGY.....</b>	<b>47</b>
<b>Thyroid function tests .....</b>	<b>47</b>
Interpretation of thyroid function tests .....	48
Goals for thyroid hormone replacement .....	49
<b>Other endocrinology tests .....</b>	<b>50</b>
<b>Ovarian profile .....</b>	<b>60</b>
<b>Anti-Müllerian Hormone (AMH).....</b>	<b>61</b>
Use of AMH in males .....	61
Use of AMH in female patients.....	61
Use of AMH for IVF treatment .....	62
Use of AMH as tumour marker for granulosa cell tumours of the ovaries .....	62
<b>Pregnancy .....</b>	<b>63</b>
Interpretation of BHCG results .....	63
Screening for fetal anomalies in the first and second trimesters of pregnancy .....	63

<b>TUMOUR MARKERS .....</b>	<b>64</b>
Tumour marker reference ranges.....	64
Intended clinical use of HE4 and ROMA Index .....	67
Interpretation of PSA Ratio (Free PSA:Total PSA) .....	68
Appropriate tumour markers for specific tumours .....	69
<b>THERAPEUTIC DRUGS .....</b>	<b>71</b>
<b>CLINICAL TOXICOLOGY.....</b>	<b>77</b>
Paracetamol overdose/toxicity .....	77
Salicylate overdose/toxicity .....	78
Organophosphate toxicity .....	79
<b>DRUGS OF ABUSE .....</b>	<b>79</b>
<b>ALCOHOL ABUSE .....</b>	<b>81</b>
<b>LABORATORY INVESTIGATIONS FOR SPECIFIC CLINICAL DISORDERS.....</b>	<b>83</b>
<b>Screening tests for endocrine disorders .....</b>	<b>83</b>
Addison's disease .....	83
Carcinoid syndrome.....	83
Cushing's syndrome.....	84
Diabetes insipidus .....	84
Hypercalcaemia and hypocalcaemia .....	84
Phaeochromocytoma .....	84
Primary hyperaldosteronism.....	85
<b>Diagnosis of Porphyria .....</b>	<b>86</b>
<b>Causes and investigation of Gynaecomastia .....</b>	<b>87</b>
<b>Investigation of Female Hirsutism .....</b>	<b>89</b>
<b>Secondary Causes and Investigation of Osteoporosis .....</b>	<b>89</b>
Osteoporosis in men.....	91
<b>Work-up for Hypertension.....</b>	<b>91</b>
<b>Late onset male Hypogonadism .....</b>	<b>92</b>

<b>HAEMATOLOGY .....</b>	<b>96</b>
Full blood count (FBC).....	96
ESR (erythrocyte sedimentation rate).....	101
Investigation of a bleeding disorder.....	106
Basic coagulation screen .....	102
Laboratory assessment for Von Willebrand disease (VWD) .....	109
Platelet aggregation studies .....	110
PFA-200 (for evaluating platelet function) .....	110
Thromboelastogram (TEG) .....	112
Other specialised coagulation tests (D-dimer and FDP) .....	105
Disseminated intravascular coagulation (DIC) screen .....	113
Tests used in the investigation of a thrombotic tendency .....	115
Testing for the presence of a lupus anticoagulant .....	119
Monitoring of anticoagulation therapy .....	120
Antifactor Xa activity (IU/ml) .....	121
Direct thrombin inhibitor (DTI).....	121
Bone marrow investigation .....	122
Flow cytometry .....	123
Tests used in the investigation of a haemolytic process.....	123
Direct Coombs test.....	124
Osmotic fragility .....	124
Testing for haemosiderin in the urine.....	125
PNH screen (paroxysmal nocturnal haemoglobinuria).....	126
Testing for inherited enzyme abnormalities .....	126
Malaria testing.....	126
JAK2 V617F PCR .....	127
<b>IMMUNOLOGY .....</b>	<b>128</b>
Autoimmune disorders.....	128
Autoimmunity and the endocrine system .....	128

Autoimmunity and the central nervous system.....	130
Autoimmunity and renal disease .....	131
Autoimmunity in gastrointestinal disease .....	131
Autoimmunity in liver disease.....	133
Autoimmune skin disorders .....	134
Autoimmune ear disease .....	134
<b>Interpretation of Anti-nuclear Antibodies.....</b>	<b>134</b>
<b>Connective tissue disease .....</b>	<b>136</b>
Basic spectrum .....	136
Rheumatoid arthritis (RA) .....	137
Systemic lupus erythematosus (SLE) .....	137
Sjögren's syndrome.....	138
Polymyositis and dermatomyositis.....	138
Systemic sclerosis (localized and systemic forms).....	139
Ankylosing spondylitis .....	139
Anti-phospholipid syndrome .....	139
Vasculitis .....	140
<b>Allergic diseases .....</b>	<b>141</b>
<b>Allergic disorders (Attachments A - D).....</b>	<b>141</b>
Immediate type hypersensitivity.....	141
Basophil mediated hypersensitivity .....	141
Delayed type hypersensitivity .....	142
<b>Allergy diagnostic tests (Attachment E) .....</b>	<b>142</b>
<b>Urticaria .....</b>	<b>142</b>
<b>Microbiology and Microbial Serology.....</b>	<b>142</b>
Antenatal screening .....	142
Chronic fatigue.....	143
CNS (meningitis/encephalitis).....	143
Congenital screening .....	144
Diarrhoea - Stool Investigations .....	144

Genital Ulcer .....	145
Haematuria .....	145
<b>Hepatitis.....</b>	<b>146</b>
<b>Respiratory Infections .....</b>	<b>150</b>
<b>Primary Immunodeficiencies: A Diagnostic Approach.....</b>	<b>152</b>
Antibody (Humoral) Deficiencies.....	154
T-Cell Defects.....	155
Tests to Determine Neutrophil Function.....	156
Tests to Determine Complement Function .....	156
Natural Killer Cells.....	157
Tests for Causes of Secondary Immunodeficiency.....	157
<b>VIROLOGY.....</b>	<b>158</b>
<b>HIV Diagnosis and Monitoring.....</b>	<b>158</b>
Possible diagnostic algorithms .....	158
HIV monitoring .....	160
Indications for starting ARV therapy.....	160
HIV and HBV .....	161
HIV and TB .....	161
HIV and pregnancy .....	161
HIV in serodiscordant couples.....	162
HIV prophylaxis after unprotected sex, rape survivors, needle sharing and occupational post exposure .....	162
<b>Hepatitis Diagnosis and Monitoring .....</b>	<b>163</b>
Hepatitis A virus (HAV) .....	163
Hepatitis B virus (HBV).....	165
Hepatitis C virus (HCV).....	172
Hepatitis D virus (HDV).....	178
Hepatitis E virus (HEV).....	179

<b>Viral Infections in Pregnancy .....</b>	<b>180</b>
Rubella .....	180
CMV.....	181
HSV .....	183
Parvovirus B19 .....	185
HBV .....	185
HCV .....	186
VZV.....	186
Measles .....	188
Enterovirus infections.....	188
<b>Viral Respiratory Tract infections .....</b>	<b>189</b>
Influenza .....	189
Other respiratory viruses .....	191
<b>Infections in the Immunocompromised Patient.....</b>	<b>193</b>
CMV.....	193
EBV.....	193
HSV .....	194
VZV.....	194
BK .....	194
Bacterial and fungus infections.....	195
Pneumocystis jiroveci.....	195
Tuberculosis .....	195
<b>Viral Central Nervous System Infections (including Rabies) .....</b>	<b>196</b>
Diagnosis .....	196
Prevention .....	197
Treatment .....	199
Infection control.....	200

## ABBREVIATIONS AND SYMBOLS KEY

Symbol/Abbreviation	Interpretation
↑	Increased / High
↓	Decreased / Low
↔	No Change
↑↑	Very High
↑↑↑	Extremely High
ACE	Angiotensin-converting Enzyme
ACTH	Adrenocorticotropic Hormone
ADH	Antidiuretic Hormone
ADP	Adenosine Diphosphate
AFP	Alpha-fetoprotein
AIDS	Acquired Immune Deficiency Syndrome
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
APC-R	Activated Protein C Resistance
APTT	Activated Partial Thromboplastin Time
ARB	Angiotensin Receptor Blocker
ARR	Aldosterone:Renin Ratio
ART	Antiretroviral Therapy
AST	Aspartate Aminotransferase
AVS	Adrenal Venous Sampling
AZT	Zidovudine
BP	Blood Pressure
CDC	Center for Disease Control
CDT	Carbohydrate Deficient Transferrin

<b>Symbol/Abbreviation</b>	<b>Interpretation</b>
CEA	Carcinoembryonic Antigen
CETP	Cholesteryl Ester Transfer Protein
CHD	Coronary Heart Disease
CHF	Congestive Heart Failure
CK	Creatine Kinase
CKD	Chronic Kidney Disease
CLL	Chronic Lymphocytic Leukaemia
CMML	Chronic Myelomonocytic Leukaemia
CMV	Cytomegalovirus
CNS	Central Nervous System
CrCl	Creatinine Clearance
CRP	C-Reactive Protein
CSF	Cerebrospinal Fluid
CT	Computed Tomography
CVD	Coronary Vascular Disease
DHEA	Dehydroepiandrosterone
DHP	Dihydropyridine
DIC	Disseminated Intravascular Coagulation
DM	Diabetes Mellitus
EBV	Epstein-Barr virus
EBNA IgG	Epstein-Barr Virus Nuclear Antigen IgG
EBV VCA IgM	Epstein-Barr Virus Viral Capsid Antigen IgM
EBV VCA IgG	Epstein-Barr Virus Viral Capsid Antigen IgG
ECG	Electrocardiography

<b>Symbol/Abbreviation</b>	<b>Interpretation</b>
EDTA	Ethylenediaminetetraacetate
eGFR	Estimated (calculated) Glomerular Filtration Rate
ESR	Erythrocyte Sedimentation Rate
F	Female
FBC	Full Blood Count
FDP	Fibrinogen Degradation Products
FH	Familial Hypercholesterolaemia
FISH	Fluorescent In Situ Hybridization
FN	False Negative
FP	False Positive
FSH	Follicle-stimulating Hormone
GGT	Gamma Glutamyltransferase
GFR	Glomerular Filtration Rate
H	High
HAART	Highly Active Antiretroviral Therapy
Hb	Haemoglobin
HBcAb	Hepatitis B Core Total (IgM & IgG) Antibody
HBcIgM	Hepatitis B Core IgM
HBeAb	Hepatitis B e-Antibody
HBeAg	Hepatitis B e-Antigen
HBIG	Hepatitis B Immune Globulin
HBsAb	Hepatitis B Surface Antibodies
HBsAg	Hepatitis B Surface Antigen
HBV	Hepatitis B Virus
HC	Hypercholesterolaemia

<b>Symbol/Abbreviation</b>	<b>Interpretation</b>
HCG	Human Chorionic Gonadotropin
HCV	Hepatitis C Virus
HDL	High-Density Lipoprotein
HDLC	High-Density Lipoprotein Cholesterol
HHV-7	Human Herpes virus-7
HHV-6	Human Herpes virus-6
HIAA	5-Hydroxyindoleacetic acid
HIV	Human Immunodeficiency Virus
HNIG	Human Normal Immune Globulin
HPLC	High-performance Liquid Chromatography
HRP-2	Histidine-rich Protein 2
HSV	Herpes Simplex Virus
HT	Hypertension
HTLV-1	Human T-cell Lymphotropic Virus-1
HVA	Homovanillic Acid
IM	Infectious Mononucleosis
ITP	Idiopathic Thrombocytopenic Purpura
L	Low
LCAT	Lecithin-cholesterol Acyltransferase
LD	Lactate Dehydrogenase
LDLC	Low-Density Lipoprotein Cholesterol
LH	Luteinizing Hormone
LMWH	Low Molecular Weight Heparin
M	Male
MA	Maximum Amplitude

<b>Symbol/Abbreviation</b>	<b>Interpretation</b>
MAOI	Monoamine Oxidase Inhibitors
MCS	Microscopy, Culture, Sensitivity
MCV	Mean Cell Volume
MDRD	Modification of Diet in Renal Disease
MELISA	Memory Lymphocyte Immuno Stimulation Assay
MRI	Magnetic Resonance Image
N	Normal
NA	Nuclear Antigen
NCEP	National Cholesterol Education Program
NNRTI	Non-nucleoside Reverse Transcriptase Inhibitors
NRTI	Nucleoside Reverse Transcriptase Inhibitors
NSAID's	Nonsteroidal Anti-inflammatory Drugs
NSE	Neuron-specific Endolase
NSTEMI	Non-ST Elevation Acute Myocardial Infarction
NVP	Nevirapine
PA	Primary Aldosteronism
PAI	Plasminogen activator inhibitor
PBC	Primary Biliary Cirrhosis
PCNSL	Primary Central Nervous System Lymphoma
PCR	Polymerase Chain Reaction
PCT	Procalcitonin
PEP	Post Exposure Prophylaxis
PHA-2	Pseudohypoaldosteronism Type 2
PML	Progressive Multifocal Leukoencephalopathy
PNH	Paroxysmal Nocturnal Haemoglobinuria

<b>Symbol/Abbreviation</b>	<b>Interpretation</b>
PSA	Prostate-specific Antigen
PT	Prothrombin Time
RA	Rheumatoid Arthritis
RF	Rheumatoid Factor
RIG	Rabies Immune Globulin
RNA	Ribonucleic Acid
RPR	Rapid Plasma Reagins
RSV	Respiratory Syncytial Virus
SCC	Squamous Cell Carcinoma
SHBG	Sex Hormone-Binding Globulin
SLE	Systemic Lupus Erythematosus
STEMI	ST-elevation Acute Myocardial Infarction
T3	Triiodothyronine
T4	Thyroxine
TAD	Tricyclic Antidepressants
TB	Tuberculosis
TC	Total Cholesterol
TEG	Thromboelastogram
TG	Triglyceride
TK	Thymidine Kinase
TPA	Tissue Plasminogen Activator
TSH	Thyroid-stimulating Hormone
TT	Thrombin Time
ITP	Thrombotic Thrombocytopenic Purpura
UFH	Unfractionated Heparin

Symbol/Abbreviation	Interpretation
UTI	Urinary Tract Infections
VAR	Variable
VCA	Viral Capsid Antigen
VMA	Vanillylmandelic Acid
VZV	Varicella Zoster Virus
WHO	World Health Organization
ZIG	Zoster Immune Globulin

**General notes:**

1. The reference ranges provided are applicable to adults only.
2. The reference ranges provided may vary according to instrument and methodology.
3. Only the most common causes are listed in the interpretation of analytes.



# BIOCHEMISTRY

## Electrolytes and renal function

Analyte	Ref. Range	Units	Interpretation
Sodium	136 – 145	mmol/l	<p>↑: Diabetes insipidus, dehydration (water loss in excess of salt loss).</p> <p>↓: Medication (e.g. diuretics, indapamide, ACE inhibitors), vomiting, diarrhoea, acute renal failure, congestive heart failure, Addison's disease, syndrome of inappropriate ADH secretion, falsely decreased (due to ↑ protein, ↑ triglycerides).</p>
Potassium	3.5 – 5.1	mmol/l	<p>↑: Acute renal failure, falsely elevated (haemolysed blood, aged blood, contamination with FBC tube anticoagulant), medication (e.g. ACE inhibitors, angiotensin receptor blockers, spironolactone, amiloride), Addison's disease, acidosis, untreated diabetic ketoacidosis.</p> <p>↓: Vomiting, diarrhoea, drugs (e.g. diuretics, indapamide, laxatives).</p>
Chloride	98 – 107	mmol/l	<p>↑: Diarrhoea, dehydration.</p> <p>↓: Diuretics, vomiting.</p>
Bicarbonate	22 – 29	mmol/l	<p>↑: Potassium depletion, vomiting, diuretics, emphysema.</p> <p>↓: Acute renal failure, diabetic ketoacidosis, diabetic hyperosmolar coma, diarrhoea, renal tubular acidosis, lactic acidosis, toxins.</p>
Urea	1.7 – 8.3	mmol/l	<p>↑: Acute or chronic renal failure, dehydration (due to vomiting, diarrhoea, sweating), intestinal bleeding, shock.</p> <p>↓: Hepatic failure, pregnancy, cachexia.</p>
Creatinine	M 64 – 104 F 49 – 90	µmol/l	<p>↑: Acute or chronic renal failure, acromegaly, meat meals, hyperthyroidism.</p> <p>↓: Pregnancy, chronic muscle wasting, immobilisation.</p>

Analyte	Ref. Range	Units	Interpretation
Urate	M 0.21 – 0.43 F 0.16 – 0.36	mmol/l	↑: Gout, renal failure, insulin resistance syndrome, alcoholism, malignancies (e.g. leukaemia, lymphoma, multiple myeloma), psoriasis, medication (e.g. diuretics, salicylates, etc). ↓: Syndrome of inappropriate ADH secretion, pregnancy.
eGFR	≥90	ml/min/ 1.73m <sup>2</sup>	eGFR = estimated (calculated) Glomerular Filtration Rate ↓: Renal impairment (see table for classification of chronic kidney disease).
Osmolality	275 – 295	mOsm/kg	↑: Hyperglycaemia, water depletion, uraemia, ethanol intoxication, hypernatraemia, diabetes insipidus, cerebral lesions. ↓: Addison's disease, water intoxication, syndrome of inappropriate ADH secretion.
Creatinine Clearance (24 hour urine and blood)	M 105 – 157 F 81 – 123	ml/min/ 1.73 m <sup>2</sup>	↑: Pregnancy, high protein diet, urine collected for >24 hours (false ↑). ↓: Prerenal (shock, haemorrhage, dehydration, congestive heart failure), intrinsic renal disease, acute tubular dysfunction, post-renal obstruction, urine collected for <24 hours (false ↓). Although a moderate decrease in GFR is expected with age, a major decrease (<60 ml/min/1.73 m <sup>2</sup> ) is uncommon in the absence of disease (e.g. diabetes) or co-morbid cardiovascular conditions (including HT, heart failure and atherosclerosis).
Urine protein (24 hour urine)	<150	mg/24 h	↑: Cystitis, pyelonephritis, glomerular disease, renal tubular disease, nephrotic syndrome, diabetes mellitus, fever, strenuous exercise, orthostatic changes.

## Diagnosis of Chronic Kidney Disease (CKD)

Chronic kidney disease (CKD) is defined as abnormalities of kidney structure or function, present for longer than 3 months, with implications for health.

The most recent Kidney Disease Improving Global Outcomes (KDIGO) Clinical Practice Guidelines (2012) recommended that CKD should be classified using the CGA staging system. This includes Cause, Glomerular filtration rate (GFR) category and Albuminuria category (as marker for kidney damage), as the combination of these factors relates to risks of adverse outcomes.

**Criteria for CKD:** Either of the following present for more than **3 months**

1. **Decreased GFR** ( $<60 \text{ ml/min}/1.73 \text{ m}^2$ ) (GFR categories G3a-G5)
2. Markers of **kidney damage** (one or more):
  - a. **Albuminuria:** Albumin excretion rate (AER)  $\geq 30 \text{ mg/day}$  or albumin-to-creatinine ratio (ACR)  $\geq 3 \text{ mg/mmol}$ .
  - b. Urine sediment abnormalities (e.g. haematuria, red cell / white cell / granular casts, etc).
  - c. Electrolyte and other abnormalities due to tubular disorders.
  - d. Abnormalities detected by histology.
  - e. Structural abnormalities detected by imaging.
  - f. History of kidney transplantation.

## Classification of CKD using the CGA system

Previous CKD guidelines included only the level of GFR for staging. By using the CGA system, the cause and albuminuria category are also used. CKD is not a disease in itself, and the assignment of cause is important for determination of prognosis and to guide treatment decisions.

### 1. **Assignment of the cause of CKD**

The cause of CKD is assigned based on the presence or absence of systemic disease and the location of observed or assumed pathology within the kidney. In developed countries, hypertension and diabetes are the most frequent causes of CKD. Examples are supplied in Table 1.

**Table 1: Classification of CKD based on systemic disease and location of pathology within kidney**

	<b>Examples of systemic diseases affecting the kidney</b>	<b>Examples of primary kidney diseases (absence of systemic diseases)</b>
<b>Glomerular diseases</b>	Diabetes mellitus, systemic autoimmune diseases, systemic infections, drugs, neoplasia (including amyloidosis)	Diffuse, focal or concentric proliferative glomerulonephritis, focal and segmental glomerulosclerosis, membranous nephropathy, minimal change disease
<b>Tubulointerstitial diseases</b>	Systemic infections, autoimmune, sarcoidosis, drugs, urate, environmental toxins (lead, aristolochic acid found in Chinese herbal medicine), neoplasia (myeloma)	Urinary-tract infections, stones, obstruction
<b>Vascular diseases</b>	Atherosclerosis, hypertension, ischaemia, cholesterol emboli, systemic vasculitis, thrombotic microangiopathy, systemic sclerosis	ANCA-associated renal limited vasculitis, fibromuscular dysplasia
<b>Cystic and congenital diseases</b>	Polycystic kidney disease, Alport syndrome, Fabry disease	Renal dysplasia, medullary cystic disease, podocytopathies

ANCA: Antineutrophil cytoplasmic antibody

Kidney International Supplements 2013: 3:19-62

## **2. Recommendations regarding GFR estimation**

- Since November 2014 Ampath is using the **2009 CKD – EPI creatinine equation**.
- The **main advantages of using the Chronic Kidney Disease Epidemiology Collaboration (CKD – EPI) equation** are the following:
  - More accurate at GFR >60 ml/min/1.73 m<sup>2</sup> and GFR >90 ml/min/1.73 m<sup>2</sup> is reportable using CKD – EPI equation.
  - Less influenced by ethnic origin and reasonable to use without correction for race and ethnicity.

- Significant reclassification from stage 3a (GFR 45 – 59) to stage 2 (GFR 60 – 89).
- CKD – EPI classification shows better accuracy compared to gold standard methods of GFR estimation than MDRD, especially at high GFRs, in younger people and women.
- Validated in older people.
- Limitations of serum creatinine (SCr) as a marker are still applicable – see Table 2.

**Table 2: Sources of error in GFR estimation using creatinine**

Source of error	Example
<b>Non-steady state</b>	<ul style="list-style-type: none"> <li>• Acute kidney injury (AKI)</li> </ul>
<b>Non-GFR determinants of SCr that differ from study populations in which equations were developed</b> <ul style="list-style-type: none"> <li>• Factors affecting creatinine generation</li> <li>• Factors affecting tubular secretion of creatinine</li> <li>• Factors affecting extra-renal elimination of creatinine</li> </ul>	<ul style="list-style-type: none"> <li>• Race / ethnicity other than US / European black and white</li> <li>• Extremes of muscle mass or body size</li> <li>• Diet and nutritional status (high protein diet / creatine supplements / ingestion of cooked meats)</li> <li>• Muscle wasting diseases</li> <li>• Decreased by drug-induced inhibition: trimethoprim, cimetidine, fenofibrate</li> <li>• Dialysis</li> <li>• Decreased by inhibition of gut creatininase by antibiotics</li> <li>• Increased by large volume losses of extracellular fluids</li> </ul>

Source of error	Example
<b>Higher GFR</b>	<ul style="list-style-type: none"> <li>Higher measurement error in low SCr and high GFR</li> </ul>
<b>Interference with creatinine assay</b>	<ul style="list-style-type: none"> <li>Spectral interferences (e.g. bilirubin, some drugs)</li> <li>Chemical interferences (e.g. glucose, ketones, bilirubin, some drugs)</li> </ul>

- GFR categories in CKD:** Please take note that the previously used Stage 3 has been subdivided into stages 3a and 3b, based on data supporting different risks and outcomes. Although some decline in GFR is expected with aging, a value below 60 ml/min/1.73 m<sup>2</sup> is also regarded as decreased.

**Table 3: GFR categories in CKD**

GFR category	GFR (ml/min/1.73 m <sup>2</sup> )	Terms
G1	≥90	Normal or high
G2	60 – 89	Mildly decreased*
G3a	45 – 59	Mildly to moderately decreased
G3b	30 – 44	Moderately to severely decreased
G4	15 – 29	Severely decreased
G5	<15	Kidney failure

\*Relative to young adult level

Kidney International Supplements 2013; 3:19-62

- In the absence of evidence of kidney damage, neither GFR category G1 nor G2 fulfil the criteria for CKD.
- eGFR from 60 to 89 is mildly decreased and not regarded as CKD unless present for longer than 3 months in the presence of moderate albuminuria (>3.0 mg/mmol). Associated risk factors

for cardiovascular disease, including hypertension, dyslipidaemia, smoking and other lifestyle factors, should be addressed.

The following additional investigations are recommended:

- Creatinine: repeat within 14 days for confirmation (after 12 hours of no meat consumption), and then annually.
- Annual urine albumin-to-creatinine ratio.

### **3. Albuminuria as a marker of kidney damage:**

- **Albuminuria** is the preferred marker for assessment of kidney damage, and should be used in preference to proteinuria, as it is the earliest marker of **glomerular diseases**. There is a graded increase in risk for all-cause and cardiovascular mortality, kidney failure, acute kidney injury (AKI) and CKD progression for higher albuminuria categories across all GFR categories.
- For **screening** purposes, an early-morning urine sample (first pass) for determination of the **albumin-to-creatinine ratio (ACR)** is preferred.
- The term microalbuminuria used for an ACR of 3 – 30 mg/mmol (category A2) should no longer be used and has been replaced by the term "**moderately increased**" **albuminuria**.
- An abnormal screening test should be **confirmed** by an ACR on an early-morning urine sample or an albumin excretion rate (AER) in a timed urine collection.
- The use of **reagent test strips are discouraged** due to poor sensitivity at lower concentrations and because the values are not adjusted for urinary concentration.
- If non-albuminuric proteinuria is suspected, use assays for **specific proteins**, e.g. determination of Bence Jones proteins in myeloma patients.

**Table 4: Albuminuria categories in CKD and relationship with proteinuria**

Measure	Categories		
	Normal (A1)	Moderately increased (A2)	Severely increased (A3)
Albumin-to-creatinine ratio (ACR) (mg/mmol)	<3	3 – 30	>30
Albumin excretion rate (AER) (mg/24h)	<30	30 – 300	>300
Protein-to-creatinine ratio (PCR) (mg/mmol)	<15	15 – 50	>50
Protein excretion rate (PER) (mg/24h)	<150	150 – 500	>500
Protein reagent strip	Negative to trace	Trace to +	+ or greater

Nephrotic syndrome: ACR >220 mg/mmol, AER >2200 mg/24h, PCR >350 mg/mmol, PER >3500 mg/24h

### Areas of controversy or confusion

**CKD with isolated GFR decrease** without markers of kidney damage may be seen with heart failure, liver cirrhosis, hypothyroidism, malnutrition and in kidney donors.

**CKD with isolated persistent albuminuria** without decreased GFR is seen in obesity and the metabolic syndrome. CKD should be excluded in patients with orthostatic (postural) proteinuria with PER >1000 mg/24h.

## Calcium, magnesium, phosphate, vitamin D and PTH

Analyte	Ref. Range	Units	Interpretation
Calcium	2.15 – 2.50	mmol/l	<p>↑: Primary hyperparathyroidism, malignancy, sarcoidosis, immobilisation, Addison's disease, thyrotoxicosis, medication, e.g. lithium, thiazides.</p> <p>↓: Renal failure, falsely ↓ due to contamination with FBC tube anticoagulant, hypoparathyroidism, hypomagnesaemia, vitamin D deficiency, blood transfusion, major surgery, trauma, sepsis, burns, pancreatitis, multiple organ failure, haemodialysis, advanced osteomalacia.</p>
Magnesium	0.66 – 1.07	mmol/l	<p>↑: Acute and chronic renal failure, untreated diabetic ketoacidosis.</p> <p>↓: Malabsorption, chronic alcoholism, diarrhoea, drugs (e.g. diuretics, laxatives), treatment of diabetic ketoacidosis.</p>
Phosphate	0.78 – 1.42	mmol/l	<p>↑: Renal failure, untreated diabetic ketoacidosis.</p> <p>↓: Acute alcoholism, primary hyperparathyroidism, drugs (e.g. diuretics, insulin), severe diarrhoea, vomiting, treatment of diabetic ketoacidosis, severe malnutrition, malabsorption, hypokalaemia, vitamin D deficiency.</p>
25(OH) Vitamin D	<20 20 – 29 30 – 100	ng/ml	<p>Severe deficiency.</p> <p>Moderate to mild deficiency.</p> <p>Optimum level.</p> <p>↑: Vitamin D intoxication, excessive exposure to sunlight.</p> <p>↓: Malabsorption, dietary osteomalacia, steatorrhoea, biliary and portal cirrhosis, anticonvulsants, renal osteodystrophy, osteitis fibrosis cystica, thyrotoxicosis, pancreatic insufficiency, coeliac disease, inflammatory bowel disease, bowel resection, rickets, hypoparathyroidism, chronic renal failure, underexposure to sunlight. Often no specific pathological cause is found, except for inadequate sunlight exposure combined with a vitamin D deficient diet (our normal diet does not contain adequate vitamin D).</p>

Analyte	Ref. Range	Units	Interpretation
Parathyroid hormone	15 – 65 (Roche)	pmol/l	<p>↑: Primary hyperparathyroidism (calcium ↑), medication e.g. lithium, thiazides.</p> <p>↑: Secondary hyperparathyroidism (calcium normal or ↓) due to e.g. renal disease, vitamin D deficiency, anticonvulsant therapy.</p> <p>↓: With ↑ calcium – due to malignancy, sarcoidosis, immobilisation, Addison's disease.</p> <p>↓: With ↓ calcium – due to hypoparathyroidism, hypomagnesaemia.</p>

## Liver function tests

Analyte	Ref. Range	Units	Interpretation
Total protein	60 – 83	g/l	<p>↑: Multiple myeloma, autoimmune disease, chronic liver disease, chronic infection (e.g. AIDS, TB).</p> <p>↓: Nephrotic syndrome, chronic liver failure, malnutrition, pregnancy.</p>
Albumin	35 – 52	g/l	<p>↑: Dehydration, prolonged tourniquet application during venipuncture.</p> <p>↓: Acute and chronic liver disease, malnutrition, malabsorption, nephrotic syndrome, acute and chronic inflammation, systemic infections, autoimmune disease, congestive cardiac failure, pregnancy.</p>
Prealbumin (Transthyretin)	200 – 400	mg/l	<p>Used for monitoring of nutritional status due to short half-life (1 – 2 days)</p> <p>↓: Protein – caloric malnutrition, inflammation, malignancy, liver cirrhosis.</p>

Analyte	Ref. Range	Units	Interpretation
Serum Cholinesterase	M 5320 – 12920 F 18 – 39y 4260 – 11250 ≥40y 5320 – 12920	U/l	↓: Organophosphorus insecticide poisoning, hepatitis, cirrhosis, hepatic metastases, hepatic congestion due to heart failure, hepatic amebiasis, malnutrition, anaemias, acute infections, burns, myocardial infarction, pulmonary embolism, after surgery, chronic renal disease, late pregnancy, conditions with low serum albumin (e.g. malabsorption), genetic cholinesterase variants.
Ammonia	M 15 – 55 F 11 – 48	μmol/l	↑: Liver failure, liver cirrhosis, gastrointestinal bleeding, portal – systemic shunting of blood.
Total Bilirubin	5 – 21	μmol/l	↑: Hepatocellular damage (e.g. hepatitis, toxic damage due to drugs or toxins), intrahepatic biliary tree obstruction (e.g. primary biliary cirrhosis), extrahepatic biliary tree obstruction (e.g. gallstones, carcinoma of the head of the pancreas), haemolytic diseases, Gilbert's disease.
Conjugated Bilirubin	0 – 5	μmol/l	↑: Hepatocellular damage (e.g. hepatitis, toxic damage due to drugs or toxins), intrahepatic biliary tree obstruction (e.g. primary biliary cirrhosis), extrahepatic biliary tree obstruction (e.g. gallstones, carcinoma of the head of the pancreas).
Unconjugated Bilirubin	0 – 18	μmol/l	↑: Mainly unconjugated bilirubin increase: - Gilbert's disease, haemolytic diseases. Combined increase of unconjugated and conjugated bilirubin: - Hepatocellular damage, intrahepatic and extrahepatic biliary tree obstruction.

Analyte	Ref. Range	Units	Interpretation
Alkaline Phosphatase (ALP)	M 40 – 130 F 35 – 105	U/l	↑: Primary and secondary hyperparathyroidism, extrahepatic biliary tree obstruction (e.g. gallstones, carcinoma of the head of the pancreas), intrahepatic biliary tree obstruction (e.g. primary biliary cirrhosis), hepatocellular disease (e.g. hepatitis), space occupying lesions in the liver (e.g. liver metastases), bone metastases, Paget's disease of bone, uraemic osteodystrophy, thyrotoxicosis, during healing of a fracture, pregnancy.
Gamma Glutamyl-transferase (GGT)	M <60 F <40	U/l	↑: Extrahepatic biliary tree obstruction (e.g. gallstones, carcinoma of the head of the pancreas), intrahepatic biliary tree obstruction (e.g. primary biliary cirrhosis), hepatocellular disease (e.g. hepatitis), fatty liver, space occupying lesions in the liver (e.g. liver metastases), induction by alcohol or medication.
Alanine amino-transferase (ALT)	M <50 F <35	U/l	↑: Acute hepatitis, chronic hepatitis, liver cirrhosis, liver cell necrosis (e.g. hypoxic shock, paracetamol overdosage), viraemia, chronic alcohol abuse, liver cirrhosis, fatty liver.
Aspartate amino-transferase (AST)	M <38 F <32	U/l	↑: Acute hepatitis, acute liver cell necrosis (e.g. hypoxic shock, paracetamol overdose), chronic hepatitis, liver cirrhosis, chronic alcohol abuse (AST:ALT ratio > 2), intrahepatic neoplasms, viraemia, fatty liver, haemolytic anaemia, megaloblastic anaemia, rhabdomyolysis, vigorous exercise, muscular dystrophy.

Analyte	Ref. Range	Units	Interpretation
Lactate dehydrogenase (LD)	100 – 250	U/l	↑: Megaloblastic anaemia, haemolytic anaemia, leukaemia, acute hepatitis, acute liver cell necrosis, liver cirrhosis, musculoskeletal disease, vigorous exercise, rhabdomyolysis, neoplastic disease, myocardial infarction.

### Interpretation of unconjugated hyperbilirubinaemia

	Gilbert's disease	Haemolysis	Megaloblastic anaemia
Total Bilirubin	↑	↑	↑
Unconjugated Bilirubin	↑	↑	↑
ALP	N	N	N
GGT	N	N	N
ALT	N	N	N
AST	N	N / ↑	↑
LD	N	↑	↑ / ↑↑

### Interpretation of liver profiles

	Hepatitis	Extrahepatic obstruction	Space occupying lesion	Alcohol
Total Bilirubin	N / ↑	↑↑↑	N	N
Conjugated Bilirubin	N / ↑	↑↑↑	N	N
Unconjugated Bilirubin	N / ↑	↑↑↑	N	N
ALP	N / ↑	↑↑↑	N / ↑	N

## Interpretation of liver profiles (continued)

	Hepatitis	Extrahepatic obstruction	Space occupying lesion	Alcohol
GGT	N / ↑	↑↑↑	↑	↑
ALT	↑ to ↑↑	N / ↑	N	N
AST	↑ to ↑↑	N / ↑	N / ↑	↑
LD	N / ↑↑	N / ↑	N / ↑	N

### Evaluation of isolated mild chronic elevation of serum ALT and AST

**Definition:** Mild (<4 times upper limit of normal), chronic ( $\geq 6$  months) elevation of one or both aminotransferases.

Disease	Tests
<b>Step 1</b>	
History	Medication, herbal remedies, recreational drugs
Alcohol abuse	AST: ALT >2:1, GGT ↑, CDT
Hepatitis B and C	Hepatitis B and C studies
Haemochromatosis	Iron profile (fasting), Haemochromatosis PCR
Fatty Liver	AST: ALT <2:1, GGT sometimes ↑, Ultrasound, CT, MRI, Liver Biopsy
<b>Step 2 – If the above are unrevealing, exclude extra-hepatic source</b>	
Hyperthyroidism and Hypothyroidism	TSH, FT4
Coeliac Disease	Anti-endomysial IgA, anti-tissue transglutaminase IgA antibodies
Adrenal insufficiency	8 – 10 am ACTH and Cortisol

Disease	Tests
<b>Step 3</b>	
Auto immune hepatitis	Serum protein electrophoresis, antinuclear antibodies, anti-smooth muscle antibodies, liver-kidney microsomal antibodies, liver biopsy
Wilson's Disease	Serum caeruloplasmin, 24-hour urine copper, Kayser Fleischer rings
Alpha-1-antitrypsin Deficiency	Alpha-1-antitrypsin level, alpha-1-antitrypsin PCR

## Pancreas

Analyte	Ref. Range	Units	Interpretation
Amylase	<110	U/l	↑: Pancreatitis, intestinal obstruction or infarction, strangulated bowel, ectopic pregnancy, perforated hollow viscus, biliary tract disease of all types, diabetic ketoacidosis, pancreatic cyst or pseudocyst, peritonitis, macroamylasemia, renal failure, abdominal trauma, viral infections, postoperative patients, alcohol, parotitis, mumps.
Lipase	22 – 51 (Beckman)	U/l	↑: Pancreatitis, gallstone colic, perforated hollow viscus, strangulated or infarcted bowel, pancreatic cyst or pseudocyst, peritonitis.
	13 – 60 (Roche)		
Stool elastase	>200	µg/g stool	↓: Chronic pancreatitis.

## Inflammatory markers

### CRP (C-Reactive Protein)

Marker	Ref. Range	Units	Interpretation
CRP	0.0 – 4.9	mg/l	↑: Inflammatory conditions including trauma, burns, surgery, autoimmune disease, neoplastic disease and infection.

### Procalcitonin

Analyte	Range	Units	Interpretation
Procalcitonin (PCT)	0.0 – 0.05	ng/ml	Normal range.
	0.06 – 0.49	ng/ml	May indicate: <ul style="list-style-type: none"> <li>- no or minor systemic inflammatory response.</li> <li>- local inflammation.</li> <li>- chronic inflammatory processes.</li> <li>- autoimmune diseases.</li> <li>- viral infections.</li> <li>- mild to moderate localised bacterial infections.</li> </ul>
	0.5 – 1.99	ng/ml	Possible bacterial infection. Non bacterial causes (such as invasive fungal infections, viral infections, severe trauma, major surgery, burns, cardiogenic shock, small cell lung cancer, <i>Plasmodium falciparum</i> malaria, etc.) have to be excluded.
	2 – 10	ng/ml	Systemic bacterial, fungal or parasitic infection is likely. Also compatible with severe polytrauma or burns.
	>10	ng/ml	Sepsis, severe sepsis or septic shock (almost exclusively bacterial) or multi organ failure most likely.

It is important to note that PCT levels below 0.5 ng/ml do not always indicate the absence of bacterial infection. Falsely low PCT levels may occur in the presence of bacterial infections e.g.:

- in the early course of localised infections and sub-acute infective endocarditis
- if PCT done very early following onset of a systemic bacterial infection (usually <6 hours)

In the above cases, PCT should be re-assessed 6 – 24 hours later.

## **Cardiac and skeletal muscle markers**

Marker	Ref. Range	Units	Interpretation
CK (Creatine kinase)	M 39 – 308 F 26 – 192	U/l	↑: Myocardial infarction, myocarditis, muscular dystrophies, polymyositis, seizures, muscle trauma, exercise, IM injection, rhabdomyolysis, surgery, hypothyroidism, congestive heart failure, tachycardia, pulmonary emboli, hypoxic shock, tetanus, extensive brain infarction, head injury.
CK-MB (mass)	M 0 – 6.4 F 0 – 6.4 (Abbott)	ng/ml	↑: Myocardial infarction, myocarditis, muscular dystrophies, polymyositis, seizures, muscle trauma, exercise, IM injection, post-operative.
	M 0 – 7.6 F 0 – 5.2 (Beckman)		
	M 0 – 7.5 F 0 – 3.9 (Minivididas)		
	M 0.9 – 11.0 F 0.6 – 6.9 (Radiometer)		
	M 0 – 7.6 F 0 – 4.7 (Roche)		

Marker	Ref. Range	Units	Interpretation
Troponin I	M/F 0 – 26.2 (Abbott)	ng/l	↑: See diagnosis of acute coronary syndrome and table for causes of troponin elevation.
	M/F 0 – 40 (Beckman Accu TnI)		
Troponin T	M/F 0 – 14 (high sensitive) (hsTnT-Roche)		
	M/F 0 – 17 (Radiometer AQT)		
Myoglobin	M 0 – 154 F 0 – 105 (Abbott)	µg/l	↑: Myocardial infarction, rhabdomyolysis, seizures, IM injection, exercise.
	M 17 – 106 F 14 – 66 (Beckman)		
	M 28 – 72 F 25 – 58 (Roche)		
	M 21 – 98 F 19 – 56 (Stratus)		
Homocysteine	Adults <15 Elderly <20 See table for interpretation	µmol/l	↑: Homocystinuria, heterozygous cystathione-β-synthase defect, vitamin B12 deficiency, folate deficiency, vitamin B6 deficiency, cigarette smoking, coffee consumption, renal failure, hypothyroidism, diabetes mellitus, psychiatric disorders. ↓: Pregnancy, hyperthyroidism, early diabetes mellitus.
NT-ProBNP	<125	pg/ml	See NT-ProBNP interpretation below.

## Homocysteine Interpretation

Classification of risk for atherosclerotic vascular disease	Homocysteine concentration ( $\mu\text{mol/l}$ )
Moderate	15 – 30 in adults 20 – 30 in elderly
Intermediate	31 – 100
Severe	>100
A follow up homocysteine level one month post initiation of treatment is indicated.	

## NT-ProBNP interpretation

### NT-proBNP <300 pg/ml

NT-proBNP values below 300 pg/ml virtually excludes ACUTE heart failure. However, an NT-proBNP result >125 pg/ml may be compatible with symptomatic heart failure in an out-patient setting.

ProBNP may be decreased by:

- Hypothyroidism
- Treatment with diuretics
- ACE-inhibitors / Angiotensin II antagonists (ARB's)
- Obesity
- Vasodilators
- Left atrial failure

## ProBNP cut-off points for patients with clinical evidence of acute (decompensated) cardiac failure

Patient age (years)	NT-proBNP values (pg/ml)	
<50	300 – 450	>450
50 – 75	300 – 900	>900
>75	300 – 1800	>1800
	<b>Acute Congestive Heart Failure (CHF) less likely</b>	<b>Acute Congestive Heart Failure (CHF) likely</b>
	Consider possible: Stable left ventricular failure (LVF), Right ventricular failure (RVF), Myocardial infarction (MI), Acute pulmonary embolism (PE), Kidney failure	

Elevated proBNP levels also occur in stable CHF (no clinical evidence of acute decompensation).

## Diagnosis and classification of Acute Coronary Syndrome (ACS) in the emergency department setting

- A **sensitive cardiac troponin** (TnT / TnI) is the **preferred biochemical marker** for diagnosis of AMI, replacing previously used biomarkers such as myoglobin and CK-MB.
- CK-MB is an acceptable alternative only when troponin is not available.
- The **99th percentile** of a reference population is used as **upper limit** of normal for troponin testing.
- The diagnosis of **ST-elevation myocardial infarction (STEMI)** is made by **typical ECG** findings in patients with a suggestive clinical presentation, and not by elevation of troponins. Treatment must be initiated immediately and not delayed until Troponin results are available.
- **Non-ST elevation myocardial infarction (NSTEMI)** is confirmed by a **troponin** above the **rule-in level** (>100 ng/l for hsTnT).

- Troponin results **above the 99th percentile but below the MI-rule in level**, should be repeated after 3 hours to show a significant change for diagnosis of acute myocardial damage / AMI in a setting of ischaemia (recent onset chest pain / ECG-changes).
- A **significant change** for hsTnT is regarded as a 50% increase for values from 15 – 52 ng/l or 20% increase for values from 53 – 100 ng/l in a follow up sample. A 50% change should be regarded as significant in the case of Troponin I.
- **NSTEMI is excluded** after 2 measurements taken at least 3 hours apart that remain negative or show an insignificant change or if a value reliably 6 hours following the onset of chest pain is negative.
- A **stable increase** in troponin level (hsTnT 15 – 100 with insignificant % change) could be due to congestive cardiac failure (CCF), severe hypertension, haemodynamic shock, pulmonary embolism, myocarditis, cardiac trauma and sepsis.

#### ST elevation acute myocardial infarction (STEMI)

A clinical scenario suggestive of evolving ischaemia  
and

One of the following ECG changes:

1. ST segment elevation (which is not transient).
2. New (or presumably new) onset left bundle branch block.
3. New onset Q waves.

Biomarkers of myocardial necrosis should still be requested but the diagnosis and treatment should not be delayed by pending results.

#### Non-ST elevation acute myocardial infarction (NSTEMI)

A rising (or falling) troponin I or troponin T concentration where at least one of the values is positive  
and one of the following:

1. A clinical scenario suggestive of evolving ischaemia.
2. ECG findings of ischaemia excluding those associated with STEMI. This includes:
  - a. ST segment depression.
  - b. T wave inversion.
  - c. Transient ST segment elevation.
3. A recent coronary artery intervention.

Unstable angina

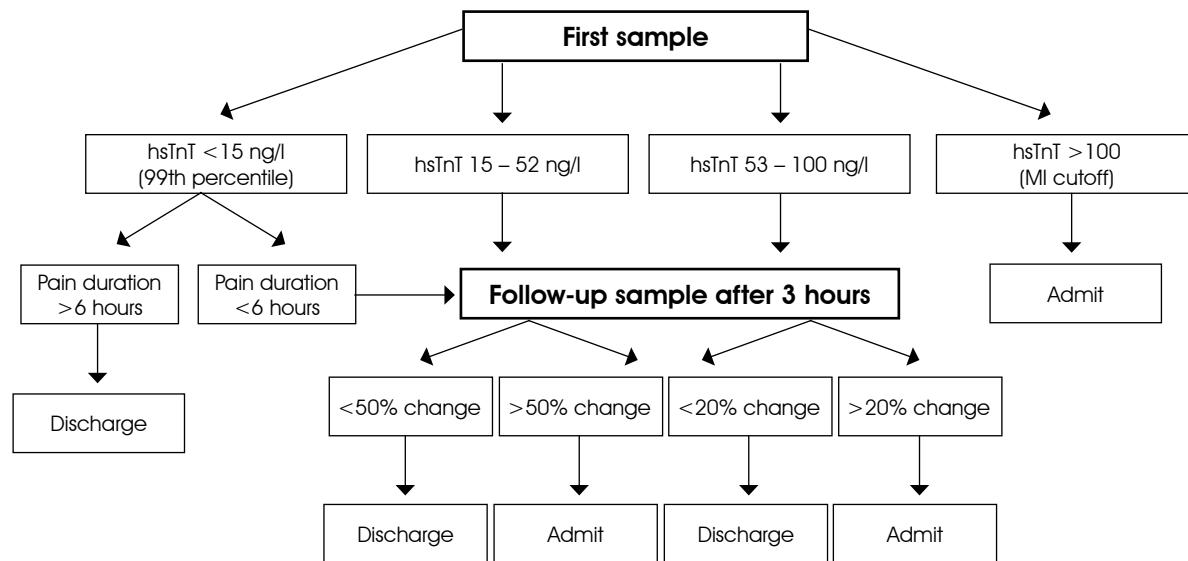
A troponin I or T concentration that does not follow a rising (or falling) pattern,  
but

A clinical scenario suggestive of evolving ischaemia at rest or that is evoked through stress testing.

The ECG may be normal or may show signs of ischaemia similar to that of a NSTEMI.

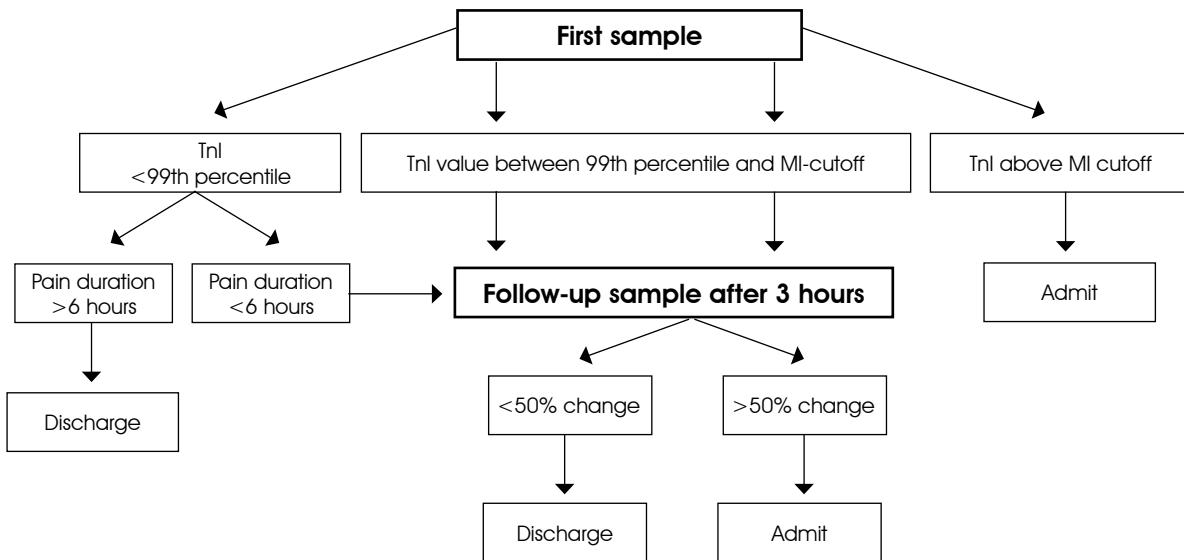
**List of contemporary sensitive troponin assays with relevant cut-off values (ng/l)**

Assay	99th percentile (upper limit of normal)	MI rule-in (using WHO-criteria)
<b>Troponin I</b>		
Abbott Architect TnI	26.2	300
Beckman AccuTnI	40	500
Siemens Stratus CS TnI	70	600
<b>Troponin T</b>		
Radiometer AQT90 TnT	17	100
Roche hsTnT	14	100

**Proposed algorithm for work-up of suspected acute coronary syndrome (ACS)****For hsTropoinin T:**

## Proposed algorithm for work-up of suspected acute coronary syndrome (ACS)

For Troponin I:



Admit = Admit and treat for ACS.

Discharge = Discharge after symptomatic treatment / stress test / investigations for other causes of chest pain.

## Causes of cardiac troponin elevation (other than acute coronary syndrome)

<b>ACUTE</b>	
<b>Ischaemic mechanisms</b>	
Acute heart failure	Hypotension / shock
Pulmonary embolism	Sepsis
Tachy-arrhythmias	Acute respiratory distress syndrome (ARDS)
Brady-arrhythmias	Aortic dissection
Accelerated hypertension	Carbon monoxide poisoning
<b>Other mechanisms</b>	
Cardiac contusion	Myo-pericarditis
Procedural trauma:	Endocarditis
Cardiac surgery	Stroke
Uncomplicated Percutaneous Coronary Intervention (PCI)	Tako-tsubo cardiomyopathy
Atrial septal defect (ASD) closure	Rhabdomyolysis
Endomyocardial biopsy	Acute renal failure
Pacing	Burns >30%
Implantable cardioverter defibrillator (ICD) shocks	Snake venoms
Radiofrequency (RF) ablation / cryo ablation	Chemotherapy: Adriamycin, 5-fluoro-uracil, herceptin
External cardiac massage	Sympathomimetic drugs
External cardioversion / defibrillation	Strenuous exertion
After non-cardiac surgery	Chronic obstructive pulmonary disease (COPD) exacerbation

## Causes of cardiac troponin elevation (other than acute coronary syndrome) (continued)

<b>CHRONIC</b>	
Stable atherosclerotic coronary artery disease	Hypertension / Left ventricular (LV) hypertrophy
Other coronary disease e.g. SLE, scleroderma, Kawasaki's disease, transplant vasculopathy	Pulmonary arterial hypertension
Atrial fibrillation	Aortic valve disease
Chronic heart failure	Hypertrophic cardiomyopathy
Chronic renal failure	Infiltration: amyloidosis, haemochromatosis, sarcoidosis
Hypothyroidism	Peri-partum cardiomyopathy
Diabetes mellitus	

## Carbohydrate metabolism

Analyte	Ref. Range	Units	Interpretation
Glucose fasting	3.9 – 6.0	mmol/l	↑: Impaired fasting glucose, diabetes mellitus type 1 and type 2, strenuous exercise, stress, severe illness, hyperthyroidism, Cushing's syndrome, acromegaly, phaeochromocytoma, corticosteroid therapy, acute and chronic pancreatitis. ↓: Insulinoma, Addison's disease, poisoning (e.g. alcohol, salicylates), medication (sulfonylureas), hepatic failure, postprandial (reactive hypoglycaemia, postgastrectomy, gastroenterostomy).
Insulin fasting	0 – 10 (Abbott)	μIU/ml	↑: Insulin resistance syndrome, obesity, diabetes mellitus type 2, insulinoma. ↓: Diabetes mellitus type 1.
	2.1 – 10.4 (Beckman)		
	0.2 – 9.4 (Roche)		
Quick index	≥0.36		<0.36 indicative of insulin resistance.

Analyte	Ref. Range	Units	Interpretation
HbA1c	4.3 – 6.1 (Biorad)	%	Reflects the mean blood glucose concentration over the past 4 – 8 weeks.
	4.0 – 6.0 (Roche)		Target for diabetic glycaemic control: Ideal <7%; Poor >8%.
Fructosamine	205 – 285 (Roche)	µmol/l	Reflects the mean blood glucose concentration over the past 2 – 3 weeks. Useful in patients with Hb variants and gestational diabetes mellitus.

### Criteria for diagnosing diabetes and categories of intermediate hyperglycaemia

Interpretation	Plasma glucose (mmol/l)		HbA1c (%)
	Fasting	OGTT (2 hr) or random	
Normal	<6.1	And <7.8	
Impaired fasting glycaemia (IFG)	6.1 – 6.9	And <7.8	N/A
Impaired glucose tolerance (IGT)	<7.0	And 7.8 – 11.0	N/A
Diabetes Mellitus (DM) (two abnormal values confirm diagnosis)	≥7.0	And / or ≥11.1	And / or ≥6.5

- An **HbA1c of 6.5%** is recommended as the cut point for diagnosing diabetes. An HbA1c value less than 6.5% does not exclude diabetes if glucose values are within the diabetic range.
- In an asymptomatic person the diagnosis should **not be based on a single abnormal glucose or HbA1c result**, but should be confirmed by at least one additional HbA1c or plasma glucose in the diabetic range, either fasting, random or 2 hours following an oral 75 g glucose load (OGTT).

- Important conditions to adhere to before performing the OGTT are ingestion of at least 150 g of dietary carbohydrate per day for 3 days prior to the test, a 10 to 16 hour fast, and commencement of the test between 7 am and 9 am.
- The use of blood glucose monitoring meters and non-laboratory HbA1c methods **for diagnosis of diabetes is discouraged.**

**WHO recommendations (2013 update) for classification of hyperglycaemia during pregnancy:**

Interpretation	Plasma glucose (mmol/l)			Plasma glucose (mmol/l)
	Fasting	OGTT 75 g 1 hour	2 hour	
<b>DM in pregnancy</b> (If either / both values are abnormal, confirm by a repeat test on another day)	≥7.0		≥11.1	≥11.1
<b>Gestational Diabetes Mellitus</b> (one abnormal value confirms the diagnosis at any gestation)	5.1 – 6.9	≥10.0	8.5 – 11.0	

**Hyperglycaemia** detected at any time **during pregnancy** should be classified as either:

- Diabetes mellitus in pregnancy
- Gestational DM in pregnancy

**NB: The use of the 50 g and 100 g OGTT tests have been abandoned in favour of the 75 g OGTT.**

Guidelines for use of HbA1c levels to diagnose DM in pregnancy are not yet established.

## Factors influencing HbA1c measurement (consider when HbA1c and glucose results are discrepant)

	<b>Increased HbA1c</b>	<b>Decreased HbA1c</b>
<b>Erythropoiesis</b>	Iron deficiency, vitamin B12 deficiency, decreased erythropoiesis	Administration of iron, B12 or erythropoietin, reticulocytosis, chronic liver disease
<b>Altered haemoglobin</b>		Haemoglobin variants do not directly cross-react with assays used, but lead to decreased erythrocyte lifespan, HbF is often measured as part of total Hb
<b>Erythrocyte destruction</b>	Increased erythrocyte lifespan: Splenectomy	Decreased erythrocyte life span: Haemoglobinopathies, haemolytic anaemia or other causes of haemolysis e.g. splenomegaly or malaria, RA or drugs (antiretrovirals, ribavirin and dapsone)
<b>Glycation</b>	Alcoholism, chronic renal failure	Aspirin, vitamin C and E, certain haemoglobinopathies, increased intra-erythrocyte pH

### HbA1c targets for glycaemic control:

- The optimal **HbA1c target** for the majority of diabetic patients is **7.0%** tested at **6 monthly intervals**.
- A lower HbA1c target of **6.5%** is advocated for younger, newly diagnosed patients at low risk for CVD.
- A higher HbA1c target of **7.5%** is applicable to the elderly, patients at high risk for CVD, those with poor short-term prognosis or hypoglycaemic unawareness.
- Although HbA1c may not be used for diagnosis of **Gestational Diabetes Mellitus**, it can be used for monitoring treatment with a lower target level of 6%.

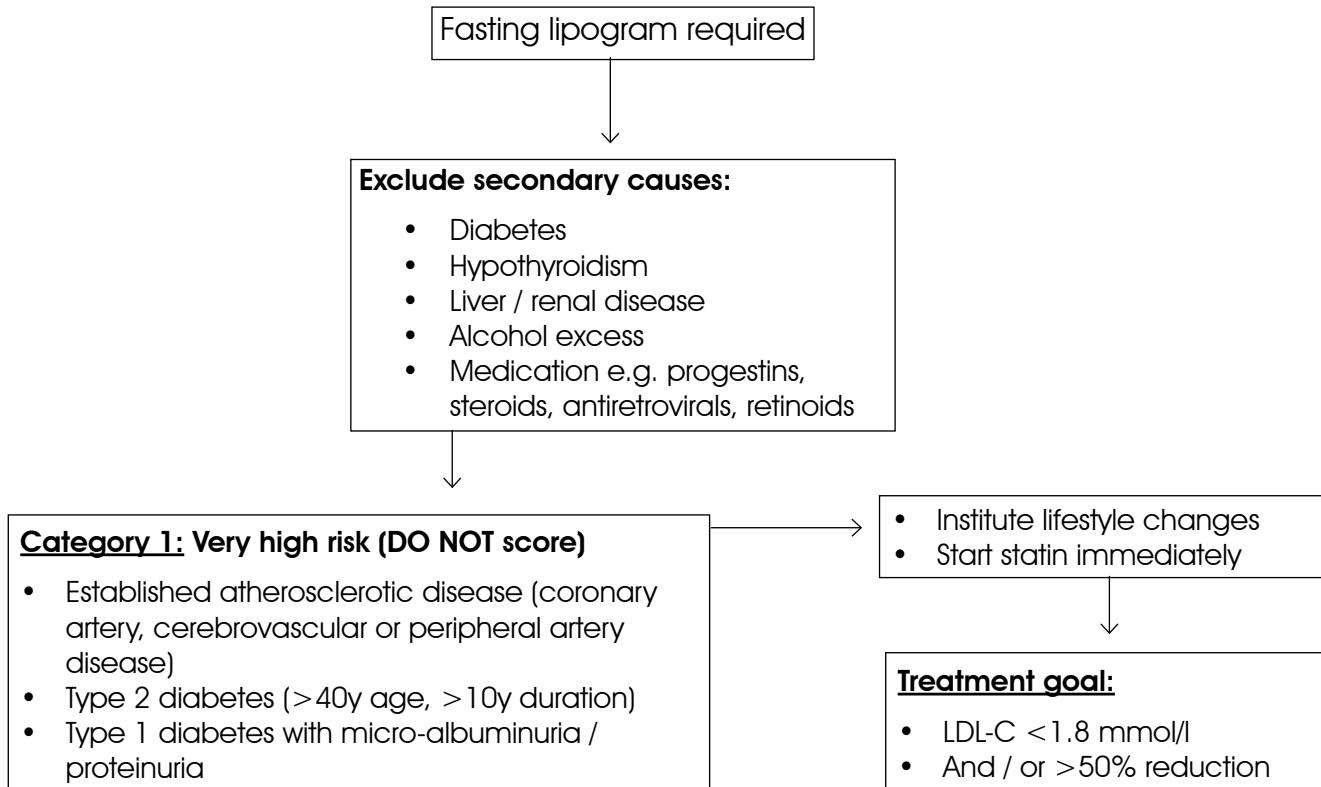
## Diagnostic criteria for insulin resistance syndrome / metabolic syndrome

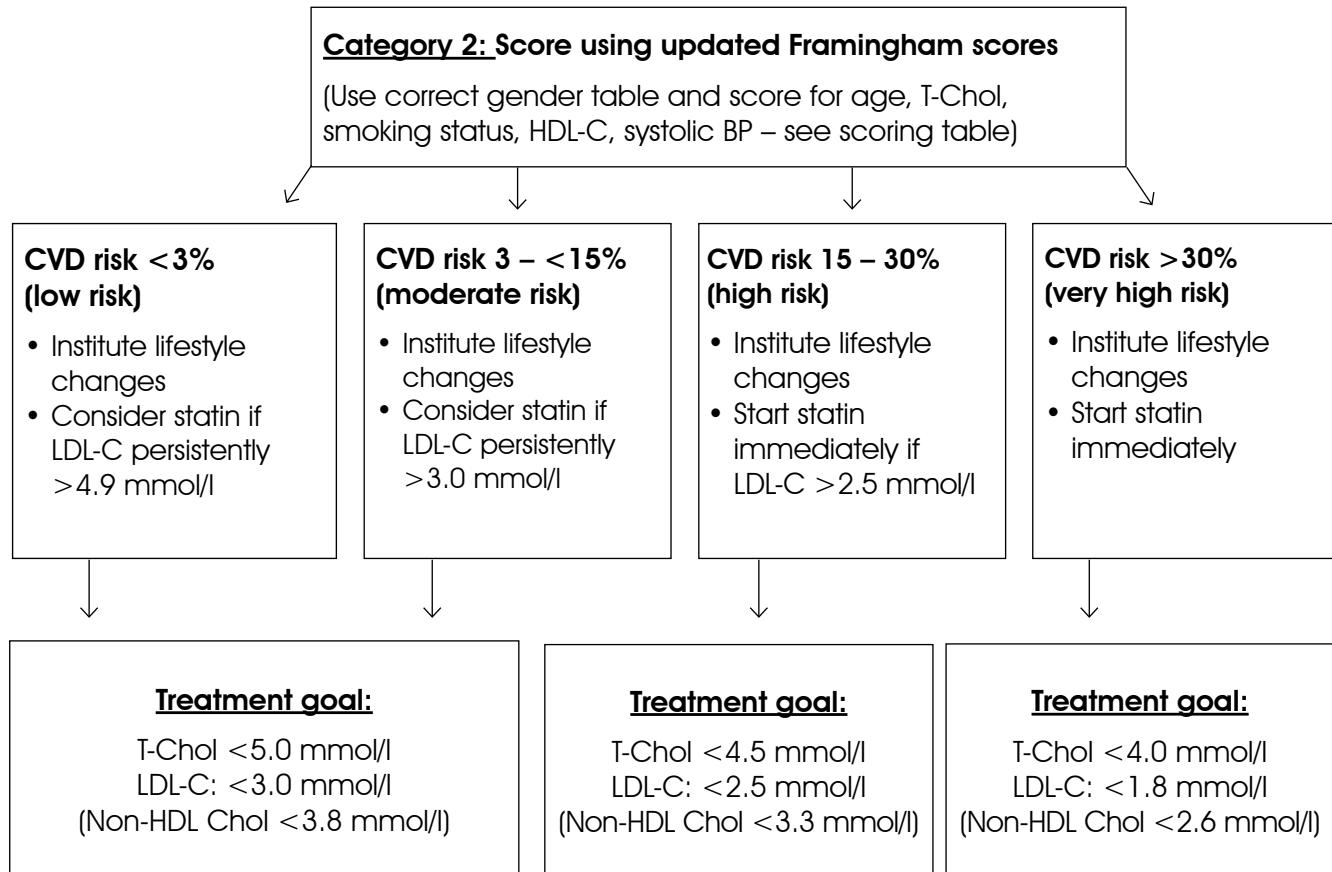
The diagnosis of the metabolic syndrome requires increased waist circumference plus any 2 of the other criteria according to the International Diabetes Federation (IDF) definition.

Parameter	Categorical cut points
Elevated waist circumference	$\geq 94$ cm in men $\geq 80$ cm women
Elevated Triglycerides	$\geq 1.7$ mmol/l or Drug treatment for elevated TG
Reduced HDL – Cholesterol	$<1.0$ mmol/l men $<1.3$ mmol/l women
Elevated blood pressure	$\geq 130$ mm Hg systolic BP or $\geq 85$ mm Hg diastolic BP or Drug treatment for hypertension
Elevated fasting glucose	$\geq 5.6$ mmol/l or Previously diagnosed type 2 diabetes mellitus

## Lipid metabolism

### CVD Risk Stratification and Cholesterol Targets (SA Dyslipidaemia Guidelines 2012)





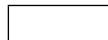
**Framingham 10-year risk assessment chart for patients without diabetes****Risk of CVD: coronary heart disease, stroke, peripheral artery disease or heart failure**

Estimate of 10-year risk of CVD for men		Estimate of 10-year risk of CVD for women	
Age (years)	Points	Age (years)	Points
30 – 34	0	30 – 34	0
35 – 39	2	35 – 39	2
40 – 44	5	40 – 44	4
45 – 49	6	45 – 49	5
50 – 54	8	50 – 54	7
55 – 59	10	55 – 59	8
60 – 64	11	60 – 64	9
65 – 69	12	65 – 69	10
70 – 74	14	70 – 74	11
75 years or older	15	75 years or older	12
Total cholesterol (mmol/l)	Points	Total cholesterol (mmol/l)	Points
<4.10	0	<4.10	0
4.10 – 5.19	1	4.10 – 5.19	1
5.20 – 6.19	2	5.20 – 6.19	3
6.20 – 7.20	3	6.20 – 7.20	4
>7.20	4	>7.20	5
HDL – cholesterol (mmol/l)	Points	HDL – cholesterol (mmol/l)	Points
≥1.50	-2	≥1.50	-2
1.30 – 1.49	-1	1.30 – 1.49	-1

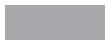
Estimate of 10-year risk of CVD for men		Estimate of 10-year risk of CVD for women	
HDL – cholesterol (mmol/l)	Points	HDL – cholesterol (mmol/l)	Points
1.20 – 1.29	0	1.20 – 1.29	0
0.90 – 1.19	1	0.90 – 1.19	1
<0.90	2	<0.90	2
Systolic BP – untreated (mmHg)	Points	Systolic BP – untreated (mmHg)	Points
<120	-2	<120	-3
120 – 129	0	120 – 129	0
130 – 139	1	130 – 139	1
140 – 159	2	140 – 149	2
≥160	3	150 – 159	4
		≥160	5
Systolic BP – on antihypertensive treatment (mmHg)	Points	Systolic BP – on antihypertensive treatment (mmHg)	Points
<120	0	<120	-1
120 – 129	2	120 – 129	2
130 – 139	3	130 – 139	3
140 – 159	4	140 – 149	5
≥160	5	150 – 159	6
		≥160	7
Smoker	Points	Smoker	Points
No	0	No	0
Yes	4	Yes	3

Points total for men		Points total for women	
Points total	10-year risk (%)	Points total	10-year risk (%)
-3 or less	<1	-2 or less	<1
-2	1.1	-1	1.0
-1	1.4	0	1.1
0	1.6	1	1.5
1	1.9	2	1.8
2	2.3	3	2.1
3	2.8	4	2.5
4	3.3	5	2.9
5	3.9	6	3.4
6	4.7	7	3.9
7	5.6	8	4.6
8	6.7	9	5.4
9	7.9	10	6.3
10	9.4	11	7.4
11	11.2	12	8.6
12	13.2	13	10.0
13	15.6	14	11.6
14	18.4	15	13.5
15	21.6	16	15.6
16	25.3	17	18.1
17	29.4	18	20.9
18 or more	>30	19	24.0
		20	27.5
		20 or more	>30

Point totals indicate the 10-year risk of cardiovascular disease  
(coronary, cerebrovascular and peripheral)



Low risk



High risk



Moderate risk



Very high risk

Adapted from D'Agostino RB, *et al.*, General cardiovascular risk profile for use in primary care: The Framingham Heart Study. *Circulation* 2008;117:743-753 and Mosca L, *et al.*, Effectiveness-based guidelines for the prevention of cardiovascular disease in women 2011 update: A guideline from the American Heart Association. *Circulation* 2011;123:1243-1262.

## Laboratory tests for dyslipidaemia

### Screening

Analyte	Indications
Random total cholesterol	Asymptomatic patient without known secondary causes for dyslipidaemia.
Fasting lipogram	Random total cholesterol $> 5$ mmol/l. Secondary causes of dyslipidaemia. Existing cardiovascular disease. Family history of premature cardiovascular disease (men $< 55$ years, women $< 65$ years). Signs of dyslipidaemia.

### Conditions for fasting lipogram

Condition	Time period
No eating, drinking (except water), smoking	12 hours before test
Usual diet, weight and activity level	At least 2 weeks
No major illness or surgery	At least 3 months
No pregnancy	At least 6 weeks

### Additional testing which might be indicated if lipogram is abnormal

1. Urine dipstick
2. Serum creatinine and eGFR
3. TSH
4. Fasting glucose and / or HbA1c

### Additional testing which might be indicated if lipogram is abnormal (continued)

5. Liver function tests
6. CK
7. Apo B especially in diabetes mellitus and metabolic syndrome

### Other lipid parameters

Analyte	Ref.Range	Units	Interpretation
Non-HDL cholesterol	Refer to risk groups for therapeutic targets (p.32)	mmol/l	Non-HDL cholesterol provides an estimate of the total number of atherogenic particles in plasma including LDL, VLDL, IDL and Lipo(a). Non-HDL cholesterol is recommended as secondary target for treatment especially in patients with diabetes mellitus, metabolic syndrome and chronic kidney disease, where hypertriglyceridaemia / mixed hyperlipidaemia is commonly found. Therapeutic targets are 0.8 mmol/l above LDL targets.
Lipoprotein (a)	<75	nmol/l	↑: Genetic. Secondary causes include uncontrolled diabetes mellitus, hypothyroidism, chronic renal failure, nephrotic syndrome.
Apolipoprotein A-I	>1.2	g/l	↓: Familial causes, hepatocellular disorders, cholestasis, nephrotic syndrome, chronic renal failure, malignancy.
Apolipoprotein B	<1.2	g/l	↑: Familial hypercholesterolaemia, familial combined hyperlipidaemia, polygenic (sporadic) hypercholesterolaemia, and other secondary causes of hypercholesterolaemia.

## Interpretation of abnormal lipid profiles

	TG	TC	HDLC	LDLC	Causes
Extreme hypercholesterolaemia	<2.5	>15	N	>13	<p><b>Primary:</b> LDL-receptor defect, Autosomal recessive hypercholesterolaemia.</p> <p><b>Secondary:</b> Nephrotic syndrome, cholestatic liver disease.</p>
Severe hypercholesterolaemia	<2.5	>7.5	N	>5	<p><b>Primary:</b> LDL-receptor defect, Apo B defect, PCSK9 defect, familial combined hyperlipidaemia (FCH).</p> <p><b>Secondary:</b> Hypothyroidism, nephrotic syndrome, chronic renal failure, diabetes mellitus type 2, pregnancy, cholestatic liver disease, primary biliary cirrhosis, acute intermittent porphyria, SLE, Cushing's syndrome.</p> <p><b>Medication:</b> Amiodarone, corticosteroids, immunosuppressants (e.g. cyclosporine), protease inhibitors, retinoids.</p>
Moderate hypercholesterolaemia	<2.5	>5	N	>3	<p><b>Primary:</b> Polygenic.</p> <p><b>Secondary:</b> Obesity, metabolic syndrome, hypothyroidism, nephrotic syndrome, chronic renal failure, diabetes mellitus type 2, pregnancy, obstructive liver disease, primary biliary cirrhosis, acute intermittent porphyria, SLE , Cushing's syndrome.</p> <p><b>Medication:</b> Amiodarone, corticosteroids, immunosuppressants (e.g. cyclosporine), loop diuretics, protease inhibitors, retinoids, thiazide diuretics, unopposed progestogens.</p>

	TG	TC	HDLC	LDLC	Causes
Hyper-alpha-lipoproteinaemia*	<2.5	>5	>2	N	<p><b>Primary:</b> Familial hyper-alpha-lipoproteinaemia.</p> <p><b>Secondary:</b> Oestrogens, alcohol, chronic hepatitis, primary biliary cirrhosis.</p>
Mixed hyperlipidaemia	1.7 – 5 1.7 – 5 1.7 – 5 1.7 – 5	>5 >5 >5 >5	L L L L	H L N H, N	<p><b>Primary:</b> FCH. Dysbetalipoproteinaemia. Metabolic syndrome.</p> <p><b>Secondary:</b> Obesity, hypothyroidism, diabetes mellitus type 2, poorly controlled diabetes mellitus type 1, nephrotic syndrome, pregnancy, SLE, chronic renal failure.</p> <p><b>Medication:</b> Combined oral contraceptives with second generation progestogens, immunosuppressants, loop diuretics, thiazide diuretics, protease inhibitors, retinoids.</p>
Moderate hypertriglyceridaemia	5 – 15	>5	L	VAR	<p><b>Primary:</b> Familial combined hyperlipidaemia, hereditary hypertriglyceridaemia.</p> <p><b>Secondary:</b> Obesity, diet, metabolic syndrome, diabetes mellitus type 2, poorly controlled diabetes mellitus type 1, chronic renal failure, hypothyroidism, alcohol abuse, nephrotic syndrome, pregnancy, viral hepatitis, biliary cirrhosis, extrahepatic biliary obstruction, infection, inflammation, HIV not on HAART.</p> <p><b>Medication:</b> Beta-blockers, clozapine, immunosuppressive drugs especially when combined with corticosteroids, loop diuretics, olanzapine, oral contraceptives, protease inhibitors, retinoids, tamoxifen, thiazide diuretics, unopposed oestrogens.</p>

	TG	TC	HDLC	LDLC	Causes
Severe hyper-triglyceridaemia	>15**	>5***	L	VAR	<p><b>Primary:</b> Familial combined hyperlipidaemia, lipoprotein lipase deficiency, Apo C2 deficiency.</p> <p><b>Secondary:</b> Obesity, diet, diabetes mellitus type 2, poorly controlled diabetes mellitus type 1, metabolic syndrome, chronic renal failure, hypothyroidism, alcohol abuse, nephrotic syndrome, pregnancy, viral hepatitis, biliary cirrhosis, extrahepatic biliary obstruction, infection, inflammation, HIV not on HAART.</p> <p><b>Medication:</b> Protease inhibitors, retinoids.</p>
Severe hypo-cholesterolaemia (low LDLC)	VAR	<2.5	VAR	<1.5	<p><b>Primary:</b> Abetalipoproteinaemia, hypobetalipoproteinaemia.</p> <p><b>Secondary:</b> Severe acute illness, infection, inflammation, malnutrition, malabsorption, malignancy, hyperthyroidism, hepatocellular necrosis, HIV not on HAART.</p>

\* HDLC >2.5 mmol/l may be associated with increased risk for atherosclerosis.

\*\* Triglyceride >15 mmol/l may trigger acute pancreatitis and is a medical emergency.

\*\*\* The most common cause of cholesterol >15 mmol/l is extreme hypertriglyceridaemia, i.e. the cholesterol is increased secondary to the increased triglycerides and often normalises when the triglycerides have normalised.

VAR Varies depending on cause

	TG	TC	HDLC	LDLC	Causes
Hypoalphalipo-proteinaemia (low HDLC)	VAR	VAR	<0.9	VAR	<p><b>Primary:</b> Tangier disease, fish eye disease, familial LCAT deficiency, familial CETP deficiency, familial Apo A1 deficiency.</p> <p><b>Secondary:</b> Obesity, metabolic syndrome, cigarette smoking, diabetes mellitus type 2, poorly controlled diabetes mellitus type 1, hepatocellular disorders, cholestasis, chronic renal failure, nephrotic syndrome, hypothyroidism, hyperthyroidism, malignancy, Cushing's syndrome, chronic illnesses, severe illnesses, Crohn's disease, coeliac disease, SLE, HIV not on HAART.</p> <p><b>Medication:</b> Beta-blockers, unopposed progestogens, anabolic steroids.</p>

VAR Varies depending on cause

## Effects of secondary causes of dyslipidaemia on the lipogram

	TC	LDLC	HDLC	TG
<b>LIFESTYLE</b>				
Alcohol	↔	↔	↑	↑
Cigarette smoking	↔	↔	↓	↔
Diet (high saturated fats and cholesterol)	↑	↑	↔	↑
Diet (high caloric intake, rapid weight gain, obesity)	↑	↑	↓	↑
Physical stress	↓	↓	↔	↑
<b>ENDOCRINOPATHIES</b>				
Diabetes mellitus type 1 (poor control)	↑	↑	↓	↑
Diabetes mellitus type 2	↑	↑	↓	↑
Hypothyroidism	↑	↑	↑ ↔ ↓	↔ ↑
Hyperthyroidism	↓	↓	↓	↓
Metabolic syndrome	↑	↑	↓	↑
Obesity	↑	↑	↓	↑
<b>GASTROINTESTINAL AND HEPATIC DISEASE</b>				
Acute Intermittent Porphyria	↑	↑	↔	↔
Cholestatic liver disease	↑	↑ ↔	↑	↑
Coeliac disease	↓	↓	↓	↔
Crohn's disease	↓	↓	↓	↔
<b>RENAL DISEASE</b>				
Chronic renal failure	↔	↔	↓	↑
Nephrotic syndrome	↑	↑	↔ ↓	↔ ↑

	TC	LDLC	HDLC	TG
<b>MISCELLANEOUS</b>				
HIV (untreated)	↓	↓	↓	↑
Pregnancy	↑	↑	↑	↑
SLE	↑	↑	↓	↑
Severe illness	↓	↓	↔↓	↑
<b>DRUGS</b>				
α-Blockers	↓	↓	↑	↓
Amiodarone	↑	↑	↔	↔
β-Blockers	↔	↔	↓	↑
Clozapine	↔	↔	↔	↑
Loop diuretics	↑	↑	↔	↑
Protease inhibitors	↑	N/A	↓	↑
Retinoids	↑	↑	↔↓	↑
Thiazide diuretics	↑	↑	↔	↑
Steroids	↑	↑	N/A	↑
Immunosuppressants	↑	↑	↑	↑
Combined oral contraceptives with:				
Second-generation progestogens	N/A	↑	↓	↑
Third-generation progestogens	N/A	↓	↑	↑
Hormone replacement therapy	↓	↓	↑ / ↔ / ↓	↑ / ↔ / ↓
Unopposed oestrogens	↓	↓	↑	↑
Unopposed progestogens	N/A	↑	↓	↓
Raloxifene	↓	↓	↔	↔
Tamoxifen	↓	↓	↔	↑

## Iron studies

Analyte	Ref. Range	Unit	Interpretation
Iron	M 11.6 – 31.3 F 9.0 – 30.4	µmol/l	↑: Pernicious and haemolytic anaemia, haemochromatosis, acute hepatitis, iron therapy, repeated blood transfusions. ↓: Iron deficiency anaemia, acute and chronic infections.
Transferrin	M 2.2 – 3.7 F 2.5 – 3.8	g/l	↑: Iron deficiency anaemia, exogenous oestrogen intake, pregnancy. ↓: Haemochromatosis, inflammation, hepatocellular disease, iron supplements.
Percentage transferrin saturation	M 20 – 50 F 15 – 50	%	↑: Haemochromatosis, secondary iron overload (liver disease, untreated pernicious anaemia, >50 blood transfusions). ↓: Iron deficiency anaemia, iron depletion, acute and chronic infections, some chronic disorders, rheumatoid arthritis.
Ferritin	M 20 – 250 F 10 – 120 (Beckman)	ng/ml	↑↑: Haemochromatosis, HIV, non-HIV chronic infection, liver disease, malignancy, renal disease, chronic transfusions. ↑: Infection, chronic inflammation, autoimmune disease (RA, SLE), megaloblastic anaemia.
	M 30 – 400 F 13 – 150 (Roche)		↓: Iron deficiency.

### Interpretation of iron profile

Condition	S-Iron	S-Transferrin	% Transferrin saturation	S-Ferritin
Iron deficiency anaemia	↓ / ↔	↑ / ↔ *	↓ / ↔	↓ / ↔ *
Iron overload e.g. Haemochromatosis	↑	↓ / ↔	↑	↑↑
Chronic disease	↓ / ↔	↓ / ↔	↔ / ↓	VAR
Acute disease	↓	↔	↓	↑

\*Concomitant infection/inflammation

Condition	S-Iron	S-Transferrin	% Transferrin saturation	S-Ferritin
Liver disease	↑	VAR	VAR	↑
Pernicious anaemia	↑	↔ / ↓	↑	↑

VAR – varies according to cause

A fasting morning specimen is preferred.

- In the earlier stages of haemochromatosis, the percentage transferrin saturation may be increased with a normal ferritin.
- Haemochromatosis PCR is available if haemochromatosis is suspected.
- Ferritin is a positive acute-phase protein.
  - In the presence of an acute-phase response a ferritin level below 50 ng/ml is regarded as an indication of depleted iron stores, whereas a level >100 ng/ml excludes iron deficiency.
  - A soluble transferrin receptor determination is recommended for ferritin levels between 50 ng/ml and 100 ng/ml to help distinguish between iron deficiency (increased) and an iron transfer block due to inflammation / chronic disease (normal).

## Folate and vitamin B12

Analyte	Ref. Range	Units	Interpretation
Serum Folate	10.0 – 45.1 (Beckman)	nmol/l	Serum folate is the test of choice for assessment of folate status. Red blood cell folate is only indicated in dialysis patients and where the serum folate is normal in the presence of clinical findings suggestive of deficiency. ↑: Excess daily intake, vitamin B12 deficiency.
	10.4 – 42.4 (Roche)		↓: Alcoholism, malnutrition, liver disease, adult coeliac disease, Crohn's disease, malabsorption, haemolytic anaemia, carcinoma, pregnancy.

Analyte	Ref. Range	Units	Interpretation
Red blood cell folate	317 – 1894 (Beckman)	nmol/l	↑: Excess daily intake. ↓: Alcoholism, malnutrition, liver disease, vitamin B12 deficiency, adult coeliac disease, Crohn's disease, malabsorption, haemolytic anaemia, carcinoma, pregnancy.
	1133 – 3413 (Roche)		
Vitamin B12	M 107 – 418 F 107 – 443 (Beckman)	pmol/l	↑: Chronic renal failure, severe congestive heart failure, diabetes mellitus, liver disease, certain leukaemias, certain carcinomas. ↓: Lack of intrinsic factor (pernicious anaemia, total or partial gastrectomy, atrophic gastritis, intrinsic factor antibody); malabsorption (pancreatic insufficiency, regional ileitis, coeliac disease); dietary deficiency (vegetarians).
	M/F 156 – 698 (Roche)		

## ENDOCRINOLOGY

### Thyroid function tests

Manufacturer	Reference ranges		
	FT4 (pmol/l)	FT3 (pmol/l)	TSH (mIU/l)
Abbott	10 – 20	2.6 – 5.7	0.35 – 4.2
Beckman	7.6 – 16.1	3.5 – 5.4	0.35 – 3.5
Roche	12 – 22	3.1 – 6.8	0.27 – 4.2

## Interpretation of thyroid function tests

Clinical condition	Combination of results		
	FT4 (pmol/l)	FT3 (pmol/l)	TSH (mIU/l)
Subclinical hypothyroidism	N	N	↑ (Treat if > 10)
Primary hypothyroidism	↓↓	N / ↓	↑
Secondary hypothyroidism	↓↓	N / ↓	N / ↓
Subclinical hyperthyroidism	N	N	↓↓ (Suppressed)
Primary hyperthyroidism / excessive thyroid hormone replacement	↑	↑↑	↓↓ (Suppressed)
Secondary hyperthyroidism / thyroid hormone resistance (both very rare)	↑	↑↑	N / ↑
Euthyroid sick syndrome	↓ / N / ↑	↓↓	↓ / N / ↑ (Recovery)
Amiodarone, salicylate, heparin, NSAID's	↑↑	↑	N
Phenytoin / glucocorticoids	N	N	↓↓ (Suppressed)
Lithium	↓	↓↓	↑
Phenytoin, Carbamazepine, Rifampicin, Tertroxin	↓	N	N

Analyte	Ref. Range	Units	Interpretation
Thyroid peroxidase antibodies	<10 (Beckman)	IU/ml	↑: Autoimmune thyroid disease (Hashimoto's thyroiditis, Graves' disease, idiopathic myxoedema), increased levels can occur in 8 – 27% of individuals with no symptoms of disease.
	<35 (Roche)		
Thyroglobulin antibodies	<116 (Roche)	IU/ml	↑: Hashimoto's thyroiditis, thyroid carcinoma, some cases of thyrotoxicosis, pernicious anaemia, SLE, De Quervain's subacute thyroiditis, 10% of the normal population may have a moderate increase.
TSH receptor antibodies	<1.75 (Roche)	IU/l	↑: Graves' disease
Thyroglobulin	3.5 – 77.0 (Roche)	ng/ml	↑: Hashimoto's thyroiditis, Graves' disease, thyroid adenoma, subacute thyroiditis. Also used as a tumour marker for monitoring recurrence of thyroid cancer.

### Goals for thyroid hormone replacement:

**Ideal TSH:** 0.50 – 2.00 mIU/l (1.50 – 3.00 mIU/l with underlying CVD / elderly).

**Eltroxin:** FT4: high normal – mildly increased.

**Tertroxin:** FT3: high normal – mildly increased.

## Other endocrinology tests

Analyte	Ref. Range	Units	Interpretation
17-hydroxy – Progesterone	M 1.9 – 6.52 F Follicular phase 0.97 – 4.45 Luteal phase 0.76 – 8.79 Oral contraceptive 0.60 – 5.75 Post menopausal 0.56 – 2.15 (Beckman RIA)	nmol/l	↑: Congenital adrenal hyperplasia, some cases of adrenal or ovarian neoplasms.
Acetylcholine receptor antibodies	0 – 0.24 (IBL RIA)	nmol/l	>0.41: Myasthenia gravis 0.25 – 0.40: Autoimmune diseases other than Myasthenia Gravis.
ACTH	1.6 – 13.9 (Roche)	pmol/l	↑: Adrenal insufficiency, Cushing's disease, ectopic ACTH-producing tumour. ↓: Pituitary insufficiency, adrenal Cushing's syndrome.
Active aldosterone: renin ratio	≤80		Ratio >80 is suggestive of primary hyperaldosteronism (Conn's syndrome).

Analyte	Ref. Range	Units	Interpretation
Aldosterone	Resting 49 – 644 Active 70 – 1087  (DiaSorin Liaison)	pmol/l	<p>↑: Primary hyperaldosteronism / Conn's syndrome (renin ↓).</p> <p>↑: <u>Secondary hyperaldosteronism</u> (renin ↑)  <i>With hypertension:</i>            Renal artery stenosis, unilateral renal disease with severe hypertension, high-renin forms of hypertension, renal parenchymal disease, oral contraceptive-induced hypertension, phaeochromocytoma.  <i>Without hypertension:</i>            Congestive heart failure, liver cirrhosis, nephrotic syndrome</p> <p>↓: Addison's disease, diabetic nephropathy, renal failure, drugs (e.g. ACE inhibitors).</p>
Androstenedione	M 2.1 – 10.8 F 1.0 – 11.5 (Siemens Immulite)	nmol/l	↑: Polycystic ovarian syndrome, congenital adrenal hyperplasia, Cushing's syndrome, hyperplasia of ovarian stroma, ovarian tumour, cyproterone acetate therapy.
Bone alkaline phosphatase	M 3.7 – 20.9  F Premenopausal 2.9 – 14.5 Postmenopausal 3.8 – 22.6  (Beckman)	µg/l	<p>↑: Postmenopausal, Paget's disease, hyperthyroidism, hyperparathyroidism, acromegaly, bone malignancies (bone forming), bone fractures (recovery phase), osteoporosis (sometimes, especially high turnover type).</p> <p>Increased S-BALP normalises 3 – 6 months after successful therapy.</p>

Analyte	Ref. Range	Units	Interpretation
Cortisol midnight (saliva)	M / F ≤9.7 (IBL)	nmol/l	↑: Cushing's syndrome – useful for screening. Samples should not be collected when any oral disease or inflammation is present (blood contamination) or when taking any exogenous corticosteroids (including oral, nasal, aerosol or topical preparations).
Cortisol (serum)	08h00 – 10h00: After 17h00: 24h00: (Abbott)	101 – 535 79 – 477 <50	↑: Cushing's syndrome, ↑ oestrogen (oral contraceptives, oestrogen administration, pregnancy), depression and other psychiatric conditions, alcohol dependence, morbid obesity, poorly controlled diabetes mellitus, physical stress (hospitalisation, surgery, pain), malnutrition, anorexia nervosa, intense chronic exercise, exogenous hydrocortisone administration (usually intravenous) due to cross-reactivity with the method.  ↓: Addison's disease, congenital adrenal hyperplasia, exogenous corticosteroids [oral (e.g. Celestamine, Meticorten); local injections with betamethasone (e.g. Celestone, Betanoid, Lenasone, Diprosone), methylprednisolone (Depo-Medrol), dexamethasone (Decasone, Dexona); inhalation].
	08h00 – 10h00: 16h00 – 20h00: 24h00: (Beckman)	185 – 624 <276 <50	
	08h00 – 10h00: 16h00 – 20h00: 24h00: (Roche)	142 – 651 51 – 424 <50	

Analyte	Ref. Range		Units	Interpretation
Cortisol (24-hour urine)	M 11.6 – 165.6 F 8.3 – 118.7  (LC-MS)		nmol/ 24h	↑: Cushing's syndrome, other causes as listed under ↑ serum cortisol except ↑ oestrogen.
DHEA-S	M 21 – 30 years 2.3 – 13.9 31 – 40 years 2.9 – 12.6 41 – 50 years 1.9 – 13.4 51 – 54 years 1.0 – 8.5 55 – 60 years 1.1 – 8.6 61 – 70 years 0.7 – 6.6 ≥71 years 0.1 – 6.9  F 21 – 30 years 0.5 – 10.6 31 – 40 years 0.6 – 7.2 41 – 50 years 0.5 – 6.3 51 – 54 years 0.2 – 5.1 55 – 60 years 0.4 – 6.0 61 – 70 years 0.3 – 3.6 ≥71 years 0.2 – 4.8  (Beckman)		μmol/l	↑: Polycystic ovarian syndrome, congenital adrenal hyperplasia, adrenal cortex adenomas and carcinomas, Cushing's disease, ectopic ACTH-producing tumors. ↓: Primary and secondary adrenal insufficiency.

Analyte	Ref. Range	Units	Interpretation	
DHEA-S	M 15 – 19 years 20 – 24 years 25 – 44 years 45 – 54 years 55 – 64 years 65 – 74 years ≥75	1.4 – 14.2 5.1 – 13.5 1.8 – 13.9 1.0 – 9.9 1.1 – 8.6 0.9 – 6.9 0.4 – 5.6	μmol/l	
	F 15 – 19 years 20 – 24 years 25 – 44 years 45 – 54 years 55 – 74 years ≥75 (Roche)	1.5 – 11.4 3.9 – 11.5 1.5 – 10.8 0.8 – 8.7 0.4 – 6.0 0.2 – 4.5		
FSH	M 1.0 – 12.0 (Abbott)  M 1.3 – 19.3 (Beckman)  M 1.2 – 15.8 (Roche)  F See ovarian profile table	IU/l	↑: Testicular absence or failure, ovarian absence or premature failure, menopause. ↓: Anterior pituitary hypofunction, hypothalamic disorders, hyperprolactinaemia, polycystic ovarian syndrome, severe illness, pregnancy, oral contraceptives.	

Analyte	Ref. Range		Units	Interpretation
Growth hormone	M 0 – 0.97 F 0.01 – 3.61 (Ultrasensitive Beckman)		µg/l	↑: Gigantism, acromegaly, exercise, stress, prolonged fasting, uncontrolled diabetes mellitus, hypoglycaemia, renal failure, liver cirrhosis, malnutrition, anorexia nervosa. ↓: Pituitary dwarfism, hypopituitarism.
IGF-1 (Somatomedin)	M / F 18 years 163 – 584 19 years 141 – 483 20 years 127 – 424 21 years 116 – 358 26 years 117 – 329 31 years 115 – 307 36 years 109 – 284 41 years 101 – 267 46 years 94 – 252 51 years 87 – 238 56 years 81 – 225 61 years 75 – 212 66 years 69 – 200 71 years 64 – 188 76 years 59 – 177 81 years 55 – 166  (Siemens)		µg/l	↑: Acromegaly. ↓: Growth hormone deficiency, hypopituitarism, malnutrition, anorexia, hepatocellular disease, hypothyroidism.

Analyte	Ref. Range	Units	Interpretation
LH	M 0.6 – 12.1 (Abbott)  M 1.2 – 8.6 (Beckman)  M 1.3 – 9.6 (Roche)	IU/l	↑: Testicular absence or failure, ovulation, ovarian absence or premature failure, polycystic ovarian syndrome, menopause. ↓: Anterior pituitary hypofunction, hypothalamic disorders, severe stress, severe illness, hyperprolactinaemia, pregnancy, oral contraceptives.
	F See ovarian profile table		
Oestradiol	M 40 – 161 (Abbott)  M 95-223 (Roche)	pmol/l	↑: Exogenous oestradiol, fertility treatment, perimenopausal, liver cirrhosis, hyperthyroidism, pregnancy. ↓: Anovulation, primary and secondary hypogonadism, postmenopausal, oral contraceptives.
	F See ovarian profile table		
Progesterone	M 0.7 – 4.7 (Abbott)  M 0.4 – 6.6 (Beckman)  M 0.7 – 4.7 (Roche)	nmol/l	↑: Pregnancy. ↓: Threatened abortion, primary or secondary hypogonadism, short luteal phase syndrome, oral contraceptives, postmenopausal.
	F See ovarian profile table		

Analyte	Ref. Range		Units	Interpretation
Prolactin	M $\geq 18$ years	3 – 13	$\mu\text{g/l}$	<p><math>\uparrow</math>: Prolactin-secreting pituitary tumours, hypothalamic-pituitary disease (e.g. craniopharyngioma), stress, primary hypothyroidism, polycystic ovarian disease, renal failure, adrenal insufficiency, anorexia nervosa, chest wall injury, medication (e.g. antipsychotics, monoamine oxidase inhibitors, opiates, tricyclic antidepressants), macroprolactinaemia.</p> <p><math>\downarrow</math>: Pituitary apoplexy (Sheehan's syndrome).</p>
	F    18 – 49 years $\geq 50$ years (Beckman)	3 – 27 3 – 20		

Analyte	Ref. Range	Units	Interpretation
Renin	Resting 2.2 – 45.4 Active 3.5 – 56  (DiaSorin Liaison)	mU/l	<p>↑: With secondary hyperaldosteronism and hypertension: Renal artery stenosis, unilateral renal disease with severe hypertension, high-renin forms of hypertension, renal parenchymal disease, oral contraceptive-induced hypertension, phaeochromocytoma.</p> <p>↑: With secondary hyperaldosteronism, oedema and normal BP: congestive heart failure, liver cirrhosis, nephrotic syndrome.</p> <p>↑: Without secondary hyperaldosteronism – Addison's disease, potassium depletion, ACE inhibitors, angiotensin receptor antagonist.</p> <p>↑: With secondary hyperaldosteronism and hypovolemia: Vomiting, diarrhoea, blood loss, diuretics, etc.</p> <p>↓: With hypertension: primary hyperaldosteronism, low-renin essential hypertension, sometimes with renal parenchymal disease.</p>
Serotonin	M / F 70 – 270  (Fast Track RIA)	ng/ml	<p>↑: Carcinoid syndrome, dumping syndrome, acute intestinal obstruction, cystic fibrosis, nontropical sprue.</p> <p>↓: Severe depression, Parkinson's disease</p>

Analyte	Ref. Range		Units	Interpretation
SHBG	M 18 – 19 years ≥20 years	13.0 – 88.0 13.3 – 89.5	nmol/l	↑: Pregnancy, exogenous oestrogen, hyperthyroidism, liver cirrhosis, advancing age. ↓: Hypothyroidism, excess testosterone, obesity, polycystic ovarian syndrome.
	F 18 – 46 years ≥47 years (Beckman)	18.2 – 135.5 16.8 – 125.2		
Testosterone Total	M ≥18 years F 18 – 49 years ≥50 years (Roche)	11.4 – 52.3 19.8 – 122 14.1 – 68.9	nmol/l	
	M 18 – 30 years 31 – 49 years ≥50 years (Beckman)	9.0 – 28.3 6.9 – 23.6 6.1 – 18.7		
Testosterone Free (Calculated)	M ≥18 years (Beckman)	<2.7	pmol/l	
	M 17 – 49 years ≥50 years F ≥17 years (Roche)	8.0 – 27.1 8.0 – 18.7 <1.9		
	M 18 – 49 years ≥50 years F 18 – 46 years ≥47 years (Beckman)	170 – 660 170 – 419 6.0 – 55.0 2.0 – 33.0		↑: Polycystic ovarian syndrome, idiopathic hirsutism, virilising adrenal tumours, virilising ovarian tumours, Cushing's syndrome, congenital adrenal hyperplasia, exogenous testosterone administration, obesity. ↓: Primary (LH / FSH ↑) and secondary hypogonadism (LH / FSH ↓).
	M 17 – 49 years ≥50 years F 17 – 50 years ≥50 years (Roche)	180 – 536 180 – 419 1.0 – 34.0 1.0 – 22.0		

## Ovarian profile

These tests should be evaluated in conjunction with the clinical picture (pre-pubertal, phase of the menstrual cycle in menstruating women, menopause, etc).

To confirm ovulation progesterone determination should be done on day 21 of the menstrual cycle.

Ref. Ranges (Abbott)	FSH	LH	Oestradiol	Progesterone
Follicular	3.0 – 8.1	1.8 – 11.8	77 – 921	<9.3
Midcycle	2.6 – 16.7	7.6 – 89.1	139 – 2382	2.4 – 9.4
Luteal	1.4 – 5.5	0.6 – 14.0	77 – 1145	4.5 – 111
Oral contraceptive		<5.7	<104	
Post menopausal	26.7 – 133.4	5.2 – 62.0	<104	<2.7
Pregnancy first trimester	<0.1	<0.1		33 – 140
Prepubertal	0.7 – 6.7	<4.0	<100	<1.8

Ref. Ranges (Beckman)	FSH	LH	Oestradiol	Progesterone
Follicular	3.9 – 8.8	2.1 – 10.9	99 – 448	1.0 – 4.8
Midcycle	4.5 – 22.5	19.2 – 103	180 – 1068	
Luteal	1.8 – 5.1	1.2 – 12.9	349 – 1589	16.4 – 59
Oral contraceptive		<5.7	<145	
Post menopausal	16.7 – 113.6	10.9 – 58.6	<148	<2.5
Pregnancy first trimester			3670 – >13216	15 – 161
Prepubertal	0.7 – 6.7	<4.0	<100	<1.8

Ref. Ranges (Roche)	FSH	LH	Oestradiol	Progesterone
Follicular	2.9 – 14.6	1.9 – 14.6	45 – 854	<9.3
Midcycle	4.7 – 23.2	12 – 118	151 – 1461	2.4 – 9.4
Luteal	1.4 – 8.9	0.7 – 12.9	82 – 1251	4.5 – 111
Oral contraceptive		<5.7	<140	
Post menopausal	16 – 157	5.3 – 65.4	<184	<2.7
Pregnancy first trimester	<0.1	<0.1	563 – 11902	33 – 140
Prepubertal	0.7 – 6.7	<4.0	<150	<1.8

## Anti-Müllerian Hormone

### Use of AMH in males

A measurable value in a boy or adult male with bilateral cryptorchidism is predictive of undescended testes, whereas an undetectable level is highly suggestive of anorchia.

### Use of AMH in female patients

Since AMH is produced continuously in the granulosa cells of small follicles, the AMH level can be used to assess the ovarian reserve. Although there are slight fluctuations of AMH during the menstrual cycle, it is not considered clinically significant enough to recommend sampling during a specific phase. Although oral or vaginal oestrogen- or progestin-based contraceptives show minimal effect, it is preferable to determine AMH levels after discontinuation of the medication for at least 4 weeks. After puberty, AMH concentration declines slowly over the reproductive lifespan as the size of the pool of follicles decrease and frequently reach undetectable levels after natural or premature menopause.

## Use of AMH for IVF treatment

Fertility studies have shown that females with higher AMH concentrations (indicating better ovarian reserve) have a better response to ovarian stimulation and tend to produce more retrievable oocytes than those with low or undetectable levels. Significantly elevated AMH levels can be used to identify females at risk of ovarian hyperstimulation syndrome following gonadotropin administration.

The following values can be used to predict the response following ovarian stimulation, but should be used in combination with clinical and ultrasound findings (antral follicular count). These values have been adjusted for the new automated method (Beckman Coulter DXI) implemented by Ampath during March 2015.

Suggested cut-off values		
ng/ml	pmol/l	Response to ovarian stimulation treatment
<0.17	<1.2	Negligible / non-responders
0.17 – 1.21	1.2 – 8.6	Reduced / poor response ( $\leq 2$ eggs at oocyte retrieval)
1.22 – 2.30	8.7 – 16.4	Normal response (3 – 20 eggs at oocyte retrieval)
>2.30	>16.4	High / excessive response ( $> 20$ eggs at oocyte retrieval)

In patients with polycystic ovarian syndrome (PCOS), serum AMH levels may be elevated due to large numbers of small follicles. AMH levels have subsequently also been shown to be affected by differences in body mass index and insulin levels.

## Use of AMH as tumour marker for granulosa cell tumours of the ovaries

Serum AMH concentrations may be increased in patients with ovarian granulosa cell tumours which comprise approximately 10% of all ovarian tumours. AMH combined with CA 125 may be useful for monitoring response to treatment and follow up of patients with these tumours.

## Pregnancy

### Interpretation of $\beta$ HCG results

Analyte	Ref. Range		Units	Interpretation
$\beta$ HCG	0 – 4.9	Negative	mIU/ml	Positive 8 – 11 days after conception.
	5 – 25	Equivocal		$\beta$ HCG reaches 25 IU/l in 50% of pregnant women on the first day of their missed period.
	>25	Positive		

### Screening for foetal anomalies in the first and second trimesters of pregnancy

- For all tests listed below, a completed Ampath Down's screening request form should be submitted, as calculated risks are dependent on the accuracy of information provided by the referring clinician.
- Calculated risks are screening tests and NOT diagnostic.

#### First-trimester screening (FTDS)

- Has better sensitivity than second-trimester screening
- Combined risk (with Nuchal Translucency (NT) measurement) has better sensitivity than biochemistry risk (without NT measurement)
- Biochemistry risk can be done 8w 0d – 13w 6d
- Combined risk can be done 10w 6d – 13w 6d
- Includes Trisomy 21 (Down's syndrome) and Trisomy 18 (Edward's syndrome)
- Does not include neural tube defects.

#### Second-trimester screening (STDS)

- Should not be done if FTDS has already been done (integrated first- and second-trimester screening is not available at Ampath yet)
- Can be done 15w 0d – 20w 6d
- Includes Trisomy 21, Trisomy 18 and neural tube defects

### Neural tube defect (NTD) screening

- Should be done if FTDS has been done
- Can be done 15w 0d – 20w 6d

### Optimal step wise approach for best sensitivity:

- Step 1: 8w 0d – 9w 6d: Blood collection for Biochemistry Risk (request FTDS)
- Step 2: 10w 6d – 13w 6d: Sonar to measure NT for Combined Risk, no blood collection, no cost (request FTDS2)
- Step 3: 15w 0d – 20w 6d: Blood collection for NTD

## TUMOUR MARKERS

### Please note:

Tumour markers should not be used for screening for malignancy. An increased marker does not necessarily indicate the presence of a tumour, whilst a normal marker does not indicate the absence of a tumour.

### Tumour marker reference ranges

Marker	Ref. Range	Units	Specimen
AFP	0 – 10	µg/l	Blood
β-2-microglobulin	1.1 – 2.5	mg/l	Blood
CA 125	0 – 35	U/ml	Blood
CA 15–3 (Abbott)	0 – 31	U/ml	Blood
CA 15–3 (Beckman)	0 – 23	U/ml	Blood
CA 15–3 (Roche)	0 – 34	U/ml	Blood

Marker	Ref. Range	Units	Specimen
CA 19-9	0 – 37	U/ml	Blood
CA 72-4	0 – 6.9	U/ml	Blood
Calcitonin	M 0 – 8.4 F 0 – 5.0	ng/l	Blood
Catecholamines	Adrenaline 0 – 109 Noradrenaline 89 – 473 Dopamine 424 – 2612	nmol/d	24-hour urine
CEA	0 – 5	ng/ml	Blood
Chromogranin A	0 – 85	ng/ml	Blood
Ferritin	M 20 – 250 F 10 – 120 (Beckman)	ng/ml	Blood
	M 30 – 400 F 13 – 150 (Roche)		
Gastrin	0 – 115 (>500 diagnostic of gastrinoma)	ng/l	Blood
HCG	0 – 5 (Values up to 11.6 may be seen in post-menopausal females)	lU/ml	Blood
HIAA	10.4 – 41.6 0 – 7.2	$\mu\text{mol}/\text{d}$ $\mu\text{mol}/\text{mmol}$	Random or 24-hour urine (with HCl as preservative)

Marker	Ref. Range	Units	Specimen	
HE4 (Human Epididymal protein 4)	18-39 y 40-49 y 50-59 y 60-69 >70	<60.5 <76.2 <74.3 <82.9 <104	pmol/l	Blood
ROMA-index	Premenopausal < 11.4 Postmenopausal <29.9 (see below)	%		
HVA	8 – 48 2 – 6.4	µmol/d µmol/mmol	Random or 24-hour urine (with HCl as preservative)	
Metanephries	160 – 2478 26 – 176	nmol/d nmol/mmol	24-hour urine (with HCl as preservative)	
Normetanephries	241 – 3418 21 – 312	nmol/d nmol/mmol	24-hour urine (with HCl as preservative)	
NSE	0 – 16	µg/l	Blood	
PSA	0 – 4	ng/ml	Blood	
Free PSA:PSA ratio	See table below		Blood	
S100-B	0.005 – 0.105	µg/l	Blood	
SCC	0.3 – 2.0	ng/ml	Blood	
Serotonin	70 – 270	ng/ml	Blood	

Marker	Ref. Range	Units	Specimen
Serum free light chains: Kappa Lambda Ratio	3.3 – 19.4 5.71 – 26.3 0.26 – 1.65	mg/l mg/l	Blood
Thyroglobulin	1.4 – 78	ng/ml	Blood
TPA	0 – 75	U/l	Blood
Urine light chains: Kappa Lambda Ratio	0 – 7.1 0 – 3.9 0.75 – 4.5	mg/l mg/l	Urine
VMA	11.5 – 34.6 0.7 – 4.3	$\mu\text{mol}/\text{d}$ $\mu\text{mol}/\text{mmol}$	24-hour urine (with HCl as preservative)

### Intended clinical use of HE4 and ROMA Index

- Risk assessment for ovarian cancer in patients with a pelvic mass (in conjunction with CA 125)
- Monitoring the treatment of patients with epithelial ovarian cancer (EOC)

### Risk assessment of ovarian cancer in patients with a pelvic mass

- When compared to CA 125, HE4 has better sensitivity in the early stages of EOC and better sensitivity to discriminate malignant from benign disease.

When combined, the ability of these markers to discriminate benign from malignant disease is further enhanced by the Risk of Ovarian Malignancy Algorithm (ROMA). The algorithm takes into account the HE4 and CA 125 values as well as menopausal status of the patient. The algorithm calculates the predictive probability of finding EOC on surgery in patients with pelvic masses.

A ROMA value of  $\geq 11.4\%$  in pre-menopausal women and a value of  $\geq 29.9\%$  in post-menopausal women are consistent with an increased risk to find malignancy on surgery.

Biomarker	Sensitivity at 95% specificity to discriminate benign from malignant disease
CA 125	43.3%
HE4	72.9%
CA 125 + HE4	76.4%

Distribution of patients in low- and high-risk groups Benign disease vs EOC and low-malignant potential tumours			
Menopausal status	Sensitivity	Specificity	Negative predictive value
Combined	88.7%	74.7%	93.9%
Pre-menopausal	76.5%	74.8%	95%
Post-menopausal	92.3%	74.7%	92.6%

### Interpretation of PSA Ratio (Free PSA:Total PSA)

1. PSA Ratio is useful with total PSA values of 2.5 – 10 ng/ml.
2. PSA Ratio is generally lower in carcinoma and higher in benign hyperplasia.  
 PSA ratio: >0.25 Probability for prostate cancer <10%.  
 <0.10 Probability for prostate cancer >80%.
3. Probability of finding prostate cancer on needle biopsy increases with increasing age and decreasing PSA ratios.

Probability of finding prostate cancer on needle biopsy:

Beckman method:

% FPSA	50 – 64 years	65 – 75 years
0 – 10 %	56%	55%
>10 – 15%	24%	35%
>15 – 20%	17%	23%
>20 – 25%	10%	20%
>25%	5%	9%

Abbott & Roche method:

% FPSA	50 – 59 years	60 – 69 years	≥ 70 years
<11%	49%	58%	65%
11 – 18%	27%	34%	41%
19 – 25%	18%	24%	30%
>25 %	9%	12%	16%

### Appropriate tumour markers for specific tumours

Tumour	Tumour marker
Breast	CEA, CA 15-3
Bladder	CEA, TPA
Biliary tract	CA 19-9
Colon	Stool occult blood, CEA, CA 19-9
Cervix	SCC, CEA
Choriocarcinoma	HCG
Carcinoid	24-hour urine 5-HIAA, Chromogranin A, Serotonin, NSE

Tumour	Tumour marker
Germ cell	AFP, HCG
Head and neck	SCC
Liver	AFP, CEA
Lung – small-cell lung cancer	NSE, TPA
Lung – non-small-cell lung cancer	TPA, CEA
Lymphoma, leukaemia	Ferritin, $\beta$ -2-microglobulin
Melanoma	S-100B
Myeloma	Serum protein electrophoresis, urine Bence Jones protein, serum free light chains
Neuroblastoma	Urine HVA, NSE, Chromogranin A
Oesophagus	SCC
Ovary	CA 125 and HE4 with ROMA-value (most common). Others CA 72-4, CEA, CA 19-9, CA 15-3
Pancreas	CA 19-9, CEA, CA 72-4 (less common)
Prostate	PSA, free PSA
Phaeochromocytoma	Urine meta- and normetanephrines, plasma meta- and normetanephrines, Chromogranin A
Stomach	CA 72-4
Testis	AFP, HCG, LDH, NSE
Thyroid	Thyroglobulin, CEA
Thyroid C-cell carcinoma	Calcitonin
Uterus	SCC, CA 125

The tumour marker list above is not a complete list and includes only the markers which occur most commonly in these tumours.

# THERAPEUTIC DRUGS

Drug	Ref. Range		Units	Time of sampling
<b>ANTIDEPRESSANTS</b>				
Amitryptyline / Tryptanol	Trough: Toxic:	100 – 250 >500	µg/l	Prior to next dose or minimum 12 hours post dose.
Fluoxetine / Prozac	Trough: Toxic:	30 – 500 >1000	µg/l	Prior to next dose.
Imipramine / Tofranil	Trough: Toxic:	150 – 250 >500	µg/l	Prior to next dose or minimum 12 hours post dose.
<b>ANTI-EPILEPTICS</b>				
Carbamazepine / Tegretol	Therapeutic: Toxic:	17 – 51 >63	µmol/l	Prior to next dose (to assess efficacy). For monitoring of possible toxicity a peak level is recommended (4 – 8 hours after oral dose).
Clonazepam / Rivotril	Therapeutic: Toxic:	20 – 80 >80	µg/l	Prior to next dose after time to steady state (7 days) has been reached.
Gabapentin / Neurontin	Therapeutic: Toxic:	2 – 20 >25	mg/l	Prior to next dose after time to steady state (1 – 2 days) has been reached.
Lamotrigine / Lamictin	Therapeutic: Toxic:	2.5 – 15 >20	mg/l	Trough (prior to next dose) after time to steady state has been reached: 3 – 6 days or 5 – 15 days with valproic acid comedication.
Levetiracetam / Keppra	Peak: Trough:	10 – 63 3 – 34	mg/l	Peak: 1 hour post dose. Trough: Prior to next dose.

Drug	Ref. Range	Units	Time of sampling
Phenobarbitone / Lethyl	Therapeutic: 65 – 172 Toxic: Slowness, ataxia, nystagmus Coma >172 >280	µmol/l	Prior to next dose (to assess efficacy). For monitoring of possible toxicity a PEAK level is recommended (4 – 10 hours after oral dose). Levels often increase after addition of valproic acid to the treatment regime, usually requiring an adjustment in dosage.
Phenytoin / Epanutin	Therapeutic: 40 – 79 Toxic: Lateral nystagmus Depressed mental capacity >79 >158	µmol/l	Prior to next dose (to assess efficacy). For monitoring of possible toxicity a peak level is recommended (4 – 8 hours after oral dose).
Topiramate / Topamax	Trough: 5 – 20 Toxic: not established	mg/l	Prior to next dose, preferably after steady state has been reached. Time to steady state: 4 – 5 days.
Valproic acid / Epilim	Therapeutic: 346 – 693 Toxic: >693	µmol/l	Prior to next dose (to assess efficacy). For monitoring of possible toxicity a peak level is recommended (1 – 4 hours after oral dose).

Drug	Ref. Range	Units	Time of sampling
<b>ANTI-INFECTIVES</b>			
<b>Antibacterials:</b>			
Amikacin	<p>PEAK Level: Intermittent dosing: 15 – 30 Single daily (pulse) dosing: 56 – 64</p> <p>TROUGH Level: Intermittent dosing: 5 – 10 Single daily (pulse) dosing: &lt;1</p>	µg/ml	<p>PEAK: 60 minutes after bolus IV / IM dose or 60 minutes after start of a 30-minute IV infusion.</p> <p>TROUGH: Immediately prior to next dose.</p>
Gentamycin	<p>PEAK Level: Intermittent dosing: 4 – 10 Single daily (pulse) dosing: 16 – 24</p> <p>TROUGH Level: Intermittent dosing: 1 – 2 Single daily (pulse) dosing: &lt;1</p>	µg/ml	<p>PEAK: 60 minutes after bolus IV / IM dose or 60 minutes after start of a 30-minute IV infusion.</p> <p>TROUGH: Immediately prior to next dose.</p>
Tobramycin	<p>PEAK Level: Intermittent dosing: 4 – 10 Single daily (pulse) dosing: 16 – 24</p> <p>TROUGH Level: Intermittent dosing: 1 – 2 Single daily (pulse) dosing: &lt;1</p>	µg/ml	<p>PEAK: 60 minutes after bolus IV / IM dose or 60 minutes after start of a 30-minute IV infusion.</p> <p>TROUGH: Immediately prior to next dose.</p>

Drug	Ref. Range	Units	Time of sampling
Vancomycin	PEAK Level: Intermittent dosing: 20 – 50  TROUGH Level: Non-serious infections : 5 – 10 Serious infections: 10 – 15 Cloxacillin-resistant S.aureus (MRSA) pneumonia: 15 – 20  CONTINUOUS INFUSION: Target Plateau level: 20 – 25	µg/ml	PEAK: 60 minutes after bolus IV / IM dose or 60 minutes after start of a 30 minutes IV infusion.  TROUGH: Immediately prior to next dose.

**Antimycotics:**

Voriconazole	Trough:	1 – 6	mg/l	Prior to next dose.
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**Antiparasitic:**

Quinine	Therapeutic: Toxic:	15.4 – 30.8 >30.8	µmol/l	
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**Antiretrovirals:**

Atazanavir / Reyataz	Minimum target trough concentration:	150	ng/ml	Prior to next dose.
Efavirenz / Stocrin	Minimum target trough concentration:	1000	ng/ml	Prior to next dose.
Indinavir / Crixivan	Minimum target trough concentration:	100	ng/ml	Prior to next dose.
Lopinavir / Kaletra / Aluvia	Minimum target trough concentration:	1000	ng/ml	Prior to next dose.

<b>Drug</b>	<b>Ref. Range</b>	<b>Units</b>	<b>Time of sampling</b>	
Nelfinavir / Viracept	Minimum target trough concentration: 800	ng/ml	Prior to next dose.	
Nevirapine / Viramune	Minimum target trough concentration: 3400	ng/ml	Prior to next dose.	
Ritonavir / Norvir	Minimum target trough concentration: 2100	ng/ml	Prior to next dose.	
Saquinavir / Invirase	Minimum target trough concentration: 100	ng/ml	Prior to next dose.	
Tipranavir / Aptivus	Minimum target trough concentration: 20500	ng/ml	Prior to next dose.	
<b>BRONCHODILATORS</b>				
Theophylline	Therapeutic: 0 – 30 Days $\geq$ 1 Month Toxic:	33 – 72 44 – 111 $>$ 111	$\mu$ mol/l	Immediately prior to next dose. After dosage adjustment, repeat sampling is recommended after 48 – 72 hours for oral dosing.
<b>CARDIAC</b>				
Digoxin	Therapeutic: Toxic:	1 – 2.6 $>$ 3.2	nmol/l	Prior to next dose ideally, but at least 8 – 12 hours after previous dose.
<b>PSYCHOLEPTICS</b>				
<b>Antipsychotics:</b>				
Clozapine / Leponex	Trough:	250 – 750	$\mu$ g/l	Prior to next dose.

Drug	Ref. Range	Units	Time of sampling	
Lithium	Therapeutic: Target concentrations: Acute mania Prophylaxis Toxic:	0.4 – 1.2 0.8 – 1.2 0.4 – 0.8 >1.5	mmol/l	24 hours after dose ideally, but at least 12 hours after previous dose.
<b>Benzodiazepines:</b>				
Chlordiazepoxide / Librium	Trough: Toxic:	400 – 3000 >3500	µg/l	Prior to next dose.
Clonazepam / Rivotril	Therapeutic: Toxic:	20 – 80 >80	µg/l	Trough or peak after time to steady state (7 days) has been reached Trough: prior to next dose. Peak: 1 – 4 hours post dose.
Diazepam / Valium	Therapeutic: Toxic:	200 – 1500 >3000	µg/l	Trough levels (prior to next dose) are most reproducible.
Flunitrazepam / Rohypnol	Peak: Toxic:	5 – 15 >50	µg/l	1 – 2 hours post dose.
Lorazepam / Ativan	Therapeutic: Toxic:	50 – 240 >300	µg/l	Trough levels (prior to next dose) are most reproducible.
Midazolam / Dormicum	Therapeutic: Toxic:	40 – 150 >1000	µg/l	Trough or peak after time to steady state (app. 6.5 – 12.5 hours) has been reached. Trough: prior to next dose Peak: 0.22 – 1.12 hours post oral dose.
Nitrazepam / Mogadon	Therapeutic: Toxic:	30 – 100 >299	µg/l	Trough levels (prior to next dose) are most reproducible.

Drug	Ref. Range	Units	Time of sampling
Oxazepam / Serepax	Peak: 200 – 1400 Toxic: >2000	µg/l	2 – 4 hours post dose.
Zolpidem / Stillnox	Therapeutic: <251	µg/l	Random. Expected concentration when taking therapeutic daily dose.

## CLINICAL TOXICOLOGY

### Paracetamol overdose / toxicity:

- The first specimen should not be taken earlier than 4 hours after paracetamol ingestion to ensure complete absorption. Serial sampling (2 – 3 samples, at 2 – 3 hour intervals) is recommended if the time of ingestion is uncertain or unreliable or if extended-release medication has been taken and may be used to estimate the paracetamol elimination half-life (probable hepatotoxicity risk if half-life more than 4 hours, hepatic coma risk if half-life more than 12 hours).

Hours after ingestion	Serum paracetamol levels (µmol/l)
	Toxic concentration
4	>993
6	>695
8	>497
10	>347
12	>248

- Acetylcysteine should be instituted if the patient's serum paracetamol concentration is above or near the toxic level for the time after ingestion.
- In substantial overdose ( $\geq 7.5$  g or  $\geq 125$  mg/kg) and in patients where the time of intake is doubtful, treatment should be started before paracetamol levels are available. The antidote may subsequently be discontinued if levels are below the toxic range.

- Patients presenting more than 24 hours after ingestion with detectable paracetamol levels should also be treated and hepatotoxicity ruled out.
- For patients on hepatic enzyme inducers (e.g. barbiturates, phenytoin, carbamazepine, rifampicin, meprobamate, alcohol abusers) or those with depleted glutathione (e.g. malnutrition and HIV) antidote therapy may be started at levels 25% lower than the above.
- The following additional investigations are recommended daily in toxic patients: Potassium (hypokalaemia), urea, creatinine, LFT, glucose and INR.

### **Salicylate overdose / toxicity:**

- Salicylate absorption may be delayed when overdose quantities are consumed, therefore serum salicylate values should be interpreted with care, especially if obtained earlier than 6 hours after ingestion.
- Repeat testing within 2 – 3 hours to ensure that absorption is complete.

### **Correlation of serum salicylate levels with level of toxicity after acute ingestion (should not be used for chronic toxicity)**

Hours after ingestion	Serum salicylate levels (mmol/l)	
	Asymptomatic	Severe toxic
6	<3.26	>6.52
8	<3.04	>6.19
10	<2.79	>5.79
12	<2.59	>5.18
24	<1.74	>3.48
36	<1.21	>2.27
48	<0.78	>1.45
60	0	>0.83

Intermediate values are associated with mild to moderate toxicity.

## Organophosphate Toxicity

Acute exposure	Serum cholinesterase (pseudocholinesterase)	↓: Organophosphates days to weeks, carbamates up to 48 hours. See other causes of decreased serum cholinesterase on page 11.
Chronic exposure	RBC cholinesterase	↓: 1-3 months (RBC lifespan)

## DRUGS OF ABUSE

Drug of abuse	Street Name(s)	Type of specimen	Approximate duration of detectability
Amphetamines	Speed, Crystal, Ice, Uppers, Ecstasy, Ephedrine, Pseudoephedrine	Urine	1 – 4 days
Barbiturates	Blue Heavens, Velvet, Devil, Red devils, Pink lady, Purple Hearts	Urine	Short acting: 1 day Long acting: up to 14 days
Benzodiazepines	Benzos, Mellow, Downers, Ativan, Rohypnol, Valium, Serepax	Blood Urine	3 hours to 3 days Average 1 – 9 days, up to 30 days
Cannabinoids / THC	Dagga, Marijuana, Pot, Weed	Urine	2 – 5 days (infrequent use) 3 – 4 weeks (chronic use) 6.5 – 11 weeks (heavy use)
CAT (Methcathinone)	CAT	Urine	At least 24 hours

<b>Drug of abuse</b>	<b>Street Name(s)</b>	<b>Type of specimen</b>	<b>Approximate duration of detectability</b>
Cocaine	Crack, Coke, Rock, Snow, Flake, Blow	Urine	Average: 2 – 3 days, up to 7 – 9 days
Ecstacy	"X"	Urine	At least 24 hours
Lysergic Acid Diethylamide (LSD)	Acid	Urine	0 – 48 hours
Metamphetamine	Tik-Tik	Urine	At least 24 hours
Methadone	Meth, Methadose	Urine	1 – 3 days
Methaqualone and Diphenhydramine	Mandrax, Soaps, Love Pill	Urine	90 – 225 hours 2 weeks after a therapeutic dose
Opiates (codeine, heroin, morphine)	<u>Morphine</u> : Junk, White Stuff, "M" <u>Heroin</u> : Horse, White Lady, "H"	Urine	Occasional use: 7 – 54 hours Chronic use: up to 11 – 12 days on confirmation method
*Opiate immunoassays do not cross-react with synthetic opioids (eg. Buprenorphine) or some commonly used opioid analgesics (eg. Fentanyl, meperidine (pethidine) and methadone).			
Phencyclidine	Angel dust, PCP	Urine	Average 1 – 14 days, up to 30 days
Propoxyphene	PPX, Doloxene	Urine	1 – 2 days

## ALCOHOL ABUSE

### Ethanol

- **Cut-off:** 0.05 g/dl (SA legal limit when driving)
- **Use:**
  - Detect acute alcohol use
  - Detect tolerance ( $>0.15$  g/dl without intoxication or  $>0.3$  g/dl at any time)
- Ingestion of  $>2$  beers by a person weighing 70 kg, would cause blood alcohol to be  $>0.05$  g/dl, 1 hour after ingestion
- **Time to normalize with abstinence** is hours, depending on the dose
- Short detection time limits the use

### %CDT (Carbohydrate deficient transferrin)

- **Cut-off:** 2.47% (N-Latex INA method)
- **Sensitivity** 93%, **Specificity** 97%
- **Use:**
  - Most useful to monitor abstinence in alcoholics
  - Detect at least 1 week of heavy drinking in alcoholics
- Ingestion of 50 – 80 g/d (4 – 6 beers/d) for at least 1 week would cause an abnormal result
- **Time to normalize with abstinence:** 2 – 4 weeks

- **Notes on %CDT:**

- Normal transferrin has 4 carbohydrate chains. With excessive alcohol use, forms of transferrin that contain no, one or two carbohydrate chains, collectively known as CDT, increase.
- In alcoholics that relapse, lower alcohol use can lead to rapid re-elevation.
- %CDT is the most accurate single serum marker for chronic alcohol use and recent heavy drinking, which is readily available.
- The main strength of %CDT is **specificity**.
- Single episodes of acute alcohol intoxication do not elevate CDT.
- %CDT is less sensitive to detect alcohol abuse in females.
- **False positive** results may occur due to non alcoholic liver disease (primary biliary cirrhosis, chronic active hepatitis, chronic Hepatitis C, hepatocellular carcinoma), carbohydrate deficient glycoprotein syndrome (rare), cystic fibrosis, pregnancy, untreated galactosaemia, rectal carcinoma, senile dementia, depression, pregnancy and solvent abuse. False positive results do not occur with genetic transferrin variants or high transferrin concentrations with the N-Latex INA (immuno-nephelometric assay) currently in use.
- %CDT methods include immunoassays, capillary electrophoresis and HPLC. Results and cut-off values from different methods cannot be used interchangeably.

# LABORATORY INVESTIGATIONS FOR SPECIFIC CLINICAL DISORDERS

## Screening tests for endocrine disorders

Disease	Screening tests
Addison's disease	8 – 10 am ACTH and cortisol
Carcinoid syndrome	<p><b>LABORATORY TESTS:</b>          24-hour urine 5-hydroxyindoleacetic acid (5-HIAA), serum serotonin, plasma chromogranin A.</p> <p><b>Preparation for 24-hour urine collection for 5-HIAA determination:</b>          48 hours before and during collection:          No tea and coffee, nicotine, avocados, dates, eggplant, butternut, fruit, nuts, tomatoes and tomato products.</p> <p><u>Medication: Avoid for 7 days if possible</u>          False positive: paracetamol, cough syrups containing guaifenesin or antihistamines, mephenesin (e.g. Spasmend), methocarbamol (Robaxin, Flexeril), reserpine, diazepam, phenobarbital.          False negative: L-dopa (Sinemet), methyldopa (Aldomet), MAOIs, isoniazid, TAD, methenamine (e.g. Hipramine), phenothiazines, salicylates, heparin, chlorpromazine (Largactil), hydrazine, prochlorperazine (Stemetil), promazine, promethazine (Phenergan).</p> <p><b>Serum serotonin:</b>  <u>Medication: Discontinue for 7 days</u>          False positive: MAOI, methyldopa, lithium, reserpine, morphine.          False negative: SSRI – escitalopram, citalopram, fluvoxamine, paroxetine, sertraline.  <u>Medication: Discontinue for 10 weeks</u>          False negative: SSRI – fluoxetine.</p> <p><b>Chromogranin A (CgA):</b>  <u>Medication:</u>          Proton Pump Inhibitors: discontinue for 2 weeks          H2-receptor antagonists: discontinue for 3 days          Corticosteroids: discontinue for 2 weeks</p>

Disease	Screening tests
Cushing's syndrome	<p><b>First line screening:</b> midnight salivary cortisol, 24-hour urinary cortisol excretion or overnight dexamethasone suppression test. Any oestrogen intake should be discontinued for 2 months before the dexamethasone suppression test.</p> <p><b>Confirmation:</b> midnight salivary cortisol (24 – 48 hours apart), 24-hour urinary cortisol excretion (at least 1 week apart) or dexamethasone suppression test.</p>
Diabetes insipidus	Serum electrolytes, urea, creatinine and osmolality, 24 hour urine volume and random urine osmolality after an 8 hour overnight fast.
Hypercalcaemia and hypocalcaemia	PTH, phosphate, magnesium.
Phaeochromocytoma	<p><b>LABORATORY TESTS:</b></p> <p>Urine meta- and normetanephines, plasma meta- and normetanephines, plasma chromogranin A.</p> <p><b>Urine meta- and normetanephines:</b></p> <p><u>Patient preparation</u></p> <p>Discontinue 1 week prior to specimen collection, if possible: TAD's, beta blockers, MAOI, phenothiazines, phenoxybenzamine, L-Dopa. Allowable drugs: hydralazine, ACE inhibitors, ARB's, diuretics (small effect).</p> <p><b>Plasma meta- and normetanephines:</b></p> <p><u>Patient preparation</u></p> <p>Discontinue for at least 5 days prior to specimen collection, if possible: Caffeine, decaffeinated coffee, nicotine, cocaine, TAD, phenoxybenzamine, MAOI Allowable drugs: diuretics, vasodilators e.g. hydralazine and minoxidil, and calcium channel blockers. Collection of the specimen after the patient has rested for 15 minutes in a supine position is recommended.</p>

Disease	Screening tests
Primary hyperaldosteronism	<p><b><u>Active aldosterone: renin ratio (ARR)</u></b></p> <p><b>Preparation for ARR measurement:</b></p> <ol style="list-style-type: none"> <li>1. Hypokalaemia must be corrected.</li> <li>2. Sodium intake should not be restricted, rather liberalise.</li> <li>3. Stop medication that markedly affect ARR for at least 4 weeks:           <ul style="list-style-type: none"> <li>• Spironolactone, eplerenone, amiloride, triamterene.</li> <li>• Potassium-wasting diuretics.</li> <li>• Products derived from licorice root (chewing tobacco, licorice).</li> </ul> </li> </ol> <p><b>If the ARR is not diagnostic and hypertension can be controlled with relatively non-interfering medications:</b></p> <p>Withdraw other medications that may affect ARR for at least 2 weeks:</p> <ol style="list-style-type: none"> <li>1. False positive ARR: <math>\beta</math>-Adrenergic blockers, central <math>\alpha</math>-2 agonists (e.g. clonidine and <math>\alpha</math>-methyldopa), nonsteroidal anti-inflammatory drugs, oestrogen.</li> <li>2. False negative ARR: Angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, renin inhibitors, dihydropyridine calcium channel blockers.</li> </ol> <p>Medication that can be used include verapamil slow-release, hydralazine (with verapamil slow release, to avoid reflex tachycardia), prazosin, doxazosin, terazosin.</p>

## Diagnosis of Porphyria

- The 3 most common forms of Porphyria in South Africa are:
  - Porphyria Variegata (VP, acute porphyria, neurovisceral and / or skin presentation)
  - Porphyria Cutanea Tarda (PCT, skin presentation)
  - Acute Intermittent Porphyria (AIP, acute porphyria, neurovisceral presentation)

### A. Acute neurovisceral symptoms (*abdominal pain, nausea, vomiting, constipation, bladder dysfunction, hypertension, tachycardia, hyponatraemia, seizures, peripheral neuropathy*)

- Request a STAT **Porphyria Screen Urine** on a **random urine specimen protected from light**
- Urine porphobilinogen (PBG) and total free porphyrins are 10-fold increased with an acute porphyria attack
- Failure to diagnose an acute porphyria attack may cause:
  - Death, as life-saving Rx will not be given
  - Administration of porphyrinogenic drugs
  - Unnecessary surgery
- Only the diagnosis is important in this setting as the initial treatment of all types of acute porphyria is the same

### B. Skin lesions OR to determine the type of porphyria

- Request a **Porphyria Study**
  - Submit urine, faeces and EDTA blood specimens protected from light, ideally while symptomatic.
  - The tests may be negative between acute attacks or when skin lesions are not present at the time of testing, therefore specimen collection should proceed if symptoms are present and not be delayed for 3 days to follow the diet below. Negative test results do not rule out a diagnosis of porphyria and follow up testing may be necessary.
  - The patient should refrain from taking the following for 3 days prior to and on the collection day: red meat, pork, biltong, meat extracts (Marmite, Oxo, soup, gravy etc.), aspirin and laxatives.

OR

- Request a **Porphyria Variegata PCR** on an EDTA blood specimen if Porphyria Variegata is suspected clinically

### C. Family of a porphyria patient (siblings and children)

- First diagnose the type of porphyria in the index patient.
- Request a **Porphyria Study** (see above) if the index patient was diagnosed with a type of Porphyria other than Porphyria Variegata. The tests may be negative in the absence of symptoms and in children. Negative results in children should be repeated when clinically indicated until 20 years of age before porphyria can be excluded.

OR

- Request a **Porphyria Variegata PCR** (see above) if the index patient was diagnosed with Porphyria Variegata.

## Causes and investigation of Gynaecomastia

<b>Physiological causes</b>	
1. Neonatal	
2. Pubertal (25%)	
3. Involutorial (mostly 50 – 80 year-old men)	
<b>Pathological Causes</b>	
1. Neoplasms Testicular (3%) – germ cell, Sertoli cell, Leydig cell Adrenal (adenoma or carcinoma) Ectopic production of HCG (especially lung, liver and kidney cancer)	HCG, oestradiol.
2. Primary gonadal failure (8%)	Testosterone, LH, FSH.
3. Secondary hypogonadism (2%)	Testosterone, LH, FSH, prolactin.
4. Liver disease	Liver function tests.
5. Renal disease and dialysis (1%)	Urea and creatinine.
6. Hyperthyroidism (2%)	TSH, free T4, free T3.
7. Hyperprolactinaemia	Prolactin, testosterone, oestradiol, LH, FSH.
8. Starvation especially during the recovery phase	
9. Medication (10 – 20%): Amiodarone, androgens and anabolic steroids, anti-retroviral therapy (some), captopril, cimetidine, cyproterone, diazepam, digitoxin, enalapril, oestrogen and oestrogen agonists, flutamide, haloperidol, isoniazid, ketoconazole, methyldopa, metronidazole, nifedipine, omeprazole, penicillamine, phenothiazines, phenytoin, ranitidine, reserpine, verapamil, tricyclic antidepressants	
10. Drugs of abuse: Alcohol, amphetamines, cannabis, heroin	
11. Idiopathic (25%)	

## Investigation of Female Hirsutism

### Differential diagnosis

1. Polycystic Ovarian Syndrome
2. Non-classic congenital adrenal hyperplasia (e.g. 21-hydroxylase deficiency)
3. Cushing's Syndrome
4. Hyperprolactinaemia
5. Adrenal tumour

### First line investigations

Serum free testosterone, androstenedione, DHEAS, 17-OH progesterone during days 3 – 7 of the menstrual cycle, prolactin and midnight salivary cortisol / 24-hour urinary cortisol excretion / overnight dexamethasone suppression test.

## Secondary causes and investigation of osteoporosis

Cause	Tests
<b>A. Endocrine</b>	
1. Hyperparathyroidism	Calcium, phosphate, PTH.
2. Cushing's syndrome	See screening tests for endocrine disorders.
3. Hypogonadism	Oestradiol, LH, FSH (females). Testosterone, LH, FSH (males).
4. Hyperthyroidism	TSH, free T4, free T3.
5. Prolactinoma	Prolactin.
6. Type 1 diabetes mellitus	Plasma glucose, oral glucose tolerance test, HbA1c.
7. Pregnancy and lactation	

**B. Gastro-intestinal and liver disease**

1. Subtotal gastrectomy	Clinical history.
2. Malabsorption	Serum total protein, albumin, oral fat loading test, xylose absorption test, faecal elastase, stool $\alpha$ -1-antitrypsin.
3. Hepatobiliary disease	Liver function tests.

**C. Bone marrow disorders**

1. Multiple myeloma	Serum protein electrophoresis, urine Bence Jones protein.
2. Disseminated carcinomatosis	Clinically appropriate tumour markers.
3. Leukaemia	Full blood count.
4. Lymphoma	Clinically, full blood count, ESR, $\beta$ -2-microglobulin.

**D. Miscellaneous**

1. Smoking	History.
2. Chronic alcoholism	Liver function tests, CDT, MCV.
3. Immobilisation	History.
4. Rheumatoid arthritis	Rheumatoid Factor.
5. Chronic obstructive pulmonary disease	X-rays lungs, lung function tests.
6. Chronic renal failure	Urea, creatinine, eGFR.

**E. Medications**

1. Corticosteroids	
2. Heparin	
3. Anticonvulsants	
4. Lithium	
5. Excess thyroid hormone therapy	TSH, Free T4, Free T3.

## Osteoporosis in men

Common causes of osteoporosis in men are hypogonadism, alcohol abuse and glucocorticoid excess. In 50% of men, a secondary cause is present and in the other 50% no cause may be found (idiopathic osteoporosis).

## Work-up for hypertension

### Laboratory tests indicated following initial diagnosis of hypertension

Serum urea, creatinine, glucose and total cholesterol.

Random urine sample for albumin-to-creatinine ratio (ACR).

### Follow up visits

- Creatinine (with eGFR), serum electrolytes, fasting glucose and fasting lipogram are recommended after the introduction of a new antihypertensive agent and then annually or more frequently if clinically indicated.
- Annual urine albumin-to-creatinine ratio if eGFR is 60-89

### When should secondary hypertension be considered?

- Onset of hypertension before age 20 or after age 50 years.
- Very high blood pressure (>200 / 120 mm Hg).

- Organ damage:

Fundoscopy: grade 2 or more retinopathy.

Serum creatinine >150 µmol/l.

Cardiomegaly.

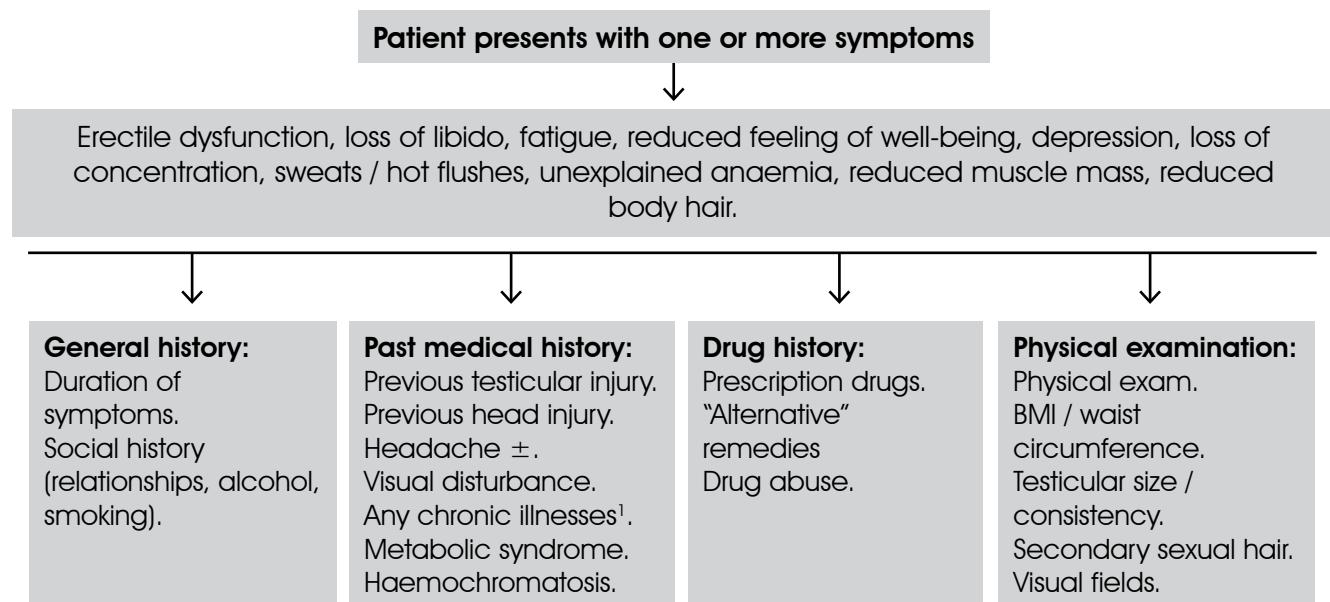
### Features of secondary hypertension:

- Spontaneous hypokalaemia (primary hyperaldosteronism, Cushing's syndrome, renal artery stenosis).
- Abdominal systolic bruit (renal artery stenosis).
- Episodic hypertension, tachycardia, sweat, tremor (phaeochromocytoma).

- Family history of renal or endocrine disease.
- Haematuria, palpable kidneys.
- Poor femoral pulse (coarctation of the aorta).
- Poor response to actually effective antihypertensive therapy.

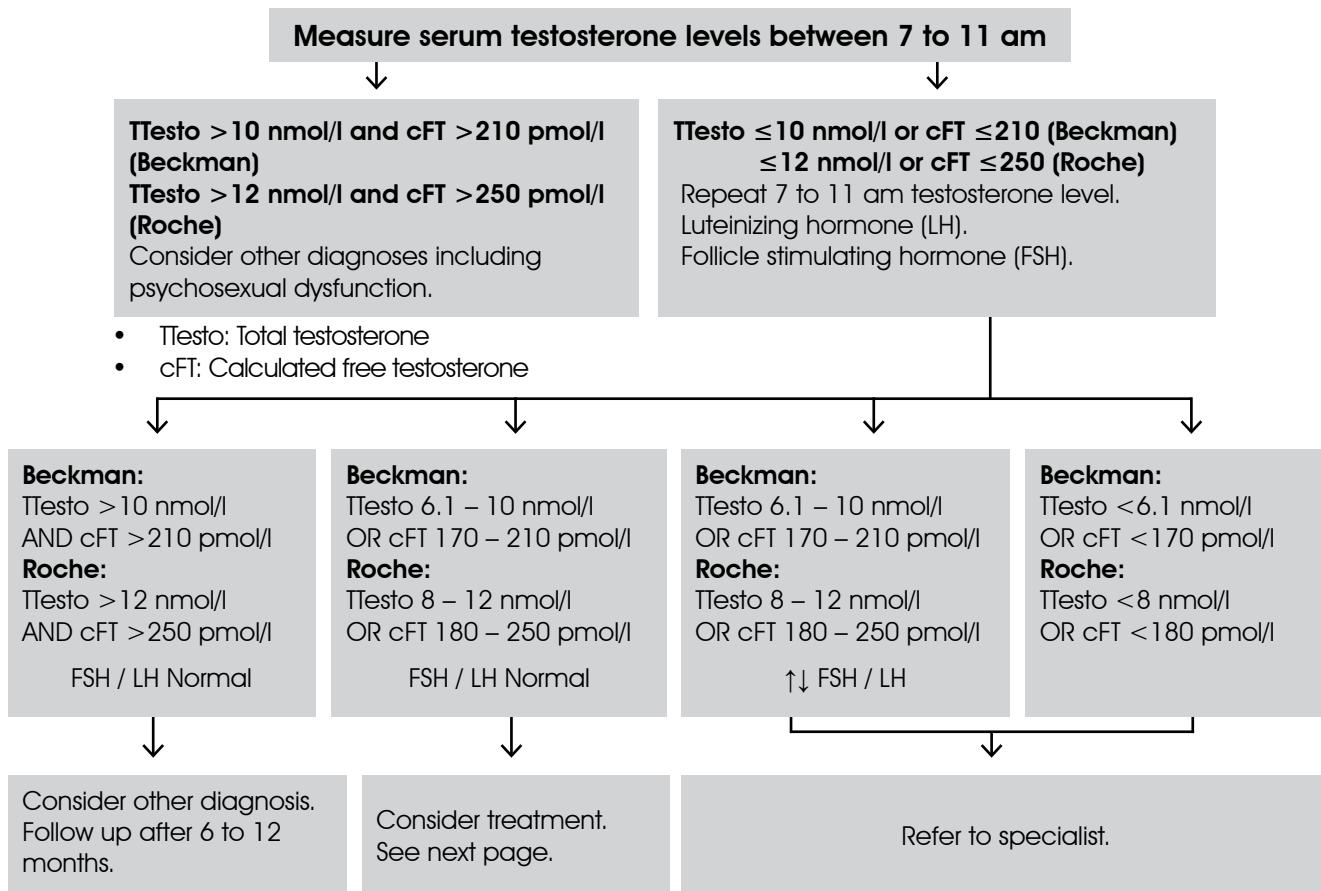
## Late-onset male hypogonadism

### Flow diagram for diagnosis of late onset male hypogonadism



1: DM, COLS, inflammatory arthritis, renal or HIV-related diseases.

## Flow diagram for diagnosis of late onset hypogonadism (continued)



All patients with erectile dysfunction must be examined for coronary heart disease.

**Contra-indications for treatment with testosterone:**

- Prostate cancer.
- Breast cancer.
- A palpable prostate nodule or induration of the prostate.
- PSA >3 ng/ml without further urological evaluation.
- Erythrocytosis (haematocrit >54%).
- Hyperviscosity.
- Untreated obstructive sleep apnoea.
- Class 3 or 4 heart failure.
- Severe lower urinary tract symptoms associated with benign prostatic hyper trophy, International Prostate Symptom Score (IPSS) >21.

**The following should be performed prior to initiating treatment, after 3 months, after 12 months and annually thereafter:**

- **Digital rectal examination:** if abnormal refer to urologist.
- **PSA:** If PSA >4 ng/ml, PSA increase >1.4 ng/ml within any 12 month period of testosterone treatment, PSA velocity >0.4 ng/ml/year using the level after 6 months of testosterone administration as the reference (only applicable if PSA data is available for a period exceeding 2 years) → refer to urologist.
- **Haematocrit:** If haematocrit is >54%, stop therapy until it normalises and re-evaluate the patient for hypoxia and sleep apnoea, reinstitute therapy with a reduced dose.

### **Monitoring of testosterone levels:**

Monitor testosterone levels 2 – 3 months after onset of therapy.

Therapy should aim to raise serum testosterone levels into the mid-normal range, supraphysiological levels should be avoided.

Adjust dose or frequency if testosterone is  $>20$  nmol/l or  $<10$  nmol/l for Beckman method

OR  $>24$  nmol/l or  $<12$  nmol/l for Roche method.

- **Injectable testosterone undecanoate:**

Measure testosterone level prior to each subsequent injection and adjust the dosing interval to maintain testosterone in mid-normal range.

- **Injectable testosterone cypionate or combined testosterone esters:**

Measure serum testosterone midway between injections.

- **Oral testosterone undecanoate:**

Measure serum testosterone level 3 – 5 hours after ingestion.

# HAEMATOLOGY

## Full blood count (FBC)

Analyte	Ref. Range		Units	Interpretation
Haemoglobin	Inland:	M 14.3 – 18.3	g/dl	↑: Polycythaemia. ↓: Anaemia (bleeding, nutritional deficiencies, malabsorption, chronic illness, haemolysis and bone marrow failure (inherited or acquired)).
	Sea level:	M 13.0 – 17.0	g/dl	
Red cell count	Inland:	M 4.89 – 6.11	$10^{12}/l$	↑: Polycythaemia, thalassaemia. ↓: Anaemia.
	Sea level:	M 4.50 – 5.50	$10^{12}/l$	
Haematocrit	Inland:	F 4.13 – 5.67	$10^{12}/l$	↑: Polycythaemia. ↓: Anaemia.
	Sea level:	F 3.80 – 4.80	%	
MCV (mean corpuscular volume)	79.1 – 98.9		fL	↑: <b>Macrocytic</b> red cells.  Check peripheral blood smear for round or oval macrocytes. Oval macrocytes are associated with megaloblastic anaemia (e.g. vit B12 / folate deficiency). Round macrocytes are associated with liver disease, hypothyroidism, antiretroviral therapy, alcohol, chemotherapy, reticulocytosis and myelodysplasia.

Analyte	Ref. Range	Units	Interpretation
			<p>↓: <b>Microcytic</b> red cells            Iron deficiency, thalassaemia, other haemoglobin defects, anaemia of chronic disease, lead poisoning, sideroblastic anaemia.</p>
MCH (mean corpuscular haemoglobin)	27.0 – 32.0	pg	<p>↑: Hyperchromatic red cells e.g. spherocytes.            ↓: Hypochromic (pale) red cells            (Causes as for microcytic cells).</p>
MCHC (mean corpuscular haemoglobin concentration)	31 – 37	g/dl	<p>↑: Spherocytes, bilirubinaemia, auto-agglutination, lipaemic sample.</p>
Red cell distribution width (RDW)	M 10 – 16.3 F 10 – 17.3	%	<p>If raised it means there are red cells of different sizes.            Often the earliest sign of a nutritional deficiency.</p>

Analyte	Ref. Range	Units	Interpretation
Platelet count	150 – 450	10 <sup>9</sup> /l	<p>↑: <b><u>Thrombocytosis</u></b>            Reactive causes should firstly be excluded e.g. iron deficiency, trauma, infection, and malignancy. If no reason is found and platelets remain increased, a chronic myeloproliferative disorder should be excluded. Platelet anisocytosis will be important to evaluate.</p> <p>↓: <b><u>Thrombocytopenia</u></b>            Production defect – bone marrow infiltration or failure            Peripheral loss mechanism e.g. peripheral destruction (ITP)            Pooling / sequestration (hypersplenism) or usage (e.g. DIC, TTP).</p>
White cell count	3.92 – 9.88	10 <sup>9</sup> /l	If abnormal, evaluate the differential white cell count.

Analyte	Ref. Range	Units	Interpretation
Lymphocytes	1.0 – 4.0	10 <sup>9</sup> /l	<p>↑: <b>Lymphocytosis</b>            Primary causes: Lymphoproliferative disorders e.g. CLL, lymphoma overspill into the blood            Reactive causes e.g. viral infections, Bordetella pertussis, stress lymphocytosis (e.g. myocardial infarction, surgery, trauma), smoking, post splenectomy and autoimmune disorders.</p> <p>↓: <b>Lymphopenia</b>            Inherited: congenital immunodeficiencies.            Acquired: e.g. viral infections, TB, lymphoma, aplastic anaemia, immunosuppressive therapy, radiation, renal failure, autoimmune diseases and negative acute-phase response.</p>
Neutrophils	2.0 – 7.5	10 <sup>9</sup> /l	<p>↑: <b>Neutrophil leucocytosis</b>            Bacterial infection, inflammation, trauma / surgery, neoplasia, haemorrhage, haemolysis, pregnancy, metabolic e.g. diabetic ketoacidosis, drugs e.g. steroids, growth factor therapy e.g. G-CSF.</p> <p>↓: <b>Neutropenia</b>            Decreased production:  <ul style="list-style-type: none"> <li>General bone marrow failure e.g. aplastic anaemia, acute leukaemia.</li> <li>Specific failure of neutrophil production e.g. congenital, cyclical, drug induced</li> </ul> </p>

Analyte	Ref. Range	Units	Interpretation
			Peripheral loss: e.g. hypersplenism, autoimmune destruction, severe infection.
Monocytes	0.18 – 1.00	10 <sup>9</sup> /l	<p>↑: <b>Monocytosis</b></p> <p>Infections e.g. TB, CMV, subacute bacterial endocarditis, syphilis</p> <p>Inflammatory and immune disorders e.g. SLE, RA, ulcerative colitis, sarcoidosis.</p> <p>Haematological malignancies e.g. CMML, AML.</p> <p>Non-haematological malignancies.</p> <p>Chronic neutropenias.</p> <p>↓: <b>Monocytopenia</b></p> <p>Haematological disorders e.g. Aplasia, hairy cell leukaemia, autoimmune e.g. RA, SLE, HIV.</p>
Eosinophils	0.0 – 0.45	10 <sup>9</sup> /l	<p>↑: <b>Eosinophilia</b></p> <p>Allergy e.g. asthma, parasites, skin disease, drug sensitivity, connective tissue disease, Hodgkin lymphoma, chronic myeloproliferative disorders, hypereosinophilic syndrome.</p>
Basophils	0.0 – 0.2	10 <sup>9</sup> /l	Usually increased in chronic myeloproliferative disorders e.g. chronic myeloid leukaemia.

## **ESR (erythrocyte sedimentation rate)**

It measures the rate of fall of a column of red cells in plasma during one hour.

Largely determined by the concentration of plasma proteins e.g. fibrinogen, other acute-phase proteins and immunoglobulins.

Raised ESR is a non-specific indicator of an acute-phase response and is of value in monitoring disease activity e.g. rheumatoid arthritis.

**Normal value:** Male 1 – 15 mm / hour and female 1 – 20 mm / hour  
(Wintrobe or Westergren method).

### **Causes of an elevated ESR include:**

- Inflammatory disorders e.g. rheumatoid arthritis, temporal arthritis, polymyalgia rheumatica, SLE.
- Infections e.g. TB, HIV.
- Malignancy.
- Myeloma.
- Anaemia.
- Pregnancy.

### **Causes of a decreased ESR:**

- Polycythaemia.
- Hypofibrinogenaemia.
- Congestive heart failure.
- Spherocytosis.
- Sickle cells.

## Investigation of a bleeding disorder

Clinical evaluation is important prior to requesting lab tests. Five important questions should be asked, if all negative, no tests may be required. The questions are:

- Is it real? Did the patient bleed with previous surgery, tooth extraction, during menstruation, circumcision, child birth, etc; keeping in mind that this may be a new cause of bleeding tendency.
- Is it a primary or secondary haemostatic dysfunction, with epistaxis, petechiae, mucosal bleeding indicating a primary (platelet / endothelial type) defect and muscle / joint bleeds pointing to a secondary (clotting factor defect).
- Is there a family history? Often this helps in requesting the correct tests.
- Does the patient have any systemic disorder that influences haemostatic function, e.g. haematological, renal or hepatic disorders.
- Which medication, including over-the-counter drugs is the patient taking that may be the cause of bleeding?

Start with the basic coagulation screen (PT, APTT, fibrinogen, thrombin time, platelet count and bleeding time). See table for interpretation of results. Normal screening values may be found with minor factor deficiencies and factor levels may then rather be done.

## Basic coagulation screen

Analyte	What is tested	Ref. Range	Units	Interpretation
Prothrombin time (PT)	Efficiency of the extrinsic clotting pathway.	9.0 – 11.2	seconds	<p><b>Prolonged:</b></p> <ul style="list-style-type: none"> <li>Warfarin therapy</li> <li>Rivaroxaban (Xeralto) therapy (neoplastin)</li> <li>Liver disease.</li> <li>Vit K deficiency.</li> <li>DIC.</li> <li>Clotting factor deficiency or defect (I, II, V, VII, X).</li> </ul>
Activated partial thromboplastin time (APTT)	Efficiency of the intrinsic clotting pathway.	22.0 – 30.7	seconds	<p><b>Prolonged:</b></p> <ul style="list-style-type: none"> <li>Heparin therapy (esp. unfractionated) or heparin contamination.</li> <li>Dabigatran (Pradaxa) therapy</li> <li>DIC.</li> <li>Liver disease.</li> <li>Massive transfusion.</li> <li>Lupus anticoagulant.</li> <li>Clotting factor defects (other than FVII).</li> <li>Also moderately prolonged in patients on warfarin and in vit K deficiency.</li> </ul>

Analyte	What is tested	Ref. Range	Units	Interpretation
Thrombin time (TT)	Fibrinogen to fibrin stage.	14.0 – 21.0	seconds	<p><b>Prolonged:</b></p> <p>Very sensitive to unfractionated heparin (LMWH can slightly prolong it at therapeutic levels).</p> <p>Dabigatran (Pradaxa) therapy</p> <p>Hypofibrinogenaemia – as with DIC or congenital defect / deficiency.</p> <p>Dysfibrinogenaemia – inherited or acquired (in liver disease) or physiologically in neonates.</p> <p>Raised concentrations of FDP as with DIC or liver disease.</p> <p>Hypoalbuminaemia.</p> <p>Grossly elevated fibrinogen.</p>
Fibrinogen	Plasma level.	1.8 – 3.5	g/l	<p>↑: Acute-phase.</p> <p>↓: DIC, liver disease, fibrinogen deficiency or dysfibrinogenaemia.</p>
Platelet count	Number of platelets in the blood.	150 – 450	10 <sup>9</sup> /l	<p>↓: Production defect – bone marrow infiltration / failure.</p> <p>Peripheral loss mechanism e.g. immune (ITP), hypersplenism, DIC, TTP, etc.</p>

Analyte	What is tested	Ref. Range	Units	Interpretation
Bleeding time (Ivy method)	<p>Platelet function and capillary integrity.</p> <p>Time measured for bleeding to stop after an incision is made on the forearm of the patient in standardised manner.</p> <p>NB: platelet count should be normal.</p>	3 – 9	minutes	<p><b>Increased:</b></p> <p>Platelet dysfunction:</p> <ul style="list-style-type: none"> <li>• Acquired e.g. aspirin, plavix, uraemia, myeloproliferative neoplasms, myelodysplasia and the presence of a paraprotein.</li> <li>• Congenital e.g. Von Willebrand's disease, storage pool defect, Bernard-Soulier syndrome etc.</li> </ul> <p>Vascular disorders e.g. Ehlers-Danlos's syndrome.</p> <p>Important: a normal bleeding time does not imply normal haemostasis and it does not correlate well with bleeding at other sites or with intra-operative bleeding.</p>

## Interpretation of basic coagulation screen

PT	APTT	TT	Fibrinogen	Platelet count	Conditions
<b>Prolonged</b>	Normal	Normal	Normal	Normal	Factor VII deficiency. Early oral anticoagulation. Mild factor II, V or X deficiency. Lupus anticoagulant with some reagents.
Normal	<b>Prolonged</b>	Normal	Normal	Normal	Factor VIII, IX, XI, XII, prekallikrein, HMWK deficiency / defect. Von Willebrand's disease. Lupus anticoagulant. Mild II, V or X deficiency. Unfractionated heparin
<b>Prolonged</b>	<b>Prolonged</b>	Normal	Normal	Normal	Vit K deficiency. Warfarin therapy. Factor II, V or X deficiency. Multiple factor deficiency e.g. liver failure. Combined V & VIII deficiency.
<b>Prolonged</b>	<b>Prolonged</b>	<b>Prolonged</b>	<b>Normal or low</b>	Normal	Heparin in large amount. Liver disease. Fibrinogen deficiency / disorder. Hyperfibrinolysis. Inhibition of fibrin polymerization.

PT	APTT	TT	Fibrinogen	Platelet count	Conditions
Prolonged	Prolonged	Prolonged	Low	Low	DIC. Acute liver disease.
Prolonged	Prolonged	Normal	Normal or low	Low	Massive transfusion. Liver disease.
Normal	Normal	Normal	Normal	Low	Thrombocytopenia. Consider causes for thrombocytopenia.
Normal	Normal	Normal	Normal	Normal	Platelet dysfunction. Factor XIII deficiency. Mild Von Willebrand's disease. Vascular disorder. Disorder of fibrinolysis. Mild or masked coagulation factor deficiency. Normal haemostasis.

Reference: Dacie and Lewis: Practical Haematology

- If PT, APTT, thrombin time, fibrinogen and platelet count are normal and the patient has a significant bleeding history, further investigation is needed to exclude a **platelet function disorder and other rarer disorders** e.g. fibrinolytic defects (tPa, PAI, antiplasmin), **Von Willebrand disease** and FXIII deficiency.

## Laboratory assessment for Von Willebrand disease (VWD)

### Initial tests:

- Von Willebrand antigen (VWF: Ag)
- Von Willebrand activity (Ristocetin cofactor activity)
- Factor VIII

### Additional tests:

- Von Willebrand multimeric analysis if antigen and / or activity is decreased.
- Platelet aggregation study with ristocetin (RIPA).
- VWF collagen-binding assay.

### Based on the results of these tests, the diagnosis can be made of:

- Type 1 Von Willebrand disease (mild quantitative defect).
- Type 2 Von Willebrand disease (subclassified as type 2A, 2B, 2M or 2N) (qualitative / functional defect).
- Type 3 Von Willebrand disease (severe quantitative defect).

NB: Testing for Von Willebrand disease should be done in the absence of any acute-phase responses (VWF is an acute-phase protein).

Systemic inflammation, infection, pregnancy, oestrogen / oral contraceptives, stress e.g. surgery can cause an increase in levels of VWF and mask lower baseline levels.

Individuals with bloodgroup O have plasma VWF levels that are 25 – 30% lower than those with bloodgroup A, B or AB. In patients with borderline results, it is often helpful to repeat the diagnostic test on two or 3 occasions and to study available family members.

## Platelet aggregation studies

Platelet aggregation responses to different agonists are measured.

It detects congenital and acquired abnormalities of platelet function.

Agonist	Ref. Range	Units	Interpretation/causes
ADP (10 µM/ml)	50 – 100	%	↓: Drugs e.g. clopidogrel and aspirin; storage pool defect.
Collagen (10 µg/ml)	50 – 100	%	↓: Storage pool defect; aspirin; cyclo-oxygenase and thromboxane synthetase deficiency.
Arachidonic acid (1.5 mM/ml)	50 – 100	%	↓: Aspirin; storage pool defect; cyclo-oxygenase and thromboxane synthetase deficiency.
Ristocetin high concentration (1.25 mg/ml)	50 – 100	%	↓: Bernard-Soulier syndrome – confirm with flow cytometry for Gp Ib Von Willebrand disease.
Ristocetin low concentration (0.5 mg/ml)	<10	%	↑: Von Willebrand disease type 2 b (functional defect).
If ↓ with ADP, Collagen and Arachidonic acid; and often primary wave only with Ristocetin			Glanzmann thrombasthenia. Confirm with flow cytometry for Gp IIbIIIa

## PFA-200 (for evaluating platelet function)

(Special acknowledgement – Dr Rita Govender; Pathchat Edition no. 23)

This test may be seen as a cross between bleeding time and quick aggregation testing.

### Availability:

Testing on the PFA 200 requires a sample <4 hours old.

Request for the test must take into account the sample's transit time to the laboratory that hosts the instrument.

Principle:

- **Citrated whole blood** is aspirated at high shear rates through disposable cartridges.
- The cartridge contains an aperture within a membrane coated with either collagen and epinephrine (Col / EPI) or collagen and ADP (Col / ADP).
- The agonists induce platelet adhesion, activation and aggregation, resulting in occlusion of the aperture and hence cessation of blood flow. Parameter is reported as closure time.

Variables affecting results:

- Test must be performed within 4 hours of blood collection.
- HCT <35% and platelet count <150 may affect closure times.
- Samples with HCT >50% and platelet count >500 have not been evaluated.
- Fatty acids, lipaemia.
- Haemolysis of sample.

Advantages:

- Small volume of citrated venous blood (therefore suitable for paediatric samples as well)
- Insensitive to clotting factor deficiency (not dependent on plasma fibrinogen or fibrin generation)
- Better standardisation
- No influence on result from oedema, loss of connective tissue, presence of fragile vessels (as in bleeding time performed in elderly patients)

Interpretation:

	<b>Normal</b>	<b>Aspirin</b>	<b>Von Willebrand Disease</b>	<b>Glanzmann thrombasthenia</b>
Col / EPI	Normal (82 – 150 s)	Prolonged	Prolonged	Prolonged
Col / ADP	Normal (62 – 100 s)	Normal	Prolonged	Prolonged

In the event of abnormal results, further testing is indicated, especially if history supports a bleeding diathesis.

Consultation with a clinical haematologist or haematopathologist is strongly recommended.

Please keep in mind: The test may be normal despite abnormal platelet function e.g. in:

- Storage Pool Disease
- Primary secretion defects
- Mild VWDx

The need for further testing must be guided by history and clinical features supportive of platelet disorder.

#### References:

Bain, B.J., Bates, I., Laffan, M.A. & Lewis, S.M. 2012.

Dacie and Lewis Practical Haematology, 11th edition, Churchill Livingstone.

British Committee for Standards in Haematology. 2011.

Postgraduate haematology: guidelines for laboratory investigation of inheritable platelet disorders  
Aug 2011

## **Thromboelastogram (TEG)**

#### What is tested:

The rate of clot formation, the kinetics of clot formation, the strength and stability of the clot.

#### Indication:

Investigation of patients with abnormal bleeding during and after surgery. It can guide in the specific treatment needed. It is also requested by some physicians as part of pre-operative work-up. Its usefulness to predict bleeding or thrombosis is however debatable.

Different aspects of TEG	Ref. Range	Units	Interpretation
<b>R-time (reaction time)</b> Measures the time between the start of the test until the first sign of fibrin formation	3 – 8	min	If prolonged, it indicates a longer time for fibrin to form and may be due to a clotting factor defect or anticoagulant therapy.
<b>K-time</b> Measures the speed to reach a certain level of clot strength.	1 – 3	min	Dependent on the availability of fibrinogen and FXIII and to a lesser extent platelets.
<b>Alpha angle</b> Measures the rapidity of fibrin build-up and cross-linking (clot strengthening)	55 – 78	min	Dependent on the availability of fibrinogen and FXIII and, to a lesser extent, platelets.
Different aspects of TEG	Ref. Range	Interpretation	
<b>Maximum amplitude (MA)</b> Direct function of the maximum dynamic properties of fibrin and platelet bonding via GPIIb / IIIa and represents the ultimate strength / stability of the fibrin clot.	51 – 69	Affected by platelet function and to a lesser extent by fibrinogen concentration. A small MA usually indicates a thrombocytopenia or platelet dysfunction.	
<b>Thromboelastogram index</b> (mathematic product of the netto effect of the R-time, K-time, alpha angle and MA measurements).	-3 – +3	<-3: hypocoagulable >3+: hypercoagulable, especially if >5.	

## Other specialised coagulation tests (D-dimer and FDP)

Analyte	What is tested	Normal values	Units	Interpretation
D-dimer (XDP)	Measures cross-linked fibrin degradation products	0 – 0.50	mg/l	<p>↑: <b>Causes:</b></p> <ul style="list-style-type: none"> <li>DIC.</li> <li>DVT/Pulmonary embolism.</li> <li>Recent surgery.</li> <li>Trauma.</li> <li>Pregnancy.</li> <li>Infection.</li> <li>Cancer.</li> <li>Elderly patients (approximately 0.5 mg/l for cut-off)</li> <li>Acute coronary syndromes.</li> <li>Cardiac or renal failure.</li> <li>Acute non-lacunar stroke.</li> </ul> <p>Circulatory half-life of D-dimer is about 12 hours. Elevated D-dimer can therefore persist for some time after the active process has ceased.</p> <p><b>D-dimer should always be interpreted in the clinical context and not on its own.</b></p>
Fibrin and fibrinogen degradation products (FDP)	Semi-quantitative detection of FDP in plasma	<5	µg/ml	<p>↑: Acute venous thromboembolism.</p> <ul style="list-style-type: none"> <li>Myocardial infarction.</li> <li>Severe pneumonia.</li> <li>After major surgery.</li> <li>Systemic fibrinolysis associated with DIC.</li> <li>Thrombolytic therapy with streptokinase.</li> </ul>

## Disseminated intravascular coagulation (DIC) screen

There is no single laboratory test that can establish or rule out the diagnosis of DIC. The diagnosis should be made based on an appropriate clinical suspicion supported by relevant laboratory tests. If clinically indicated, repeat testing should be done if initial results are negative.

### Tests used in the diagnosis and evaluation of patients with possible DIC:

Prothrombin time (PT) ↑

Activated partial thromboplastin time (APTT) ↑

Thrombin time (TT) ↑

Fibrinogen normal or ↓

XDP (D-dimer) ↑

FDP ↑

Platelets ↓

Blood smear: red cell fragments

### ISTH diagnostic scoring system for DIC (International Society of Thrombosis and Haemostasis):

#### **Scoring system for overt DIC:**

**Risk assessment:** Does the patient have an underlying disorder known to be associated with overt DIC?

If yes: proceed.

If not: do not use this algorithm.

#### **Score**

Platelet count ( $>100 \times 10^9/l = 0$ ,  $<100 \times 10^9/l = 1$ ,  $<50 \times 10^9/l = 2$ ).

Elevated fibrin marker (e.g. D-dimer, fibrin degradation products).

(no increase = 0, moderate increase = 2, strong increase = 3).

Prolonged PT ( $<3 s = 0$ ,  $>3 s$  BUT  $<6 s = 1$ ,  $>6 s = 2$ ).

Fibrinogen level ( $>1 g/l = 0$ ,  $<1 g/l = 1$ ).

**Calculate score:**

≥5 compatible with overt DIC: repeat score daily.

<5 suggestive of non-overt DIC: repeat within 1 – 2 days.

**Tests used in the investigation of a thrombotic tendency**

Testing should be done prior to starting anticoagulation or 7 – 10 days after completion of anticoagulation therapy.

Analyte	What is tested	Ref. Range	Units	Interpretation
Protein S	<p>Free Prot S level.</p> <p>Prot S is a vit K dependent natural anticoagulant that potentiates the function of protein C.</p>	<p>M 60 – 140 F 70 – 140</p>	%	<p>↓:Congenital deficiency. Acquired deficiencies:</p> <ul style="list-style-type: none"> <li>• Warfarin therapy.</li> <li>• Oestrogen-containing oral contraceptives.</li> <li>• Pregnancy.</li> <li>• Hormone replacement therapy.</li> <li>• Acute-phase reaction.</li> <li>• Liver disease.</li> <li>• Nephrotic syndrome.</li> <li>• L-asparaginase chemotherapy.</li> <li>• DIC.</li> <li>• Some patients with antiphospholipids.</li> </ul>

Analyte	What is tested	Ref. Range	Units	Interpretation
Protein C	<p>Prot C function</p> <p>Prot C is a vit K dependent anticoagulant. After activation by thrombin, it forms complexes with prot S and phospholipids to degrade factors Va and VIIIa.</p>	70 – 140	%	<p>↓:Congenital deficiency. Acquired deficiencies:</p> <ul style="list-style-type: none"> <li>• Warfarin therapy.</li> <li>• Liver disease.</li> <li>• DIC.</li> <li>• Early post-operative period.</li> </ul> <p>The presence of aprotinin may result in an underestimation of protein C level.</p>
Antithrombin	<p>Antithrombin function.</p> <p>It is an inhibitor of thrombin and its action is enhanced by heparin.</p> <p>It also inhibits factor Xa and, to a lesser extent, IXa, XIa, XIIa, plasmin and kallikrein.</p>	80 – 120	%	<p>↓:Congenital deficiency. Acquired deficiency:</p> <ul style="list-style-type: none"> <li>• DIC</li> <li>• Nephrotic syndrome.</li> <li>• Liver disease.</li> <li>• L-asparaginase chemotherapy.</li> <li>• Current massive thrombosis.</li> <li>• Inflammatory bowel disease .</li> </ul> <p>Some test procedures may be affected by heparin therapy. (The current method used by our lab is not affected by therapeutic doses of heparin).</p> <p>Thrombin inhibitors may lead to an over-estimation of antithrombin level.</p>

Analyte	What is tested	Ref. Range	Interpretation
Activated protein C resistance (APC-R).	Resistance against activated protein C.	≥ 120 seconds If < 120 seconds sample is regarded as APC-R positive  In these cases, PCR for factor V Leiden is important.	<b>Congenital:</b> <ul style="list-style-type: none"><li>Factor V Leiden (mutation in factor V).</li></ul> <b>Acquired:</b> <ul style="list-style-type: none"><li>Lupus anticoagulant.</li><li>Increased factor VIII levels.</li><li>Oestrogen-containing oral contraceptives.</li><li>Hormonal replacement therapy.</li><li>Pregnancy.</li></ul> The presence of thrombin inhibitors may lead to false negative results and aprotinin may lead to false positive results.  Insensitive to heparin (UFH and LMWH up to 1 IU/ml).
Factor V Leiden PCR	Detect a mutation Arg 506Glu in factor V. This mutation destroys the cleavage site of FVa by activated protein C and results in a slower inactivation of factor Va by activated prot C.		Detects heterozygotes and homozygotes for Factor V Leiden with a higher risk of thrombosis.

Analyte	What is tested	Ref. Range	Interpretation
Prothrombin gene mutation PCR	Detect a mutation G20210A in the 3' untranslated region of the prothrombin gene. This mutation is associated with elevated levels of prothrombin.		Detects heterozygotes and homozygotes for this mutation with a higher risk of thrombosis.
Plasminogen	Fibrinolytic activity.	75 – 150%	↓: DIC. Liver cirrhosis. During and after fibrinolytic therapy, Newborn. ↑: Acute-phase and malignant disease.
Lupus anticoagulant		See separate table.	

## Testing for the presence of a lupus anticoagulant

Test	What is tested	Ref. Range	Interpretation
Dilute Russell's viper venom test	The venom activates FX directly and triggers the coagulation pathway downstream.	<1.2 ratio If >1.2, a confirmatory test based on the addition of phospholipids is done.	<p><b><u>Increased:</u></b></p> <ul style="list-style-type: none"> <li>• Lupus anticoagulant.</li> <li>• Factor deficiency (X,V, II and fibrinogen).</li> <li>• Other clotting factor inhibitor.</li> </ul> <p>The presence of a lupus anticoagulant is confirmed if the patient time is shortened by the addition of phospholipids. (screen test ratio / confirmed ratio &gt; 1.15).</p> <p>If a clotting factor deficiency or oral anticoagulation, correction will be obtained with addition of normal plasma and not with phospholipids.</p> <p>If other clotting factor inhibitor, no correction will be obtained with either normal plasma or phospholipids.</p>
Kaolin clotting time	Modified aPTT test without added phospholipid.	<1.2 ratio	<p><b><u>Increased:</u></b></p> <ul style="list-style-type: none"> <li>• Lupus anticoagulant.</li> <li>• Other clotting factor defects.</li> </ul> <p>If correction with platelet neutralization procedure, it supports the presence of a lupus anticoagulant.</p>

Test	What is tested	Ref. Range	Interpretation
Lupus sensitive PTT	The reagent has been sensitized to aid the detection of lupus anticoagulants.	31.6 – 44 seconds	<p><b>Prolonged:</b></p> <ul style="list-style-type: none"> <li>• Lupus anticoagulant.</li> <li>• Intrinsic pathway factor deficiencies or inhibitors.</li> <li>• Dysfibrinogenaemia.</li> <li>• Presence of heparin and warfarin.</li> <li>• Treatment with thrombin inhibitors.</li> <li>• DIC.</li> </ul> <p>If correction is obtained with platelet neutralization procedure it confirms the presence of a lupus anticoagulant, while correction with normal plasma supports a clotting factor deficiency.</p>

## Monitoring of anticoagulation therapy

### INR (International normalised ratio)

Standardised way of reporting the Prothrombin time (To compensate for different laboratories using different reagents and instruments).

Developed for the standardisation of oral anticoagulant treatment. Using the INR system, the patient's INR should be approximately the same in any laboratory worldwide.

Used for monitoring of warfarin therapy.

Therapeutic target varies according to the indication for warfarin, but generally aimed at 2 – 3.5

INR >4 – usually over anticoagulated

INR <2 – usually under anticoagulated

## **Antifactor Xa activity (IU/ml)**

Used for monitoring of low molecular weight heparin therapy.

The medication type (pharmaceutical name) and the time of the last dose should always be supplied.

Sample should be taken 3 hours after the last dose on Day 2 of treatment, especially if renal dysfunction is present.

Expected Anti-Xa activity levels for patients on therapy are as follows:

Prophylaxis: 0.3 – 0.5 IU/ml

Therapeutic: 0.5 – 1.2 IU/ml

Valve replacement: 1.0 – 1.2 IU/ml

## **Direct thrombin inhibitor (DTI):**

### **Haemoclot: For the measurement of Dabigatran (Pradaxa) levels.**

Haemoclot Thrombin Inhibitors is an in vitro diagnostic test intended to be used for the quantitative measurement of direct thrombin inhibitors, such as dabigatran, in human citrated plasma. It is a clotting method based on the inhibition of a constant and defined concentration of thrombin. It is intended for prescriptive use. Measuring DTI concentrations in patient's plasma may be used as an aid in the management of patients receiving DTIs who are suspected of having excess anticoagulant activity.

Pradaxa is a direct thrombin inhibitor with a mean half-life of 12 – 17 hours. It is eliminated unchanged primarily in the urine (85%). The rate of elimination by the kidney depends on the individual patient's kidney function; the half-life of Pradaxa is prolonged in cases of renal impairment.

The Haemoclot direct inhibitor assay determines dabigatran plasma concentration:

## Expected plasma levels

	<b>Two hours after dosing ng/ml</b>	<b>10 - 16 hours after dosing ng/ml</b>
150 mg bd	<b>175</b> (117 – 275)	<b>91</b> (61 – 143)
110 mg bd	<b>126</b> (85 – 200)	<b>65</b> (43 – 102)

## Bone marrow investigation

Bone marrow aspirate and trephine biopsy can be done to assess the bone marrow status. It is usually done under local anaesthetic or under conscious sedation from the posterior iliac crest of the pelvic bone. Aspirated cells and bone marrow particles are spread onto slides, which allow evaluation of cytological detail, and iron stores can be assessed. The trephine biopsy is fixed and processed for histology assessment.

### Indications for bone marrow investigation

- Unexplained cytopenias e.g. anaemia, thrombocytopenia, neutropenia and pancytopenia.
- Suspected bone marrow disorders or infiltration e.g. leukaemia, chronic myeloproliferative neoplasm, myeloma, storage disease, metastatic disease, myelodysplasia, aplastic anaemia.
- As part of a staging procedure in lymphoma.
- Suspected infection e.g. TB, fungi.

### Special investigations that can be done on bone marrow aspirate

- Chromosome analysis (cytogenetics), FISH (fluorescent in situ hybridization) for specific molecular abnormalities and PCR analysis.
- Immunophenotyping of abnormal populations of cells with flow cytometry.
- These tests are used to aid in the diagnosis, sub-classification and prognostification of haematological malignancies. Also used to detect evidence of residual disease after treatment.
- Microbiological cultures e.g. tuberculosis, MCS and fungus culture.

## Flow cytometry

Automated technique where cells are incubated with different monoclonal antibodies that are conjugated to different fluorochromes. The labelled cells are then passed in a fluid stream past a laser light source, which allows quantification of antigen expression on the population of interest.

Depending on the underlying pathology, various panels of antibodies can be used to identify the immunophenotype of an abnormal population of cells.

The technique is very important in the diagnosis and classification of acute and chronic leukaemias and lymphomas, to detect minimal residual disease during disease follow-up and may provide valuable prognostic information. It is also used in the diagnosis of PNH (paroxysmal nocturnal hemoglobinuria).

Flow cytometry can be performed on blood samples, bone marrow aspirate, FNA samples and body fluids e.g. pleural effusions, ascites fluid and CSF.

Sample needed: EDTA (purple top) or heparin (green top).

At our laboratory, flow cytometry testing and data analysis are being performed in accordance with EuroFlow protocols.

The EuroFlow protocol is a standardised method of flow cytometric testing for the diagnosis and classification of haematological malignancies. It is currently the only available protocol that is completely validated and standardised at every stage of flow cytometry testing. The adoption of this protocol ensures that our laboratory is able to maintain an international standard in haematological flow cytometry.

## Tests used in the investigation of a haemolytic process

### Reticulocyte count

Reticulocytes are young red cells, which contain remnants of RNA.

Normal range: 0.5 – 2.5 % (absolute count 50 – 100 x 10<sup>9</sup>/l)

Increase with increased erythropoietic activity e.g. blood loss, haemolysis and response on haematinic replacement therapy.

Decreased: red cell production defect e.g. bone marrow disorders and dietary deficiencies.

### **Reticulocyte production index (RPI)**

The reticulocyte production index (RPI) can be calculated to assess whether the reticulocyte response is appropriate for the degree of the anaemia.

**RPI: 1** – when Hb is normal and marrow activity normal

**RPI >2.5** – when anaemia is due to haemolysis, bleeding or hypersplenism and bone marrow response is normal

**RPI <2** – when anaemia is due to marrow failure for any reason e.g. iron deficiency, vit B12 / folate deficiency or bone marrow infiltration

### **Direct Coombs test**

Detect the coating of red cells by immunoglobulins and / or complement.

#### **Causes for a positive Direct Coombs include:**

Autoimmune haemolytic anaemia (warm type and cold type).

Alloimmune haemolytic anaemia (mismatched transfusion, haemolytic disease of the newborn and following solid organ or bone marrow transplantation).

Drug induced immune haemolytic anaemia.

False adsorption of antibodies to the surface of the red cells (occurs in some cases of HIV).

### **Osmotic fragility**

Red cells are suspended in different concentrations of saline and the degree of haemolysis is assessed by spectrophotometry. Spherocytes have an increased volume / surface area ratio and are therefore more susceptible to lysis than normal red blood cells.

If the blood smear shows a picture of spherocytic haemolysis, it is usual practice to start with a Direct Coombs test. If negative, an osmotic fragility could be done and if that is increased, a sample should be sent for red cell membrane protein electrophoresis to confirm a possible congenital spherocytosis.

### **Hb electrophoresis / Abnormal haemoglobin screen**

A lysate of red cells is applied to a gel and an electronic current is applied at an acid and alkaline pH. Different haemoglobins show different migration patterns.

Indication: to detect the presence of inherited haemoglobin defects e.g. Hb S (sickle cell disorders), Hb E, Hb C, Hb D, Hb H and thalassaemia.

Our laboratory has changed to a new method of using ion-exchange high-performance liquid chromatography (HPLC) to detect abnormal haemoglobin in whole blood and to determine the percentages of Hb A2 and HbF.

### **Testing for haemosiderin in the urine**

It can be used as a marker of intravascular haemolysis.

With intravascular haemolysis, haemoglobin is released from the red blood cells into the bloodstream in excess of the binding capacity of haptoglobin. The excess haemoglobin is then filtered by the kidney and reabsorbed in the proximal tubule. The iron portion then gets stored as ferritin or haemosiderin.

When the tubule cells get sloughed off, the haemosiderin gets excreted into the urine. It can remain in the urine for several weeks.

Haptoglobin assay can also be used as a more reliable marker for intravascular haemolysis. A decreased level of haptoglobin will support the presence of intravascular haemolysis.

## **PNH screen (paroxysmal nocturnal haemoglobinuria)**

Haemosiderin in the urine – confirms the presence of intravascular haemolysis.

Flow cytometry: at our laboratory, we perform immunophenotyping of the neutrophils for the expression of FLAER and CD24 and monocytes for the expression of CD14 and FLAER. In PNH, there is a decreased expression of these antigens.

Sample needed: peripheral blood (EDTA or heparin).

## **Testing for inherited enzyme abnormalities**

- Glucose-6-phosphate dehydrogenase deficiency (G6PD).  
A screening test is done and if positive, a quantitative test should be done for confirmation.  
Reticulocytes have higher G6PDH levels and therefore false negative results may be obtained if testing is done during a reticulocytosis.
- Pyruvate kinase deficiency screen.

## **Malaria testing**

- Thin smear – used for species identification and quantification of parasite load.
- Thick smear – higher detection rate, but cannot be used for species identification or quantification.
- Fluorescent microscopy/Quantitative Buffy Coat method (malaria parasites fluorescent after staining with acridine orange). Fairly sensitive, but false positives may occur in the presence of Howell Jolly bodies and reticulocytes. May be used as an additional screening test.  
Increases the detection rate and is more sensitive than the thick smear.
- Malaria antigen testing based on the presence of certain malaria antigens (pLDH and HRP-2). It aids in the detection and identification of malaria and is usually used in conjunction with the thin smear.
- Malaria PCR – parasite nucleic acids are detected. Not suitable for routine diagnostic use, but may be useful in selected cases – most useful for species identification.
- If malaria profile is negative, but clinical suspicion is high, repeat testing is advised.

## JAK2 V617F PCR

Principle: A real-time polymerase chain reaction assay which is used to identify the JAK2 V617F mutation.

Indication: It is usually requested where a chronic myeloproliferative neoplasm is suspected from the clinical picture, peripheral blood and / or bone marrow findings.

Sample type: Blood or bone marrow collected in EDTA (purple top).

Clinical use and interpretation:

The presence of the Jak-2 V617F mutation will support the diagnosis of an underlying chronic myeloproliferative neoplasm.

A bone marrow investigation will usually be performed to confirm the diagnosis and to identify which type of myeloproliferative disorder is present.

The highest incidence of the JAK2 V617F mutation is found in polycythaemia vera (65-97% of cases will be positive). The mutation may also be present in approximately 50% of cases of essential thrombocythaemia and primary myelofibrosis.

It is negative in CML (Chronic myelocytic leukaemia).

If polycythaemia vera is suspected clinically and the Jak-2 V617F PCR is negative, further screening for JAK2 exon 12 mutations can be arranged.

Please take note that a negative JAK2 screen does not exclude the presence of a chronic myeloproliferative disorder.

# IMMUNOLOGY

## AUTOIMMUNE DISORDERS

### Autoimmunity and the endocrine system

<b>Diabetes Mellitus:</b>	
<b>Antibodies</b>	<b>May be increased in the following conditions:</b>
<b>Islet cell antibodies:</b>	<ul style="list-style-type: none"> <li>• Type 1 diabetes mellitus. Can be detectable several years before the onset of disease</li> <li>• Latent autoimmune diabetes in adults (LADA)</li> <li>• Close relatives of affected patients</li> <li>• Autoimmune gestational diabetes</li> <li>• Normally not detectable in other forms of diabetes such as maturity onset diabetes of the young (MODY)</li> </ul>
<b>Glutamic acid decarboxylase (GAD)-65 antibodies</b>	<ul style="list-style-type: none"> <li>• Newly diagnosed type 1 DM</li> <li>• First degree relatives of patients with type 1 DM</li> <li>• Stiffman syndrome</li> <li>• LADA</li> <li>• Gestational DM</li> <li>• Organ-specific autoimmune diseases</li> <li>• Autoimmune polyendocrine syndrome type II</li> <li>• A small percentage of non-insulin dependent diabetic patients: A positive titre is also predictive of insulin requirement within 2 – 3 years in elderly type II diabetics</li> </ul>

<b>Diabetes Mellitus:</b>	
<b>Antibodies</b>	<b>May be increased in the following conditions:</b>
<b>Islet antigen 2 (IA-2) antibodies</b>	<ul style="list-style-type: none"> <li>• Newly diagnosed type 1 DM</li> <li>• LADA</li> <li>• First degree relatives</li> <li>• There is an overall risk in IA2 antibody positive relatives in developing IDDM within 5 years.</li> </ul>
<b>Insulin receptor antibodies</b>	<ul style="list-style-type: none"> <li>• Antibodies against the insulin receptor can have an inhibitory or stimulatory influence and can therefore cause hyper- or hypoglycaemia</li> <li>• Paraneoplastic hypoglycaemia has been described in patients with Hodgkin's lymphoma</li> </ul>

### Thyroid disease – refer to interpretation of thyroid antibodies (page 49)

#### Addison's disease

- Adrenocortical auto-antibodies: found in two thirds of patients.
- 21-Hydroxylase antibodies
- 17 α-Hydroxylase antibodies
- TPO antibodies, Parietal cell antibodies: Monitor for development of associated endocrinopathies and B12 deficiency

#### Poly-endocrinopathy

- Adrenal cortex antibodies
- 21-Hydroxylase antibodies
- Ovarian antibodies
- Testis antibodies

- Steroid hormone-producing cell antibodies
- Parathyroid gland antibodies
- Islet cell antibodies
- TPO antibodies
- Parietal cell antibodies
- Intrinsic factor antibodies

### **Infertility**

- Anti-phospholipid antibodies
- Ovarian antibodies
- Testis antibodies
- Spermatozoa antibodies
- Pituitary gland antibodies
- Placenta antibodies
- Prostate antibodies

### **Chronic atrophic gastritis (autoimmune gastritis) / Pernicious anaemia (PA)**

- Parietal cell antibodies
- Intrinsic factor antibodies

### **Autoimmunity and the central nervous system**

Syndrome	Antibodies
Autoimmune polyneuropathy	Ganglioside antibodies
Paraneoplastic syndromes	Neuronal antibodies, including anti-amphiphysin, anti-CV2, anti-Hu, anti-Ma2, anti-Ri, anti-VGCC, anti-Yo, anti-NMDA receptor antibodies etc.
Neuromyelitis optica	Anti-Aquaporin 4 Antibodies (NMO IgG)

Autoimmune encephalitis	NMDA antibody profile
Demyelinating neuropathy	Myelin associated glycoprotein (MAG) antibodies
Myasthenia Gravis	Acetylcholine receptor antibodies, MuSK antibodies, Titin antibodies

### **Autoimmunity and renal disease**

- Initial laboratory assessment of auto-antibodies should include tests for ANCA, ANA and dsDNA-antibodies since GBM disease, systemic vasculitis and SLE may produce similar clinical features.
- Glomerular basement membrane (GBM) antibodies.
- PLA2R antibodies.

### **Autoimmunity in gastrointestinal disease**

#### **Inflammatory bowel diseases**

##### **Faecal calprotectin**

Elevated in organic bowel disease with bowel inflammation, eg:  
Colitis, Crohn's, ulcerative colitis, ulcers, diverticulitis, polyps, adenomas, malignancies or infections.

##### **Crohn's disease**

ANCA

Anti-saccharomyces cerevisiae antibodies (ASCA)

Pancreas acinar cell antibodies

##### **Ulcerative colitis**

ANCA

DNA-bound lactoferrin (pANCA)

Goblet cell antibodies

## Coeliac disease

The definitive diagnosis is usually identification of villous atrophy by examination of small intestinal biopsy followed by a clear improvement once the patient is on a gluten-free diet.

- Total IgA
- Endomysial IgA
- Tissue Transglutaminase IgA
- Gliadin IgA
- If total IgA <0.3 g/l do:
  - Endomysial IgG
  - Tissue Transglutaminase IgG
  - Gliadin IgG
- HLA DQ2 / HLA DQ8

Positive anti-tissue transglutaminase (TTG) antibodies and endomysial antibodies (EMA) are associated with a high probability for Coeliac Disease.

HLA DQ2 / DQ8 typing is a useful tool to determine if the patient is genetically susceptible to Coeliac Disease (CD). If HLA DQ2 / DQ8 testing is negative, CD is excluded or highly unlikely. The HLA DQ2 allele is found in 90 – 95% of individuals with CD and the remaining 5 –10% possess the HLA DQ8 allele.

In symptomatic patients with high anti-TTG IgA levels (> 10x ULN), verified by EMA positivity and who are HLA DQ2 and / or HLA DQ8 positive, histological assessment may be omitted.

If a diagnosis of CD has been made, a gluten-free diet (GFD) should be instituted. Follow up regularly for symptom improvement and normalisation of CD-specific antibodies – in general this is achieved within 12 months of starting a GFD.

## Autoimmunity in liver disease

### **Screening auto-antibodies in suspected autoimmune liver disease:**

- Smooth muscle antibodies
- Mitochondrial antibodies
- Liver kidney microsome (LKM) antibodies

## Primary biliary cirrhosis (PBC)

### **Liver western blot:**

- Anti-mitochondrial M2 (AMA-M2) antibodies
- AMA-M4 and AMA-M8
- SP100, PML: These antibodies can occur in AMA-negative PBC
- Gp210

## Autoimmune hepatitis (AIH)

### **Liver western blot:**

- Soluble liver antigen / liver pancreas (SLA/LP) antibodies
- Liver cytosol (LC) antibodies
- Liver kidney microsome (LKM) antibodies

## Primary sclerosing cholangitis (PSC)

- pANCA
- Liver western blot
- Biopsy

## Autoimmune skin disorders

### Tests for diagnosis

- Skin biopsy (H&E, direct immunofluorescence, EM)
- Skin antibodies: Indirect immunofluorescence detect antibodies against desmosomes and basal membrane
- IgA EMA / tTG (dermatitis herpetiforme)
- Vitiligo: strong association with other autoimmune diseases, including autoimmune endocrinopathies, pernicious anaemia, autoimmune hepatitis, alopecia, psoriasis, SLE, RA, myasthenia gravis

## Autoimmune ear disease

Heat shock protein (hsp)-70 antibodies: autoimmune sensorineural hearing loss

## Interpretation of anti-nuclear antibodies

Antibody	Disease association
<b>ANA pattern</b>	
Homogenous	SLE, exclude mixed connective tissue disease (MCTD), Drug-induced Lupus, RA, Systemic Sclerosis, Juvenile chronic arthritis.
Speckled	Sjögren's syndrome, Scleroderma with or without Polymyositis overlap, MCTD, Raynaud's phenomenon, Psoriasis.
Nucleolar	Scleroderma, Polymyositis-Scleroderma overlap, SLE.
Centromere	CREST syndrome, also other auto-immune disorders.
Rim	SLE.
Multiple nuclear dots	Various autoimmune diseases, especially Sjögren's syndrome, SLE and Primary Biliary Cirrhosis (PBC).
Few nuclear dots	Autoimmune and viral liver disease.

Golgi	SLE, Sjögren's syndrome, other undefined rheumatic diseases.
Lysosomal	SLE.
Centriole	Viral infection, Raynaud's, Scleroderma, hyperthyroidism, non-specific rheumatic disease, Sjögren's Syndrome.
Midbody	Scleroderma, Raynaud's.
Mitotic spindle	Unknown significance, associated with respiratory tract tumours.
PCNA (Proliferating cell nuclear antigen)	SLE.
High avidity dsDNA-antibodies	Highly specific for SLE, associated with renal involvement.
<b>ENA (extractable nuclear antigens)</b>	
Sm	SLE.
RNP	Mixed connective tissue disease, SLE.
SSA	Sjögren's, SLE, Scleroderma, RA, Polymyositis.
SSB	Sjögren's, SLE.
Scl-70	Scleroderma, SLE, Raynaud's phenomenon.
PM-Scl	Polymyositis, dermatomyositis and Scleroderma overlap syndrome, Scleroderma, dermatomyositis and Polymyositis.
Jo-1	Polymyositis, often associated with interstitial lung fibrosis.
Nucleosome	SLE.
Histone	Drug-induced SLE, SLE, Rheumatoid arthritis.
Ribosomal-P	SLE.
Anti-mitochondrial M2	Primary Biliary Cirrhosis, RA, Scleroderma.

CENP B	CREST syndrome, mild variant of progressive systemic sclerosis, primary biliary cirrhosis, Raynaud's phenomenon and infrequently in Sjögren's syndrome.
PCNA	SLE.
Mi-2	Steroid responsive dermatomyositis, rarely polymyositis.
Ku	SLE, MCTD, Sjögren's syndrome, scleroderma (often with myositis).
Fibrillarin	Fibrillarin antibodies occur in 5 – 10% of patients with systemic sclerosis, occurring in the diffuse or limited cutaneous forms. The clinical phenotype associated with this antibody include pulmonary arterial hypertension, cardiac and skeletal muscle involvement.

## **CONNECTIVE TISSUE DISEASE**

### **Basic spectrum**

- FBC
- Platelet Count
- ESR
- CRP
- Anti-nuclear antibodies
- Double stranded DNA antibodies
- ENA Screening Test: If positive:
  - ENA profile: Include antibodies to RNP, Sm, SSA, SSB, Scl70, PM-Scl, Mi-2, Fibrillarin, Jo-1, CENP-B, PCNA, ribosomal P-protein

### **OR**

- ENA Western blot: Include antibodies to double-stranded DNA, RNP, Sm, SSA, SSB, Scl70, PM-Scl, Jo-1, CENP-B, PCNA, ribosomal P-protein, Histone, Nucleosome, AMA M2
- RF
- AMA (anti-mitochondrial antibodies)

- ASMA (anti-smooth muscle antibodies)
- ANCA

### **Rheumatoid arthritis (RA)**

- FBC and platelets
- ESR
- CRP
- Anti-nuclear antibodies (ANA)
- ENA Screening Test: If positive:
  - ENA profile / Western blot
- Rheumatoid factor (RF)
- Anti-cyclic citrullinated peptide (CCP) antibodies
- ANCA may be found in RA vasculitis
- CRP most sensitive marker of disease activity
- Immunoglobulins: Hypergammaglobulinaemia often present

### **Systemic Lupus Erythematosus (SLE)**

- FBC and platelets
- ESR: Raised in active disease
- CRP: Normal or slightly elevated. Increased levels associated with intercurrent infections
- ANA
- dsDNA-antibodies
- ENA screen – if positive:
  - ENA profile / ENA Western blot: Sm (SLE specific), SSA (Ro), Ro-52, SSB (La), Ribosomal P-proteins (SLE specific), U1-nRNP, histones, nucleosome (SLE specific), PCNA
- ANCA
- Complement 4: C4 reduction is common and does not relate to disease activity reliably
- Complement 3: Active disease: levels are reduced, but owing to increased levels within an acute-phase response, levels may not drop below normal range

- Immunoglobulins: Polyclonal increase, IgA deficiency may occur
- Regular monitoring of UKE, LFT, TFT and urine
- Monitoring of established disease: FBC, UKE, LFT, TFT, Urine, ESR / CRP, C3 / C4, anti-dsDNA / Sm
- Recheck full serology every 6 – 12 months: patterns may change, half life of antibodies is 3 weeks, do not repeat more frequently than monthly

### **Sjögren's syndrome**

- ANA
- dsDNA
- ENA screen – if positive:
  - ENA profile / ENA Western blot : SS-A (Ro) – Antibodies to SSA are found in approximately 50 – 75% of patients with primary Sjögren's syndrome, SSB – SSB antibodies are primarily found in patients with Sjögren's syndrome (40 – 80%) but may also occur in patients with SLE (6 – 21%)
- RF
- Thyroid antibodies: Strong association with thyroid disease
- Mitochondrial antibodies: May be found in those going on to develop PBC
- C3 / C4
- Serum protein electrophoresis and Cryoglobulins
- IgG subclasses: May have an increase in IgG1 with reduced IgG2, IgG3 and IgG4
- β2-microglobulin: should be monitored as marker of lymphoproliferation

### **Polymyositis and dermatomyositis**

- ANA
- ENA screen – if positive:
  - ENA profile / ENA Western blot: Jo-1, PM-Scl75
- Myositis profile: Mi-2, Jo-1, PL-7, PL-12, PM-Scl, RO-52, Ku

### **Systemic sclerosis (localised and systemic forms)**

- ANA: Anti-centromere antibodies (very specific for CREST)
- ENA screen – if positive:
  - ENA profile / ENA Western blot: CENP B, Scl-70, Pm-Scl
- Systemic sclerosis profile: Scl-70, CENP A, CENP B, RP11, RP155, Fibrillarin, NOR90, Th/To, PM-Scl100, PM-Scl75, KU, PDGFR, Ro-52
- FBC
- U&E and creatinine: Systemic sclerosis may lead to major renal and lung involvement
- TFT: association with thyroid disease
- LFT
- Anti-mitochondrial antibodies: association with PBC
- Cryoglobulins: All patients with Raynaud's phenomenon
- CRP, ESR

### **Ankylosing spondylitis:**

- HLA B27

### **Anti-phospholipid syndrome**

- Anti-cardiolipin antibodies
- $\beta$ 2-glycoprotein antibodies
- Prothrombin antibodies
- Clotting studies
- Lupus anticoagulant
- Thrombophilia screen (exclude other thrombophilic disorders)

## Vasculitis

- Biopsy
- Imaging
- Immunological tests
  - Immunoglobulin levels: may be non-specifically raised
  - Serum protein electrophoresis: to look for paraproteins, albumin will be reduced (negative acute-phase protein) with elevated  $\alpha_2$  band
  - Consider possibility of cryoglobulins
  - ANA
  - ENA
  - dsDNA-antibodies
  - Anti-neutrophil cytoplasmic antibodies (ANCA)
- Acute-phase markers: mostly high, regular monitoring provides useful information on response to treatment
  - CRP
  - ESR
  - C3, C4: levels will be elevated
  - Caeruloplasmin: often significantly elevated in some vasculitides
  - Ferritin: levels may be very high ( $> 1000$  ng/ml) in adult Still's disease
- FBC: often anaemia of chronic disease with thrombocytosis and often a lymphopaenia

## **ALLERGIC DISEASES**

**Allergic disorders (also refer to Ampath's "An approach to the diagnosis of an Allergy)" – \*\*refer to attachments A - D**

### ***Immediate type hypersensitivity***

Total IgE

Phadiatop inhalant screen

- Breakdown into individual allergens if positive

Food mix screen (FX5)

- Breakdown into individual allergens if positive
- Individual food allergen component IgE if positive

Allergen specific IgE (Immunocap®) ; eg latex, bee venom, individual allergens

ISAC: 112 Allergen component IgE testing

Skin Prick Testing

Nasal mucus examination for eosinophils

***Basophil mediated hypersensitivity (can present like immediate type hypersensitivity or with delayed type onset of up to 48 hours after exposure)***

CAST inhalant screen

CAST food screen

CAST colourants and preservatives

Allergen-specific CAST

Drug-specific CAST

### ***Delayed type hypersensitivity reaction***

Allergen-specific MELISA testing

Metal MELISA testing

Drug-specific MELISA

\*\*For a complete list of allergy diagnostic tests available at Ampath, refer to attachment E

### **Urticaria**

FBC + diff

ESR

ANA, ENA

Total IgE

CAST colourants and preservatives or medication

Allergy tests dependent on history

Complement (C3, C4)

Helicobacter pylori antibodies

Thyroid antibodies

LFT + protein electrophoresis

Mast cell tryptase

Urine for PGD2 (elevated in mast cell activation syndrome)

Urine for Methyl Histamine

(elevated in mast cell activation syndrome – this is sent away to the Mayo clinic)

### **MICROBIOLOGY AND MICROBIAL SEROLOGY**

#### **Antenatal screening**

FBC

Indirect Coombs

ABO Group

Rh type

HBs Ag

HIV

Rubella IgG Ab

Syphilis serology including RPR, *Treponema pallidum* screen

### **Chronic fatigue**

FBC

Glucose

LFT

TSH

U & E

Coxsackie B Ab

EBV Ab

Brucella Ab

Enterovirus PCR (CSF)

Lyme serology (Borrelia ELISA)

Q-fever serology

Immunoglobulins

Total IgE and allergic work-up if clinically indicated

### **CNS (meningitis / encephalitis)**

Autoimmune encephalitis antibodies including:

- NMDA
- Aquaporin 4 antibodies

ANA

Bacterial antigens

Blood culture

CMV PCR  
Cryptococcus antigen  
CSF MCS  
Enterovirus PCR  
HSV PCR  
HIV serology  
Lyme serology  
Mumps PCR  
Syphilis serology on CSF (including VDRL, *Treponema pallidum* IgM and IgG) **and** serum (including RPR, *Treponema pallidum* antibody screen)  
TB culture and PCR  
Toxoplasma PCR  
Varicella zoster PCR  
Viral Meningitis Multiplex PCR

### **Congenital screening**

CMV Ab  
HSV 1 & 2 Ab  
Rubella Ab  
Syphilis serology including RPR, *Treponema pallidum* IgM and IgG  
Toxoplasma Ab  
Rubella PCR  
CMV PCR

### **Diarrhoea – stool investigations**

Microscopy including parasites  
Culture for pathogens  
*Clostridium difficile* PCR

- Bacterial gastroenteritis multiplex PCR panel for detection of:
  - Campylobacter spp. (jejuni and coli)
  - Salmonella spp
  - Shigella spp
  - Enteroinvasive E. coli (EIEC)
  - Shiga toxins (stx1 and stx 2) found in Shiga toxin-producing E. Coli (STEC) and Shigella dysenteriae
- Viral gastroenteritis multiplex PCR panel for detection of:
  - Rotavirus
  - Norovirus genogroup 1 and 2
  - Adenovirus
  - Astrovirus
  - Sapovirus
- Parasite gastroenteritis multiplex PCR panel for detection of:
  - Entamoeba histolytica
  - Cryptosporidium spp
  - Giardia spp

Rotavirus and adenovirus rapid antigen test

### **Genital ulcer**

MCS

HSV PCR

Syphilis serology including RPR, *Treponema pallidum* antibody screen

### **Haematuria**

Urine MCS

Urine microscopy for parasites

Bilharzia Ag + Ab  
HBsAg  
ANA, ENA  
ASOT  
Complement (C3, C4)  
ALT  
Glucose  
Protein electrophoresis  
U & E  
FBC  
PNH flow cytometry

## **HEPATITIS**

### **Hepatitis A, B, C**

#### **Acute disease**

Hep A IgM  
Hep B acute profile (HBsAg, cAb, sAB)  
HCV PCR

#### **Immunity / post vaccination**

Hep B sAb  
Hep A IgG

#### **Other hepatitides**

Amoebic Ab  
Brucella Ab/PCR  
CMV Ab

EBV Ab

Hep E Ab

HSV Ab / PCR

Hydatid Ab

Leptospira Ab/PCR

Q-fever Ab

Syphilis serology RPR, *Treponema pallidum* antibody screen

Mitochondrial Ab

Smooth muscle Ab

LKM Ab

Autoimmune hepatitis western blot

Alpha-1 antitrypsin

Iron studies

Serum Caeruloplasmin

24-hour urine copper

### **Lymphadenopathy / mononucleosis syndrome serology**

Cytomegalovirus Ab

HIV ELISA

TB spot test / Mantoux

Bartonella Ab

Brucella Ab

EBV Ab

Mumps Ab

Rubella Ab

Syphilis serology including RPR, *Treponema pallidum* antibody screen

Toxoplasma Ab

Other:

Bone marrow

Lymph node biopsy

- Histology
- TB culture
- TB PCR

ANA, ENA

Serum ACE

### **Proteinuria**

Urine MCS

Urine protein / creatinine ratio

24-hour urine protein

FBC

Albumin

Complement (C3, C4)

ANA, ENA

ASOT

HIV ELISA

Hep B sAg

Glucose

Protein electrophoresis

Urine for Bence Jones proteins

### **Pyrexia of Unknown Origin (PUO)**

Blood cultures (Large vol – 60 ml)

Culture of any suspected source e.g. sputum

Malaria films / QBC / PCR

Mantoux skin test

Urine micro and culture

Serology:

- EBV Ab
- CMV Ab
- Arbovirus Ab
- Bartonella Ab
- Brucella Ab
- Brucella PCR
- Dengue Ab
- Leptospira Ab
- Q-fever Ab
- Rickettsia Ab
- Toxoplasma Ab

ANA, ENA

Bone marrow if indicated

### **Rash – vesicular**

MCS

Serology:

- Bartonella Ab
- CMV Ab
- Coxsackie B Ab
- Dengue and other arbovirus Ab
- EBV Ab
- HIV Ab
- Human herpes virus 6 Ab
- Human herpes virus 7 Ab

- Measles Ab
  - *Mycoplasma pneumonia* Ab
  - *Mycoplasma pneumonia* PCR
  - Parvovirus B19 Ab
  - Rickettsia Ab
  - Rickettsia PCR
  - Rubella Ab
  - Syphilis serology including RPR, *Treponema pallidum* antibody screen
- ANA, ENA

### **Respiratory infections**

#### **Upper**

Culture (throat swab)

*Bordetella pertussis* PCR (throat Swab / NPA)

Multiplex Respiratory Virus PCR

Respiratory virus DFA

Serology:

- *Bordetella pertussis* toxin IgA and IgG
- ASOT / Anti DNase B

#### **Lower (acute)**

Blood cultures x 2

Sputum MCS

*Legionella* PCR (sputum)

*Mycoplasma pneumoniae* PCR (sputum)

*Chlamydophila pneumoniae* PCR (sputum)

Influenza PCR

*Bordetella pertussis* PCR

Multiplex Respiratory Virus PCR

Multiplex Bacterial Pneumonia PCR

Serology:

- *Bordetella pertussis* toxin IgA, IgG
- *Chlamydophila pneumoniae* Ab
- *Chlamydophila psittaci* Ab
- Legionella Ab
- *Mycoplasma pneumoniae* Ab
- Q-fever Ab

**Lower (non-resolving):**

Sputum and / or BAL

- MCS
- Fungal culture x 3
- MTB micro and culture x 3
- MTB PCR

T Spot TB Test / Quantiferon Gold / Mantoux Skin Test

Total IgE

Serology:

- *Bordetella pertussis* toxin IgA, IgG
- Aspergillus precipitins / IgG
- Aspergillus IgE / skin prick test
- HIV Ab
- Legionella Ab
- *Mycoplasma pneumoniae* Ab

Sweat test

ANCA

*Pneumocystis jiroveci* PCR

## **Urethral discharge**

MCS

*Chlamydia trachomatis* PCR

*Neisseria gonorrhoea* PCR

## **Urinary tract infection**

Urine MCS

Blood cultures x 2 (systemic symptoms)

Serology:

ASOT / Anti DNase B (Nephritis)

## **PRIMARY IMMUNODEFICIENCIES: A DIAGNOSTIC APPROACH**

### **Features suggestive of a primary immunodeficiency**

(First three features most predictive)

- Family history of immunodeficiency or unexplained early death (< 30 years)
- Failure to thrive
- Need for IV antibiotics and / or hospitalisation to clear infection
- Six or more new infections in one year
- Two or more sinus infections or pneumonia in one year
- Four or more new ear infections in one year
- Two or more episodes of sepsis or meningitis in a lifetime
- Two or more months of antibiotics without an effect
- Recurrent or resistant Candida infections
- Recurrent tissue or organ abscesses
- Infection with an opportunistic pathogen
- Structural damage due to infections

- Complications from a live vaccine
- Chronic diarrhoea
- Non-healing wounds
- Extensive skin lesions
- Persistent lymphopaenia
- Unexplained fever or autoimmunity

Additional features in infants include:

- Delayed umbilical separation (> 30 days)
- Congenital heart defects
- Hypocalcaemia
- Absent thymic shadow on CXR

Most children referred for recurrent infections do not have an underlying immunodeficiency. The majority of children will have increased exposure, allergy (~30%), other chronic diseases (~10%) or an anatomic defect. Only about 10% will have an immunodeficiency.

### **Laboratory investigations**

Abnormal results need to be verified at a later stage, as external factors like infections and medication may have a transient influence on the test results.

- Test for HIV, CMV, EBV, TB, disseminated BCGosis, PCP and other infections where relevant
- FBC and differential count
- Quantitative immunoglobulins PRIOR to administration of immunoglobulins: IgG, M, A & E
- CRP and ESR
- Screen for Cystic Fibrosis where indicated

## **1. ANTIBODY (HUMORAL) DEFICIENCIES – these accounts for about 70% of primary immunodeficiencies**

Patients suffering from antibody deficiencies have a problem with recurrent, severe upper (recurrent pneumonia), and lower respiratory tract infections (i.e. recurrent otitis media, sinusitis) especially with pyogenic organisms such as *S. pneumoniae* and *H. influenzae*.

Appropriate screening tests would be:

- Full blood count and differential
- IgG, IgA, IgM
- IgG subclasses

If any abnormality is detected, additional tests should be done:

- Lymphocyte immunophenotype: To determine total B-cell numbers
- Specific antibody titres to *S. pneumoniae*, *H. influenzae*, Tetanus and Diphtheria
- If specific antibody levels are low, booster immunisations should be administered and titres measured again 3 – 4 weeks later
- B-cell function testing for B-cell activation markers
- Memory B-cells: Flow cytometric assay to assess germinal B-cell function in a patient with a humoral immunodeficiency.
- B-cells are associated with more serious / frequent infections, autoimmune disease
- KREC PCR
- Diagnostic tests:
  - a. Bruton's Tyrosine Kinase: Flow cytometric assay in patients with suspected X-linked agammaglobulinaemia
  - b. CD 40 Ligand: Flow cytometric assay in patients with suspected hyper-IgM syndrome

## **2. T-CELL DEFECTS – these account for about 15% of primary immunodeficiencies**

**T-cell** defects usually present at a very young age with life-threatening infections due to a wide range of different pathogens, often opportunistic infections. Infections are persistent and severe and viruses, fungi and intracellular bacteria are often involved. A family history of unexplained, especially infective deaths should prompt further investigation.

HIV ELISA or PCR in babies: Performed on all patients suspected of having a T-cell deficiency.

- FBC and differential: Patients are usually lymphopenic with persistent absolute lymphocyte counts  
 $< 1.5 \times 10^9/l$  in older children and  
 $< 2.5 \times 10^9/l$  in younger children
- Lymphocyte immunophenotyping to enumerate the lymphocyte subtypes (B- and T-cells and NK cells)
- TREC PCR
- Lymphocyte proliferation tests to mitogens
  - a. PHA
  - b. PMA
  - c. PMA + ionophore
  - d. CD3
  - e. CD3 + IL-2
  - f. CON A
  - g. PWM
- Lymphocyte proliferation tests to recall antigens:
  - a. Varicella zoster
  - b. Candida
  - c. Tetanus

- Specialised immunophenotyping:
  - a. Naïve and memory CD4 and CD8 cells, recent thymic emigrants
  - b. Alpha / beta, gamma / delta T-cells
  - c. Common gamma chain / IL-7RA
  - d. CD3+ CD25+ Fox P3
  - e. Th17
- Genetic testing (contact genetic counsellor Sarah Walters, 012 678 1362)

For infants and young children, all of the lymphocyte proliferation tests to mitogens should be ordered, whereas only LPT to candida, varicella zoster and PHA should be ordered in adults.

### **3. TESTS TO DETERMINE NEUTROPHIL FUNCTION**

These tests should be requested in patients with recurrent skin, soft tissue or deep abscesses, or recurrent infections with *S.aureus*, coagulase negative staphylococci, *Serratia marcescens*, *P. aeruginosa*, *Chromobacterium violaceum* or *Aspergillus spp*

- FBC with differential count
- Neutrophil oxidative burst, phagocytosis and chemotaxis
- Leukocyte adhesion studies: CD11 and CD18: in babies with delayed umbilical cord separation (> 30 days) and patients with recurrent bacterial infections, mainly involving the skin and mucous membranes, periodontitis, absent pus formation, impaired wound healing
- Neutrophil antibodies for suspected autoimmune neutropenia

### **4. TESTS TO DETERMINE COMPLEMENT FUNCTION**

The total Haemolytic Complement activity should be requested in patients with recurrent Neisserial and pyogenic infections. It is therefore important that symptomatic patients with normal antibody and neutrophil tests be further evaluated by complement function testing.

- Classic and alternate pathways (CH100 and ACH100)
- Complement 3 and 4 levels
- Mannan Binding Lectin (MBL)

## **5. NATURAL KILLER CELLS**

NK cells play a crucial role in the host defence against herpes virus infections, especially Herpes simplex virus and Varicella zoster virus reactivation and latency.

- Total NK cell numbers
- NK cell function.

## **6. TESTS FOR CAUSES OF SECONDARY IMMUNODEFICIENCY**

Secondary causes for immunodeficiencies should always be excluded.

Includes HIV, UKE, LFT, glucose, albumin, urinalysis, serum protein electrophoresis, stool  $\alpha$ 1-antitrypsin levels etc.

## VIROLOGY

### HIV (Human immunodeficiency virus) diagnosis and monitoring:

HIV infection is usually diagnosed by means of serology testing. This includes the 4th generation ELISA based technology, which detects both p24 antigen and HIV antibody; 3rd Generation ELISA-based technology, which detects only antibodies, and the p24 antigen testing ELISA, which is useful in the diagnosis of acute infection with HIV. Serological diagnosis includes the use of western blot tests which may be used to confirm positive ELISA results, or to differentiate HIV-1 from HIV-2. HIV infection in infants <18 months of age is normally diagnosed using HIV PCR testing where viral DNA is detected, as persistent maternal HIV antibodies in the baby may be detected with HIV ELISAs up until 18 months of age. Over the age of 18 months, routine diagnostic algorithms may be used.

Possible diagnostic algorithms include:

Algorithm	Interpretation
<ul style="list-style-type: none"> <li>Screening assay (usually 4th generation HIV ELISA or HIV rapid test) non-reactive</li> </ul>	Patient not infected with HIV, or in early infection pre-seroconversion. If the latter is suspected, HIV nucleic acid testing should be requested. This is likely to be positive approximately 5 days prior to p24 antigen becoming detectable.
<ul style="list-style-type: none"> <li>4th-generation HIV ELISA reactive</li> <li>HIV western blot positive</li> </ul>	Confirmed HIV infection. A second sample should be tested to ensure that there have been no sample misidentification problems.
<ul style="list-style-type: none"> <li>4th-generation HIV ELISA reactive</li> <li>3rd-generation HIV ELISA reactive</li> </ul>	Confirmed HIV infection. A second sample should be tested to ensure that there have been no sample misidentification problems.

Algorithm	Interpretation
<ul style="list-style-type: none"> <li>• 4th-generation HIV ELISA reactive</li> <li>• HIV viral load detectable (HIV VL should be above 5000 copies/ml). Bear in mind that the use of HIV VL assays to confirm HIV infection is off-label use of these assays, as they are only licensed for use in monitoring HIV infection once diagnosis has been made.</li> </ul>	Confirmed HIV infection. A second sample should be tested to ensure that there have been no sample misidentification problems.
<ul style="list-style-type: none"> <li>• 4th-generation HIV ELISA reactive</li> <li>• HIV western blot or 3rd-generation ELISA indeterminate or non-reactive</li> <li>• HIV p24 antigen positive</li> </ul>	Acute infection with HIV. This infection may be confirmed with the use of HIV VL (viral load) testing as the HIV VL is usually high in these circumstances. One may also confirm infection simply by monitoring the course of seroconversion with repeat serology at a later stage.
<ul style="list-style-type: none"> <li>• 4th-generation HIV ELISA (Enzyme Linked Immunosorbent Assay) reactive</li> <li>• HIV western blot or 3rd -generation ELISA indeterminate or non-reactive</li> <li>• HIV p24 antigen non-reactive</li> </ul>	This result combination may be due to false reactivity in the 4th-generation ELISA assay. Molecular testing such as HIV viral load or PCR (Polymerase chain reaction) may be useful in determining whether or not infection is present. HIV -2 should also be considered.
<ul style="list-style-type: none"> <li>• 4th-generation HIV ELISA reactive</li> <li>• HIV Wwestern blot indeterminate or negative</li> <li>• HIV p24 antigen non-reactive</li> <li>• HIV VL/PCR negative or undetectable or unexpectedly low (&lt;5000 copies/ml).</li> </ul>	Ensure that there are no laboratory / specimen errors. Exclude HIV-2 infection. Check CD4 count. If high, consider that the patient may be an elite or viraemic controller of HIV. Consult with a pathologist.

### HIV monitoring:

Once a diagnosis of HIV has been confirmed in a patient, it is then necessary to commence with staging the infection and making treatment and prophylaxis decisions with the patient. The staging is clinical, as well as laboratory based, with the use of CD4 (cluster of differentiation 4) and viral load testing. In general, antiretroviral treatment should be commenced in any patient with confirmed HIV infection regardless of CD4 count.

SMX / TMP (Bactrim) prophylaxis should be used in any patient with a CD4 of less than 200 cells/ $\mu$ l in order to reduce the risk of pneumocystis pneumonia developing in the patient. In addition, all patients with CD4 counts <100 cells/ $\mu$ l should be tested for cryptococcal antigenaemia by means of a serum cryptococcal antigen test (CRAG) and positive asymptomatic antigenaemic patients must be given fluconazole 800 mg daily for 2 weeks, then 400 mg daily for 8 weeks followed by fluconazole 200 mg daily for at least 10 months (or until the CD4+ T-cell count rises to >200 cells/ $\mu$ l, twice, 6 months apart) to prevent the development of cryptococcal meningitis.

### Indications for starting ARV therapy

#### **1. Symptomatic patients (irrespective of CD4 count)**

- WHO clinical stage 3 and 4
- Any severe HIV-related disorder should be considered an indication for ART such as:
  - Immune thrombocytopaenia
  - Thrombotic thrombocytopaenia
  - Polymyositis
  - Lymphocytic interstitial pneumonitis
- Non-HIV-related disorders, e.g.:
  - Malignancies
  - Hepatitis B

- Hepatitis C
- Symptomatic vascular disease or diabetes mellitus
- Any condition that requires long term immunosuppressive therapy

## 2. Asymptomatic patients

- Start ART regardless of CD4 count

### HIV and HBV (Hepatitis B virus):

Screening for HBV should occur at the time of diagnosis of HIV with the use of HBsAg (hepatitis B surface antigen) testing. If the patient is HBsAg negative and not immune, HBV vaccine should be offered. If HBsAg positive, ARV (antiretroviral) therapy should be commenced irrespective of CD4 count. First and second line ARV regimens should contain Tenofovir and Emtricitabine, or Tenofovir and Lamivudine for the treatment of both the HBV and HIV infections, together with a third drug active against HIV.

### HIV and TB:

WHO recommends that all patients are started on ARV as soon as possible after starting TB treatment, regardless of CD4 count (within 8 weeks of starting TB treatment).

- CD4 <50 cells/mm<sup>3</sup>: Start ARV 2 weeks after starting TB treatment
- CD4 >50 cells/mm<sup>3</sup> but with severe clinical disease (low Karnofsky score, low BMI, low Hb, low albumin, organ system dysfunction): start ARV within 2 – 4 weeks of starting TB treatment
- CD4 >50 cells/mm<sup>3</sup> without severe disease clinically: start ARV within 8 weeks of TB treatment.

### HIV and pregnancy:

All pregnant women should start triple drug ARV treatment regardless of CD4 count or viral load. This treatment should be started with a view to lifelong therapy.

### HIV in serodiscordant couples:

The HIV-infected partner should start ARV treatment regardless of CD4 count and WHO stage in order to prevent transmission to the non-infected partner.

### **HIV prophylaxis after unprotected sex, rape and needle sharing, and occupational post exposure prophylaxis**

HIV PEP is of critical importance in a high prevalence setting such as South Africa. PEP has proven efficacy if taken correctly and without interruption **and for the full 28 days**. PEP should be started as soon as possible following exposure. The longer the delay in starting PEP, the less likely it is to be effective. Beyond 7 days, PEP is no longer given. In South Africa, we recommend a triple drug regimen, although this should never be at the expense of adherence. HIV, HBV and HCV testing should be performed at baseline, at 6 weeks, and at 3 months following exposure. HCV testing should be repeated at 6 months. Some sources also recommend repeat HIV ELISA at 6 months post exposure.

- In high prevalence regions such as South Africa, a 3 drug regimen is usually recommended:
  - The backbone NRTI regimen can include either of the following combinations:
    - Truvada (Emtricitabine 200 mg and Tenofovir 300 mg) taken once daily
    - AZT (Azithromycin) 300 mg and Lamivudine 150 mg (Combivir) taken twice a day
  - The third drug can include one of the following:
    - Lopinavir 400 mg boosted with Ritonavir 100 mg (Aluvia) twice daily
    - Lopinavir 800 mg boosted with Ritonavir 200 mg (Aluvia) once daily
    - Raltegravir (Isentress) 400 mg twice daily
    - Atazanavir (Reyataz) 300 mg boosted with Ritonavir 100 mg daily. It is very important to use Ritonavir boosted Atazanavir if using it in combination with Tenofovir.

A good “go to” PEP regimen is Truvada once daily and Raltegravir 400 mg BID for 28 days. The side effect profile is favourable, and these drugs work early in the life cycle of HIV.

HIV PCR or viral load testing is NOT recommended following exposure to determine possible early HIV infection. This is because the time points following exposure when HIV PCR and viral loads become positive, should infection occur, are not well defined. In addition, PEP can delay infection, and a negative PCR or viral load performed early after exposure does not exclude the possibility of HIV infection. Routine HIV ELISA testing at 6 weeks and 3 months are the only HIV tests that should be performed.

For detailed information on the management of HIV in South Africa, check the latest guidelines on the website of the SA HIV clinician’s society <http://www.sahivsoc.org>

## **Hepatitis diagnosis and monitoring**

### **Hepatitis A virus (HAV)**

Diagnosis:

HAV IgG positive, IgM negative: immune to HAV

HAV IgG negative, IgM positive: acute infection with HAV.

HAV IgG positive, IgM positive: acute infection with HAV. IgM persists for 3 – 6 months, while IgG persists lifelong.

Management for HAV-infected patients is largely supportive. A small percentage of patients may develop fulminant hepatitis, but this is not a common manifestation of infection with this virus.

Some laboratory HAV IgG assays have been replaced by total antibody assays that detect both IgG and IgM antibodies. Total antibody assays may be a more sensitive marker of acute HAV infection even in the absence of a positive HAV IgM result. If acute HAV infection is suspected and HAV total antibodies are positive, repeat the HAV IgM after 3 – 5 days. If the IgM is then positive, a diagnosis of acute HAV is confirmed.

### Post exposure prophylaxis for HAV can be achieved in 2 ways

HAV is oral-faecal transmitted. Rare cases of transmission in blood products have been described. Sexual transmission may occur, but is also rare.

- Hepatitis A vaccine alone is favoured unless >2 weeks since contact or if the contact is immunocompromised or has pre-existing liver disease where vaccine plus immune globulin should be given. The efficacy of vaccine alone in persons over 40 years is not well established and HNIG (human immunoglobulin) should be given in addition to vaccine.
- People who may need PEP after contact with an infected patient include:
  - Household and sexual contacts
  - Children in day-care centres
  - Cases in institutions where hygiene is likely to be poor e.g. with physically or mentally disabled people, and where incontinence and nappy use is likely to be encountered.
  - Workers who may have been exposed to faecal material without use of adequate protective equipment.
- Dose
  - Vaccine (Havrix®, Avaxim®) – 2 doses a month apart
  - Human normal immune globulin (HNIG) (Beriglobin®, Intragam®) – 0.02 – 0.04 ml/kg.
- Vaccine and immune globulin should be given at different sites.

### Pre-exposure prophylaxis:

- HAV inactivated vaccine is the method of choice for pre-exposure prophylaxis for HAV. It is highly immunogenic and almost 100% of adults will develop immunity within 4 weeks of vaccination. A booster provided within 6 – 12 months provides long lasting, and probably lifelong, immunity to HAV.
- Should travel to a highly endemic area be anticipated and there is insufficient time available for immunity to the vaccine to develop, normal human immunoglobulin may be administered at a dose of 0.02ml/kg IM. It is important to note that this only provides immunity for 3 months and so the vaccine

should be administered at the same time (administered into the opposite Deltoid muscle to the one used for immunoglobulin). It takes approximately 2 – 4 weeks for immunity to develop to the vaccine. Immunity provided by the immunoglobulin is almost immediate.

### Infection control

- Standard and contact precautions are adequate
- Strict hand hygiene
- Proper and careful disposal of faecal waste

For detailed information on HAV, refer to the guidelines on “Guidelines for the control of hepatitis A in South Africa” on the NICD website: <http://www.nicd.ac.za>. Click on the publications tab, and then on guidelines.

### Treatment

Management is mainly supportive.

### Hepatitis B Virus:

#### Diagnosis

HBsAg	HBeAg	Anti HBc IgM	Anti HBc	Anti HBe	Anti HBs	Interpretation
Pos	Neg	Neg	Neg	Neg	Neg	Incubation, Early disease
Pos	Pos	Pos	Pos	Neg	Neg	Acute disease
Pos	Neg	Pos	Pos	Pos	Neg	Acute disease
Neg	Neg	Pos	Pos/Neg	Neg	Neg	Diagnostic window
Pos	Pos	Pos/Neg	Pos	Neg	Neg	Super carrier
Pos	Neg	Neg	Pos	Pos	Neg	Simple carrier

HBsAg	HBeAg	Anti HBc IgM	Anti HBc	Anti HBe	Anti HBs	Interpretation
Neg	Neg	Neg	Pos	Pos	Pos	Convalescence
Neg	Neg	Neg	Pos	Neg	Pos	Immune following wild virus infection
Neg	Neg	Neg	Neg	Neg	Pos	Immune due to vaccine or immunoglobulin
Neg	Neg	Pos	Neg	Neg	Pos/Neg	Fulminant HBV infection

- In general, HBsAg should clear within 6 months following an acute infection. If this does not occur, the patient is classified as a chronically infected or a carrier of HBV and remains potentially infectious. The rate of chronic infection varies depending on the age at which infection is acquired. An adult infected with HBV generally has a risk of chronicity of around 10%. A child infected in the perinatal period has a risk of 80 – 90% if no PEP (post exposure prophylaxis) is available.

#### Post exposure prophylaxis

HBV is transmitted parenterally. Contamination with infected blood or blood products, IVDU (intravenous drug use), and vertical transmission usually during the birth from an infected mother to the baby are the common routes of transmission. HBV is not transmitted in breast milk. Sexual transmission also occurs. Transmission has been described in some contact sports where blood contamination may occur.

- A previously vaccinated healthy individual with proven seroconversion requires no PEP
- Contacts are infectious if they are hepatitis B surface antigen (HBsAg) positive
- Mucocutaneous exposure to blood and body fluids requires PEP with hepatitis B immune globulin (HBIG) and vaccine
- Institutional or household contacts (no defined exposure) require vaccine only
- Newborns of HBsAg-positive mothers
  - If mother eAg-positive: HBIG and vaccine (first dose at birth)

- If mother eAg negative: vaccine only (first dose at birth). Then continue with the normal EPI schedule.
- Dose (vaccine and immunoglobulin to be given at different sites)
  - HBIG (200 IU/2ml) (Hebagam®)
    - Newborn – <5 years: 200 IU
    - 5 – 9 years: 300 IU
    - 10 years: 500 IU
  - Vaccine (Engerix-B®, H-B-Vax II® Heberbiovac HB®): 3 doses at 0, 1 and 2 months with a booster at 12 months.

### Pre-exposure prophylaxis

HB vaccine is administered now as part of our EPI programme in South Africa. In addition to the children vaccinated in the EPI schedule, the following people should also be vaccinated against HBV:

- Healthcare workers at risk of exposure to blood or blood contaminated fluids
- Patients in renal failure in need of chronic dialysis
- Patients with underlying liver disease or HCV (hepatitis C virus) infection
- HIV-infected people who are not immune to HBV
- People who present to medical care with a sexually transmitted infection
- Men who have sex with men
- Sexual partners of those with a chronic HBV infection
- Household members of a person chronically infected with HBV
- Residents and staff of institutions for developmentally disabled people
- Non-immune travellers

Higher doses of vaccine may be required in immunocompromised people and people on dialysis. People requiring post vaccine serological testing for immunity (anti-HBs – hepatitis B surface antibody):

- Healthcare workers
- Infants born to HBsAg-positive mothers
- Immunocompromised and dialysis patients
- Sexual partners of people with chronic hepatitis B.

People who require booster vaccine:

- Immunocompromised patients should be monitored annually, and when their HBsAb (hepatitis B surface antibody) levels drop below 10mIU/mL, booster dose of vaccine should be administered.
- Otherwise healthy individuals with recorded seroconversion to vaccine do not need ongoing monitoring or boosters.

### HBV treatment

- Treatment of acute infection is largely supportive. However, Lamivudine may be used in severe infection as it reduces viral load and therefore reduces the risk of recurrent infection should liver transplantation be necessary. It may also be used in patients with prolonged severe acute infection (jaundice >4 weeks and INR >1.5). The elderly, immunocompromised patients, and patients coinfected with HCV should also be treated if they acquire acute HBV disease, as they are more likely to follow a fulminant or sub-fulminant course.

### Treatment for chronic HBV infection

#### Assessment and monitoring

- Assessment of liver disease
  - Full LFTs
  - FBC
  - PTT

- Liver ultrasound
  - Declining trend in albumin, increase in globulins, prolonged PTT and declining platelet counts are often seen as cirrhosis develops
- Hepatitis VB viral load
  - Essential for diagnosis and decision to treat and subsequent monitoring
- Exclude other causes of liver disease
  - HCV, HIV, HDV, HAV IgG (if negative, vaccinate against HAV)
  - Alcoholic, autoimmune, and metabolic liver disease
- Liver biopsy
  - Determines degree of fibrosis and necroinflammatory disease and can assist in the decision to start treatment. Usually not required in patients with other evidence of cirrhosis, or in those in whom treatment is indicated irrespective of what histology would show

### End goals of therapy

The goal of therapy is to achieve sufficient viral suppression so that biochemical and histological improvement occurs and complications are prevented. Ideally one would like to achieve sustained loss of HBsAg, but this is not often reached with current treatment options. The aim presently therefore is sustained biochemical and virological remission.

- In HBeAg (hepatitis B e antigen) positive and negative patients sustained off therapy loss of HBsAg even without serological evidence of seroconversion to HBsAb. This is associated with complete remission of chronic hepatitis B and improved long-term outcome.
- Sustained off therapy virological and biochemical response in HBeAg-negative patients. This has been associated with improved prognosis.
- A maintained virological remission with undetectable HBV DNA under long-term antiviral therapy in HBeAg-positive patients who do not achieve HBeAg seroconversion and in also in HBeAg-negative patients who do not necessarily achieve the above end points.

## Indications for therapy

Factors to consider:

- HBV DNA levels >2000IU/mL
- Serum ALT levels above the upper limit of normal
- Severity of liver disease indicating moderate to severe necroinflammatory disease, or moderate fibrosis on the liver biopsy. If there is evidence of severe liver disease and elevated HBV DNA levels, treatment is indicated even if ALT is normal
  - HBeAg-positive patients, <30 years of age, normal ALT levels, high HBV VL (HBV viral load) levels, no evidence of liver disease or family history of HCC or cirrhosis:
    - No need for immediate therapy or biopsy
    - Follow up 3 – 6 monthly
  - HBeAg-positive patients >30 years of age, and/or family history of HCC (hepatocellular carcinoma) or cirrhosis:
    - Consider biopsy
    - Consider therapy even if biopsy not available
  - HBeAg-negative patients, normal ALT levels (determined 3 monthly for at least 1 year), HBV VL >2000IU/ml but <20 000IU/ml, no evidence of liver disease:
    - No need for immediate liver biopsy or therapy
    - ALT 3 monthly for first year
    - HBV VL 6 monthly for first 3 years
    - Thereafter, follow up as for all inactive chronic HBV carriers
  - HBeAg-positive and -negative patients with obvious chronic active Hepatitis,
  - ALT > 2 times upper limit of normal, HBV VL >20 000 IU/ml:
    - Start treatment even without liver biopsy. Biopsy or a non-invasive method to determine presence of cirrhosis would be useful, but would probably not change the decision to treat.

- Detectable HBV DNA and compensated cirrhosis:
  - Treat even if ALT levels are normal
- Detectable HBV VL and decompensated cirrhosis:
  - Urgent treatment with nucleot(s)ide analogues
  - Liver transplantation may be necessary, although some patients are rescued by use of urgent antiviral intervention

Available drugs (adefovir and telbivudine not yet available in South Africa):

- Interferon (IFN) and pegylated interferon (PEG-IFN)
- Nucleoside analogues (lamivudine, entecavir, emtricitabine, telbivudine)
- Nucleotide analogues (tenofovir, adefovir)

### Treatment strategies

- Finite duration treatment:
  - IFN, PEG IFN, or a nucleoside/nucleotide analogue (NA): This treatment is designed to achieve a sustained off-therapy virological response.
    - IFN/PEG IFN (latter is preferred) for 48 weeks has several advantages including finite duration of therapy, absence of resistance, and immune mediated control of the virus allowing the chance of achieving a sustained virological response with HBsAg loss once treatment is concluded. However, the use of IFN demands subcutaneous injections and has a high rate of side effects and is contraindicated in patients with decompensated cirrhosis, autoimmune disease, pregnancy, and severe depression or psychotic conditions. Combination with a NA is currently not recommended. IFN works best on genotypes A and B HBV.
  - Entecavir or Tenofovir are the only NA recommended for first line monotherapy as they both have a high barrier to resistance. The other 3 drugs should be avoided unless the above 2 are not available or contraindicated. Treatment duration is unpredictable, and close virological monitoring of patients is required after

treatment is stopped, as relapses are common. Once HBeAg seroconversion occurs, treatment should be continued for at least another 12 months. If HBeAg seroconversion persists during these 12 months, a durable off-treatment response will be found in 40 – 80% of such patients.

- Long-term treatment with a nucleoside/nucleotide analogue:
  - This strategy is necessary in patients who fail finite therapy or who are not expected to achieve a sustained off-treatment response (e.g. patients who fail to achieve HBeAg seroconversion or who are HBeAg negative at the start of therapy). This strategy is also recommended for patients with cirrhosis regardless of HBeAg status or seroconversion. Tenofovir or Entecavir are recommended as first line monotherapies in this situation. Treatment for >3 years is associated with maintained virological remission in the majority of patients.

Before treatment is considered, all patients with chronic HBV infection should be screened for HIV. If HIV is found, treatment using 2 active NA's against HIV and HBV should be used, together with a third ARV drug for HIV. HBV should never be treated in isolation in this situation as HIV will develop resistance to the NA used for HBV. Similarly, HIV should never be treated without using drugs active against HBV, as immune reconstitution will be associated with severe flare ups of HBV disease.

For complete guidelines on the management of HBV infection, please refer to EASL clinical practice guidelines: Management of hepatitis B virus infection. Journal of Hepatology 2012; vol 57:167-185.

South African Guideline for the Management of Chronic Hepatitis B:2013 S Afr Med J 2013;103(5):335-349. DOI:10.7196/SAMJ.6452

## **Hepatitis C virus**

### Diagnosis

If the HCV ELISA (combined IgG and IgM) is negative then the patient is unlikely to be infected with HCV. The seroconversion window period may however be prolonged, anything from 6 weeks to 3

months, and testing should be repeated to exclude later seroconversion. Alternatively, PCR is helpful in that window period to determine an acute HCV infection. In HIV infected persons with chronic HCV, the ELISA may be negative and a PCR should be requested should a person have risk factors for HCV or if it is suspected on clinical grounds.

HCV ELISA serology: positive. The patient is seropositive for HCV. This result should be confirmed on an alternative technology as we have a low prevalence of HCV in South Africa and the positive predictive value of a single ELISA result is poor. The seropositive result may be confirmed with the use a recombinant blot (RIBA) assay, or PCR. Most patients infected with HCV will become chronic carriers of the virus and therefore PCR confirmation is more useful than additional serological assays, and a positive result will indicate the need for further monitoring and treatment.

HCV ELISA	HCV PCR	Interpretation
Positive	Negative	Seroconversion and cleared infection, OR early infection with low-level viraemia.
Positive	Positive	Seroconversion and chronically infected. May also be a recent infection depending on clinical context.
Negative	Positive	Early acute infection prior to seroconversion.
Negative	Negative	Unlikely to be infected.

#### Pre- and post exposure prophylaxis

At this stage there is no vaccine available for HCV. Prevention includes avoiding exposure to the virus by preventing percutaneous injury or mucosal exposure and contamination with infected blood. The use of gloves and PPE (personal protection equipment), proper and safe use and disposal of sharps,

appropriate testing of the blood supply, and use of clean needles/needle exchange in IVDU are the best methods of preventing infection. Sexual transmission of HCV does occur particularly in receptive MSM (men who have sex with men) and can be prevented with condom use.

Similarly there is no proven post-exposure prophylaxis for HCV exposure. Should an exposure occur with a known HCV infected source then a baseline ALT and anti-HCV should be performed on the exposed person. Follow-up testing at 6, 12 and 24 weeks is advised and if the ALT is elevated then request a HCV PCR to determine an early infection in the exposed person.

#### Management of acute HCV infection

Identification of acute HCV infections is not common as most acute infections are asymptomatic. Acute infections may be confirmed in patients who are symptomatic or when there has been laboratory monitoring for HCV infection following an exposure to an infected source such as from a needle stick injury.

There is growing evidence that interferon alpha therapy given during the acute-phase will reduce the rate of chronicity to 10% or less.

Thus, all patients with acute HCV infections, where detected, should be considered for interferon-based therapy. The excellent responses seen in clinical trials of antivirals for acute HCV were with standard interferon monotherapy, however, because of the ease of administration Pegylated interferon is preferred. Treatment should be delayed for 8 – 12 weeks to allow for spontaneous resolution first.

No definite recommendations can be made regarding the optimal duration of therapy but most guidelines recommend treating for up to 24 weeks. In addition no recommendations can be made at this stage for or against the addition of Ribavirin given the high rates of a sustained virological response with interferon, and the decision to add Ribavirin is made on a case by case basis.

**Dose:**

Standard interferon alpha 2b- 5 MU subcutaneously daily for 4 weeks then 5 MU subcutaneously 3 times a week for 20 weeks.

Pegylated interferon alpha 2b- 1.5 µg/kg subcutaneously given weekly for 12 – 24 weeks.

**Management of chronic HCV infection****Workup prior to starting therapy**

- HCV VL as a baseline for future monitoring once on treatment
- HCV genotyping as this determines duration of treatment and likelihood of virological response
- Liver biopsy if information is needed on fibrosis stage for prognostic reasons, or to detect the presence of liver disease in the subset of patients in whom ALT levels are normal

**Indications for therapy**

- **All** adults with confirmed chronic HCV infection.
- Particular consideration should be given to those most likely to progress to fibrosis and other complications of HCV infection: patients with HIV infection, male gender, older people with HCV infection, obese individuals, and the use of more than 50 g of alcohol daily all predict quicker progression to fibrotic liver disease. Obesity also adversely affects treatment outcomes and patients with BMI >25 should be encouraged to lose weight before commencing treatment. Excessive alcohol use also increases the rate of fibrosis, and while not an absolute contraindication to antiviral therapy, should be stopped before treatment is considered.
- Patients with advanced fibrosis should be considered for treatment although this is not an absolute indicator for antiviral treatment.
- Symptomatic cryoglobulinaemia.
- Occupations in which transmission to others is likely.

### Predictors of good response to treatment

- Female gender; age <40; genotype 2 or 3 infections; HCV VL <600 000 IU/ml; body weight <75 kg; ALT >3 times upper limit of normal; absence of bridging fibrosis or cirrhosis; absence of insulin resistance

### Treatment

- PEG IFN in combination with ribavirin.
- Genotype 2 and 3 infections:
  - PEG IFN α2a 180 ug per week subcutaneously + Ribavirin 800 mg daily OR
  - PEG IFN α2b 1.5 ug/kg/week subcutaneously + Ribavirin 800 mg daily
  - Duration of treatment: 24 weeks
- Genotype 1 and 4 infections:
  - PEG IFN α2a 180 ug per week subcutaneously + Ribavirin 15 mg/kg daily OR
  - PEG IFN α2b 1.5 ug/kg/week subcutaneously + Ribavirin 15 mg daily
  - Duration of treatment: 48 weeks. This may be extended to 72 weeks if RNA clearance is delayed.
- Genotype 5 and 6 infections:
  - There is little data on treatment of these 2 genotypes, and while some evidence is emerging to show a good response to treatment in Genotype 5, the recommendation is rather to treat as per Genotype 1 and 4 infections until more definitive data is available.
- The newer antiviral treatments for HCV, while not yet easily obtained in South Africa, are extremely successful in curing HCV across various genotypes. An example likely to be available shortly is Gilead Science's Harvoni® (Sofosbuvir and Ledipasvir). A 12 week course of one dose a day has demonstrated cure rates of up to 98%. Even at special public sector prices though, this drug is likely to cost around R17 000 for the full treatment course.

### Types of response to therapy encountered

RVR (rapid virological response): Negative HCV RNA at Week 4 of treatment. RVR may allow shortening of treatment period for Genotypes 2 and 3

cEVR (complete early virological response): Negative HCV RNA at week 12 of treatment

pEVR (partial early virological response): >2log reduction in VL from baseline

ETR (end of treatment response): HCV RNA negative at end of treatment (week 24 or 48)

SVR (sustained virological response): HCV RNA negative at 24 weeks after treatment is stopped. SVR is the best predictor of a sustained response to HCV treatment

Breakthrough: Reappearance of HCV RNA while still on therapy

Relapse: Reappearance of HCV RNA once therapy has been stopped

Non responder: failure to clear HCV RNA after 24 weeks of therapy

Null responder: failure to decrease HCV RNA by > 2 logs by week 24 of therapy

Partial responder: >2 log decrease in HCV RNA level by week 24, but failure to clear infection

### Contraindications to therapy

- Major uncontrolled depressive illness
- Solid organ transplant
- Autoimmune disease which may be exacerbated by IFN (interferon)
- Untreated thyroid disease
- Pregnancy or not on adequate contraception
- Severe concurrent medical disease e.g. hypertension, COPD (chronic obstructive airway disease), uncontrolled diabetes, CAD (coronary artery disease)
- Age less than 2 years
- Known hypersensitivity to the drugs for treatment of HCV

For full guidelines of the management of HCV, please refer to the guidelines below:

AASLD guidelines: Hepatology 2009; April:1335–1373

EASL clinical practice guidelines: management of hepatitis C virus infection. Journal of Hepatology 2011; vol 55: 245–264

South African Hepatitis C management guidelines: The South African Gastroenterology Review April 2010.

### **Hepatitis D virus**

HDV is a defective viral particle that cannot cause infection in the absence of HBV. Therefore, infection with HDV either occurs simultaneously with HBV infection (co-infection), or is superimposed on existing chronic HBV infection (superinfection). Severe/fulminant hepatitis is seen more frequently in HBV-HDV coinfection than in HBV infection alone, while chronic HDV infection is more commonly encountered when HDV superinfects an existing HBV chronically infected patient. HDV is found mainly in the Mediterranean countries and in South America. It is infrequently encountered in South Africa except in patients of Mediterranean extraction and occasionally from the former Portuguese colonies (Mozambique and Angola).

### **Diagnosis**

Diagnostic assays are not commonly available for HDV infection, so please contact the laboratory should you wish to test for this virus.

Assays that can be used to make a diagnosis:

HDV IgM serology

HDV RNA PCR (none are standardised at this stage)

## Prevention

- Infection control
- HBV vaccine

## Treatment

- Treatment of the underlying HBV infection.
- HDV does not respond to the NA, and only responds to PEG-IFN or IFN. Not much is known about the efficacy, or how long to treat, or how long to monitor post treatment, but more than a year of treatment may be necessary.

## **Hepatitis E virus**

HEV is mainly faecal orally transmitted, and is associated with contaminated water sources. HEV is commonly encountered in India and parts of Africa. We do not often see disease due to HEV in South Africa except in travellers, but there is evidence that the virus is present in this country. It causes clinical illness similar to that encountered in HAV infection and most people will recover with supportive management. Patients particularly vulnerable to severe infection with a mortality rate of 20% include pregnant women.

## Diagnosis

Diagnostic assays are not freely available in South Africa, so please contact the laboratory should you wish to test for HEV.

HEV serology

HEV PCR

### Prevention and PEP

- None known or proven to work
- Ensure that only clean water is consumed, particularly when travelling
- Contact precautions and careful disposal of faecal material in the hospital setting.

### Treatment

Mainly supportive

### Other viral causes of Hepatitis

- HSV which can cause a rapidly fulminant hepatitis and needs to be treated with IV acyclovir as a matter of urgency. A HSV PCR must be requested on a blood sample in any patient with an unexplained hepatitis where an acute hepatitis A and B has been excluded.
- VZV frequently causes a low grade hepatitis that is seldom severe.
- CMV may cause hepatitis particularly in immune-compromised patients. This may occasionally be encountered in otherwise healthy patients too during a primary CMV infection.
- EBV may cause fulminant hepatitis. This is a well-described complication in people with X-linked lymphoproliferative disorder and certain other primary immunodeficiency conditions. It can occur in otherwise healthy people during a primary EBV infection.
- Arboviral infection and Viral Haemorrhagic Fevers.

## Viral infections in pregnancy

### Rubella

Rubella in the first 4 months of pregnancy carries a severe risk to the foetus of Congenital Rubella Syndrome. Diagnosis of Rubella in pregnancy is predominantly serological.

## Interpretation of serology

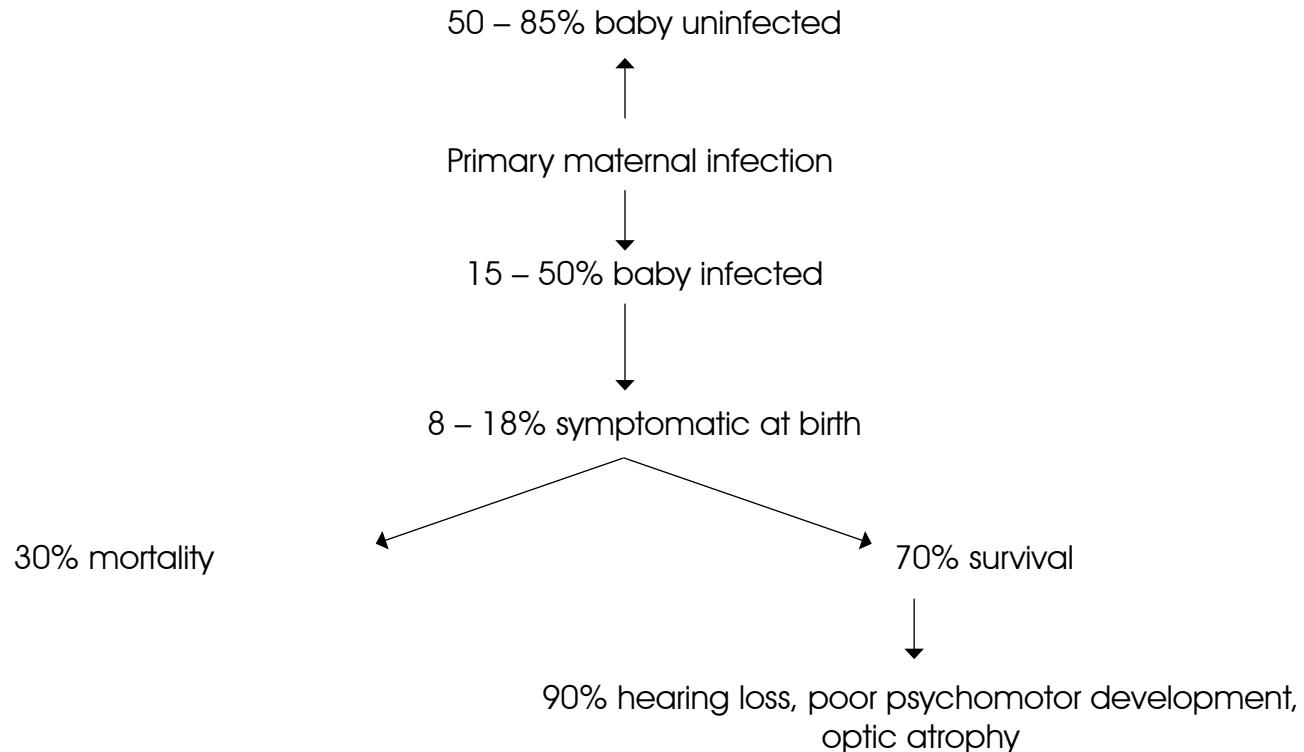
Maternal IgM	Maternal IgG	Avidity	Interpretation	% Risk to the foetus
-	+		Maternal immunity	0
+ (always confirm on a second different assay)	-		Primary infection in the mother	>80%
+	+	>70%	Pre-existing immunity with re-exposure to rubella	3 – 8%
+	+	<30%	Recent primary infection	>80%
-	-		No evidence of infection serologically, but monitor for seroconversion	0, but recheck in 2 weeks. <b>Immunize mother once baby is delivered!</b>

## CMV:

### Serological interpretation

Maternal IgM	Maternal IgG	Avidity	Interpretation
-	+		Previous maternal infection
+ (always confirm on a second different assay)	-		Primary infection in the mother
+	+	>50%	Prior infection with CMV and most likely reactivation of CMV or second infection with a different genotype of CMV
+	+	<50%	Recent primary infection
-	-		No evidence of infection serologically, but monitor for seroconversion

### Risks to the foetus in primary maternal infection



It is important to note that CMV may cause foetal infection in primary infection of the mother, which is the highest risk situation, but also with reactivation or secondary CMV infection. Additional tests that may be performed include looking for evidence of maternal viraemia, and examining the CMV VL in amniotic fluid after 21 weeks gestation. If a primary CMV infection is detected antenatally then mother needs to be treated with CMV hyper immune globulin to reduce the possibility of CMV damage to the foetus.

Infected babies can be treated with Ganciclovir 6mg/kg 12 hourly IVI for 6 weeks. Evidence accumulating in the literature suggests that this treatment limits the damage caused by CMV in the neonate.

## **HSV**

### Diagnosis

In the infant:

- HSV PCR performed on lesions or CSF (cerebrospinal fluid). May be positive also in blood in babies with disseminated infection.

In the mother:

- Serology can be confusing for diagnosis of HSV disease.
  - IgG negative, IgM positive: most likely primary infection with HSV.
  - IgG positive, IgM negative: most likely prior exposure to HSV.
  - IgG positive, IgM positive: this could be due to late primary infection or reactivation disease or a false positive IgM result.
  - IgG negative, IgM negative: IgM responses can be significantly delayed in some people. So in general this result may indicate no prior infection with HSV, but should not suggest that HSV is not present at the time serology is performed.
- PCR of lesion fluid.

Primary genital infection with HSV poses a 30% risk of HSV transmission to the neonate. The risk is considerably lower in reactivation disease being closer to 3%. The risk to the baby occurs in being delivered through an infected genital tract, or with HSV ascending into the uterus (which is rare). Maternal treatment with acyclovir does not seem to be protective for the infant.

### Disease manifestations in the infant

- True congenital infection following in-utero transmission is rare
  - Presents within 48 hours.
  - Triad of skin vesicles or scarring, eye disease, and microcephaly or hydrancephaly.
- Disseminated disease
  - Multi-organ involvement in which 80% have skin vesicles (IMPORTANT -20% have no skin lesions) and 75% will have encephalitis as part of the dissemination.
  - Presents at 9 to 11 days of life.
- Encephalitis
  - Found in 30% of babies with neonatal HSV infection.
  - May not have evidence of rash .
  - Presents within 9 to 11 days if part of disseminated disease, or 16 to 17 days if part of retrograde axonal spread to the brain.
  - It is important to note that the CSF may be cytologically and biochemically normal in these babies! A high index of clinical suspicion is required, and treatment should be started empirically and not wait on laboratory results.
- Skin, eye and mouth disease
  - Presents with typical HSV lesions within 10 to 11 days of birth.
  - Should be treated because there is a high rate of subsequent neurological delay in these children if they are not managed with acyclovir.

### Treatment

- Acyclovir 20mg/kg slow IV 8 hourly for 21 days.

## **Parvovirus B19**

Parvovirus infection occurs in approximately 5% of pregnant women exposed to this virus, and about 8% of these pregnancies will show an adverse outcome. In general, infection with this virus is not an indication for TOP, as many babies will recover before delivery. Parvovirus can cause anaemia in the foetus with subsequent cardiac failure and the development of hydrops foetalis. This frequently recovers spontaneously in utero. There are accounts of successful treatment with the use of foetal blood transfusion.

### Diagnosis

Serology:

- IgG positive, IgM negative: previous infection with parvovirus, foetus not at risk.
- IgG negative, IgM negative: no previous infection with parvovirus. Check serology again in 2 weeks.
- IgG negative, IgM positive: primary infection with parvovirus, monitor foetus.
- IgG positive, IgM positive: may be primary infection or repeated exposure on a background of maternal immunity. Monitor foetus.

Parvovirus PCR on maternal blood.

## **HBV infection in pregnancy**

This is generally encountered in countries with high or intermediate endemicity where chronic infection rates with HBV are high. Diagnosis in the mother is usually serological as in the earlier section on HBV diagnosis.

### Risks to the infant

- HBeAg positive mother: the risk of the baby acquiring the infection during the birth is 70 – 90%.
- HBeAg negative mother: the risk of the baby acquiring infection during the birth is 10 – 40%.
- If the baby becomes infected, the risk of becoming chronically infected is 80 – 98% with risk of early manifestation of progressive disease such as cirrhosis and hepatocellular carcinoma.

### Management of the exposed infant

- HBIG administered within 24 hours of birth AND
- Vaccine administered within 24 hours of birth. After this initial dose, the normal EPI schedule is followed.
- 85 – 90% of babies will be protected if this combination is used.
- In some countries, HBIG is not easily obtained and vaccine alone is used. This is efficacious, but carries a slightly increased risk of infection of the baby.

HBV is not transmitted in breast milk, so babies born to infected mothers can be safely breast fed in the absence of other contraindications.

### HCV infection in pregnancy

South Africa has a relatively low prevalence of HCV (<2%) in the general population. Transmission from the mother to the foetus/newborn is relatively rare. No immunoprophylaxis is available. However, it seems that babies tend to clear the HCV at a higher rate than adults, so monitoring of an exposed baby is usually sufficient. Treatment is usually not indicated until the child reaches 2 years of age if infection persists. HCV transmission is not associated with breast feeding. Diagnosis is as described in the previous section on HCV infection.

A higher risk of infant infection is associated with dual HCV/HIV infection of the mother, and higher HCV VL in the mother ( $>10^6$  IU/ml).

### VZV (varicella zoster virus) infection in pregnancy

Most in utero infections occur with primary rather than reactivation disease in the mother. The highest risk to the foetus is in the first 4 months of pregnancy. In utero injury to the baby is rare in this infection, and the overall risk to the foetus is around 2% in primary maternal infection (chicken pox). Infection with this virus is therefore usually not regarded as an indication for TOP (termination of pregnancy).

## Diagnosis

Serology:

- IgG positive, IgM negative: prior exposure with immunity to VZV.
- IgG negative, IgM positive: primary infection with VZV.
- IgG positive, IgM positive: either recent primary infection or reactivation of VZV – correlate clinically.
- IgG negative, IgM negative: no prior immunity to VZV. Monitor for serological conversion.

PCR on blister fluid if lesions are present.

If chicken pox occurs in the mother in the last 5 days of pregnancy or first 2 days following delivery, the baby is unprotected (maternal IgG has not had time to form, cross the placenta and protect the baby). Such infants are very vulnerable to severe chicken pox with a 30% associated mortality rate. These babies should receive varicella-zoster immune globulin (VZIG) administered as close to delivery as possible, and acyclovir should be used at the first sign of break-through disease.

Chicken pox in pregnancy is also far more dangerous for the mother than in adults generally. Any pregnant woman or woman in the perinatal period who develops chicken pox should be treated with acyclovir (800 mg orally 5 times daily for 7 days, or 10mg/kg 8 hourly IVI for 7 to 14 days). This increased risk is present during the pregnancy and for up to 2 months following delivery of the baby. Acyclovir has not been associated with foetal abnormalities when used in pregnancy, although consent for its use should be obtained from the mother.

Strict infection control and isolation of the mother and baby is required to protect other susceptible patients/staff in the hospital.

## **Measles in pregnancy**

Diagnosis:

Serology:

- Measles IgG positive, IgM negative: immune to measles.
- IgG positive, IgM positive: could be either late primary infection or re-exposure in an immune individual. Correlate clinically.
- IgG negative, IgM positive: primary infection with measles. Notify the local authorities and hospital infection control.
- IgG negative, IgM negative: non-immune and no evidence of acute infection. Repeat if suspect acute disease, although usually IgM is present when the patient presents.

Measles infection in pregnancy is not associated with foetal abnormalities. If the mother develops measles in the last week of pregnancy though, disease in the infant can be severe. The mortality rate associated with neonatal measles approaches 30%. Immunoglobulin prophylaxis should be administered to the infant as close to delivery as possible.

Exposed contacts should be offered measles vaccine within 72 hours of exposure. This measure is an excellent PEP in most cases. Measles vaccine is often not effective in young babies due the presence of maternal antibody. Strict infection control and isolation must be practiced in the ward/ICU in which the mother and baby are nursed.

## **Enterovirus infections in pregnancy**

There is no consensus at this stage regarding the development of foetal abnormalities if enteroviral infections occur during pregnancy. However, if the mother is infected with an enterovirus in the last part of the pregnancy and does not have time to develop antibodies that can cross the placenta and protect the infant before delivery, infection in the neonate can be severe. The disease is usually multi-system and resembles gram-negative septicaemia. Diagnosis may be made with the use of

PCR testing on throat swabs, stool, blood, CSF etc. Treatment is supportive. Some reports indicate that the use of high titre immunoglobulin against the specific enterovirus is useful therapeutically. Serological diagnosis of enteroviral infections is seldom helpful.

## **Viral respiratory tract infections**

Acute respiratory disease accounts for an estimated 75% of all morbidity in developed countries, and 80% of these are due to viral infections. Up to 8 URTI (upper respiratory tract infections) will be encountered in young children per year and up to 5 in adults, making URTI the commonest disease encounter human beings experience!

### **Influenza**

Influenza virus is a member of the *Orthomyxoviridae* family.

Annual epidemics in temperate climates generally lasting 3 – 8 weeks are encountered in the winter months.

Influenza has pandemic potential when novel viruses emerge into the human population either from a zoonotic source, or from genetic reassortment resulting in a major antigenic change and the emergence of a virus to which there is little or no population immunity. Further genetic changes may then accumulate which render the virus more suitable to human transmission and disease.

Many respiratory infections are indistinguishable clinically, and influenza is an important pathogen to diagnose accurately as treatment is available in severe cases and in persons at risk of developing a severe infection, and infection control a priority in institutional or hospital settings.

Available diagnostic tests include the following:

DFA (direct immunofluorescence assay):

The sensitivity of this assay is 70 – 100%, and the specificity 80 – 100% (compared with culture) PCR for influenza virus is the gold standard of diagnosis. The sensitivity is 91 – 100% for influenza B, and 96% – 100% influenza A. The specificity 96% for both influenza A and influenza B.

### Influenza prevention

- Annual vaccination with WHO (World Health Organization) recommended strains for that season.
- Infection control in hospital settings.
- Antiviral agents such as Oseltamivir are no longer recommended as PEP. Use of these drugs as PEP has been associated with the development of antiviral resistance in influenza.

### Influenza treatment

Treatment decisions should be based on clinical presentation and NOT delayed pending laboratory confirmation.

- Oseltamivir (Tamiflu):
  - Standard adult treatment: 75 mg bd for 5 days. Longer duration of therapy may be considered in critically ill patients. In these patients, higher dosages (150 mg 12 hourly) have no proven efficacy, and the side effect profile is increased at these dosages.
  - In critically ill ventilated patients, the tablets can be crushed and given via the nasogastric (NG) tube. The NG tube must be flushed initially with saline and then flushed again after the oseltamivir is given with 50 mL saline.
  - Renal adjustment is required depending on GFR.
  - Children:
    - Premature infants; 1 mg/kg 12 hourly for 5 days
    - 0 – 12 months: 3 mg/kg 12 hourly for 5 days
    - ≤15 kg body weight: 30mg/kg 12 hourly for 5 days
    - 15 – 23 kg: 45 mg/kg 12 hourly for 5 days
    - 24 – 40 kg: 60 mg/kg 12 hourly for 5 days
    - >40 kg: adult doses.
  - Dosing should be adjusted in children with renal impairment.

- Zanamivir (Relenza):
  - Two inhalations (5 mg per inhalation) bd for 5 days
  - Poorly suited for treatment in children due to difficulty in coordination of inhalational drugs.
  - Should not be used in patients on mechanical ventilation as it damages the ventilator, and also must not be administered via nebulisation.

### **Other respiratory viruses**

These include parainfluenza viruses, respiratory syncytial virus, human metapneumovirus, adenovirus, rhinoviruses, enteroviruses, parechoviruses, bocavirus and coronaviruses.

These are generally best diagnosed by means of PCR assays. PCR technologies are generally more sensitive assays for respiratory pathogen detection and permit more frequent and accurate diagnoses of RTI. This is particularly important in the case where shedding of virus may be low in respiratory specimens. The advantages of PCR:

- Appropriate drugs may be prescribed to manage the viral condition.
- Assists infection control in the hospital.
- Prevents unnecessary investigations.
- Provides accurate epidemiological information.
- Can be multiplexed, thus testing for a range of pathogens.
- Allows for the detection of pathogens that are not easily cultured or for which mAb (monoclonal antibodies) are not easily available for the performance of DFA.
- Generally more sensitive than other detection technologies.
- Allows the documentation of multiple viral aetiologies, which may account for an average of 3 – 30% of positive samples. There is also some evidence that mixed viral infections may contribute to more severe disease in the patient.

Disadvantages:

- Increased costs associated with PCR.
- These costs may well be offset by the benefit to the patient, the hospital and epidemiologically.

Infection control for most viral RTI

- Standard precautions.
- Droplet precautions.
- Isolation/cohort isolation.
- Be aware that many of these viruses can be transmitted via fomites.

## **Infections in the immunocompromised patient**

Infections encountered in this population depend on the degree and type of immunosuppression, and the use of prophylactic agents such as TMP/SMX, and antivirals. The more commonly encountered pathogens include:

### **CMV (cytomegalovirus)**

- Initial screening can be serological. In transplant patients this will allow some determination of the risk of acquiring or reactivating CMV infection. For example, if the recipient is found to be CMV seronegative and the donor seropositive, a significant risk exists for CMV infection developing in the post-transplant period in the immunocompromised recipient.
- Thereafter, CMV is best diagnosed using molecular technology. PCR is useful in terms of qualitative positive/negative results, but quantitative viral load offers more information in terms of the significance of a positive result. In addition, VL trends may be monitored over time and either therapeutic or pre-emptive management strategies followed when CMV is seen to be increasing or reaching a pre-determined threshold.
- Treatment usually includes the use of Ganciclovir 5 mg/kg 12 hourly IV. Oral management and maintenance with Valganciclovir 900 mg 12 hourly may be used under certain circumstances.

### **EBV (Epstein-Barr virus)**

- In certain patients, particularly transplant patients, EBV is associated with the development of post-transplant lymphoproliferative disorder. This presents as pyrexia, a mononucleosis type of illness, GIT bleeding/obstruction/perforation, hepatocellular/splenic infiltrative disease, CNS disease. If allowed to continue unchecked, there is a high chance of malignant disease occurring in these patients.
- EBV VL monitoring is helpful in following trends in these patients. Diagnosis is aided with the use of flow cytometry and histology including staining for EBV RNA.
- Management includes decreasing the level of immunosuppression, chemotherapy, Anti-CD20 antibody. Some research indicates that adoptive immunotherapy may be useful.

### HSV (Herpes simplex virus)

- Systemic disease due to HSV or local reactivations can be dangerous and troublesome in immunosuppressed patients.
- Diagnosis is usually easily achieved with PCR testing of lesion fluid, or of other appropriate samples such as CSF if encephalitis is suspected, or blood in the case of hepatitis.
- Treatment: acyclovir 10 mg/kg 8 hourly IV for 14 – 21 days for severe systemic illness. Oral treatment (acyclovir 200 mg 5 times daily or 400 mg 8 hourly) may be considered for localised disease. Valacyclovir 1000 mg 12 hourly may also be used.

### VZV (Varicella zoster virus)

- Primary infection with VZV can be extremely dangerous in patients who are immunocompromised, and should always be treated with IV acyclovir 10 mg/kg 8 hourly IV for 7 – 14 days.
- Reactivation or zoster should be treated with valacyclovir 1000 mg 8 hourly for 7 days or acyclovir 800 mg 5 times daily for 7 days. If the zoster is disseminated, then IV treatment is recommended with acyclovir 10 mg/kg 8 hourly for 7 days IV.
- Diagnosis may be serological if primary infection is found. The VZV IgG will ideally be negative and IgM positive. Otherwise PCR of lesion fluid will provide a quick and sensitive result.

### BK virus

- Diagnosis by PCR/VL on either urine and blood. Although there is no precise viral load level fully predictive of disease, useful cut off values seem to be  $10^7$  copies/ml in urine, and  $10^4$  copies/ml in plasma. A diagnosis of BK nephropathy should always be confirmed with a renal biopsy.
- Associated with BK nephropathy and ureteric stricture in renal transplant patients
- Associated with haemorrhagic cystitis in HSCT transplant patients.
- Management includes decreasing the level of immunosuppression. At times, it may be necessary to allow the renal graft to be lost and then to consider re-transplantation once the BK viraemia is under control.

### Bacterial and fungal infections

- Usually MCS serves for diagnosis
- Antigen testing such as for Cryptococcus is useful for some pathogens

### Pneumocystis jiroveci

- Diagnosis is most usefully achieved with PCR in correlation with clinical findings.

### Tuberculosis

- The gold standard of diagnosis is still the use of culture.
- Molecular PCR methods are useful, but of limited sensitivity on smear negative samples. They average around 70% sensitivity when compared with culture on smear negative TB samples at this stage.
- Genotypic methods may also be used to determine drug resistance in *Mycobacterium tuberculosis*.

## **Viral central nervous system infections** (including rabies)

Viral infections may cause injury to the CNS either due to the infection itself, or following post-infectious phenomena. Commonly encountered CNS viral infections responsible for meningitis / encephalitis include the following:

- Enteroviruses (EV)
- Mumps
- HSV 1 and 2
- VZV

Rarer cases are caused by arbovirus infections (arthropod borne) such as West Nile virus, and rabies transmitted from infected animals.

### Diagnosis

- PCR on CSF is the most sensitive and specific method. As these viruses often cause clinically indistinguishable signs and symptoms, multiplex viral PCRs are most useful in excluding at least the EV, mumps, HSV and VZV as causes of the infection.
- Arbovirus infection can also be diagnosed by means of PCR on CSF. These requests are sent to the NICD, as most diagnostic laboratories lack the facilities to safely handle these pathogens.
- Blood serology may be requested for mumps diagnosis, but is not helpful for the other viral causes of meningo-encephalitis.
- Arbovirus serology may be useful, but one often has to look for a 4-fold rise in titre over a period of 2 weeks, so it does not always allow the quickest diagnosis.
- Rabies may be diagnosed by means of PCR on CSF, on 3 saliva samples collected at different times, and on nuchal skin biopsy samples. Fluorescent antibody testing may also be performed on corneal impression slides or nuchal skin biopsies, but PCR is the most reliable ante-mortem diagnostic testing available. Serology is not helpful, as antibodies are only detectable in blood approximately 5 days after the onset of clinical disease. If rabies vaccine or immunoglobulin has been given, the serology

will be positive and is not predictive of disease. Post-mortem sampling of the brain is mandatory in rabies cases, particularly those in whom diagnosis was not confirmed ante-mortem. Ideally, the entire brain should be removed, half preserved in 50% glycerol saline, and half in 10% neutral buffered formaldehyde solution. If post-mortem sampling of the brain is strongly opposed by the surviving relatives, a Tru-Cut biopsy taken by inserting a Tru-Cut needle through the superior orbital fissure into the cranial cavity may be taken. This has the added advantage of minimizing exposure of healthcare workers to the virus. It should be noted that consent is NOT required to perform a post-mortem examination of a possible rabies patient. Brain samples should be packaged in screw-top secure plastic containers, packaged in secure secondary containers with lots of absorbant material to cushion the specimens and absorb any spills, carefully labelled and sent to the NICD via secure courier. The NICD should be notified in advance that a sample is en route.

#### Prevention of viral CNS infections

- Vaccination against measles and mumps and chicken pox.
- PEP.
  - VZIG (Varicella-zoster immunoglobulin) under appropriate conditions for exposure to VZV. The live attenuated VZV vaccine is also a reasonably good PEP measure if administered within 72 hours to non-immunocompromised exposed people.
  - Immunoglobulin or live attenuated measles vaccine administered within 72 hours of exposure provides good protection against measles infection.
  - There is little PEP available for enteroviruses, mumps, HSV and arboviruses.
  - Rabies following contact with an infected or suspected animal:
    - Determine category of exposure and treat accordingly.

Risk category	Type of exposure	Recommended action
1	<ul style="list-style-type: none"> <li>• Handling or feeding the animal</li> <li>• Licking of intact skin</li> </ul>	<p>None if the history is reliable. If the history is not reliable, manage as for Category 2.</p>
2	<ul style="list-style-type: none"> <li>• Nibbling of uncovered skin</li> <li>• Superficial scratch with no bleeding</li> </ul>	<ul style="list-style-type: none"> <li>• Quarantine animal. If well after 10 days, can stop PEP.</li> <li>• Wound care.</li> <li>• No need for RIG (rabies immunoglobulin).</li> <li>• Administer rabies vaccine.</li> </ul>
3	<ul style="list-style-type: none"> <li>• Licking of mucous membranes</li> <li>• Licking of broken skin</li> <li>• Bites or scratches that penetrate skin and cause bleeding</li> </ul>	<ul style="list-style-type: none"> <li>• Wound care</li> <li>• Administer RIG 20IU/kg as much as possible into the wound, and the remainder into the deltoid muscle.</li> <li>• Administer rabies vaccine into the opposite deltoid muscle on day 0, 3, 7, 14.</li> </ul>

- Special categories:
  - Previously immunized people:
    - No need for RIG.
    - Administer a single dose of vaccine on day 0 and day 3.
  - Immunocompromised people:
    - Administer full course of RIG and vaccine for category 2 and 3 exposures.
  - Bat exposure:

- Any close contact with bats is regarded as Category 3 exposure. Bites can be inapparent and should not be treated lightly.
- Late presentation:
  - Treat as if the exposure was recent and according to category of exposure. RIG and vaccine should be used when appropriate and the schedule should be adhered to rigidly.
- If RIG is not immediately available in Category 3 exposures, start the vaccine. RIG can still be administered within 7 days of starting the vaccine.

#### Treatment of viral CNS infections

- HSV: acyclovir 10 mg/kg IVI 8 hourly for 14 to 21 days (neonates 20 mg/kg IVI 8 hourly for 21 days)
- VZV: acyclovir 10 mg/kg IVI 8 hourly for 14 days
- Enteroviral infection: supportive. In severe cases, high titre virus-specific immunoglobulin may be effective.
- Mumps: supportive
- Arbovirus infection: mainly supportive
- Rabies virus: No curative treatment is available. Rabies is fatal once clinical disease starts.
  - Honestly counsel relatives and prepare them for the course of the disease.
  - High-level supportive care
  - Sedation.
  - Pain relief.
  - Anxiolytics.
  - Hydration and nutrition.
  - Prevent bedsores and bacterial complications.
  - Antiviral medications, corticosteroids, rabies vaccines and RIG have no proven efficacy in management of rabies once clinical disease is established.

## Infection control

This depends on the virus:

- Enterovirus:
  - Standard precautions.
  - Contact precautions when handling children in nappies or incontinent adults.
  - Careful disposal of faecal waste.
  - Strict hand hygiene/washing. Alcohol disinfectants are not sufficient for enteroviruses.
- HSV:
  - Standard precautions.
  - Contact precautions.
- VZV:
  - Contact precautions.
  - Airborne precautions.
- Measles
  - Airborne precautions.
- Mumps:
  - Droplet precautions.
- Arbovirus
  - Mostly standard precautions depending on the virus concerned.
- Rabies
  - Standard precautions.
  - Respiratory precautions.
  - If significant exposure occurs in a staff member, e.g. saliva contamination of mucous membranes, copious washing with water, and rabies vaccine should be administered.

## ImmunoCap® IgE

## LIST OF ALLERGY TESTS



INHALANT ALLERGENS	INHALANT ALLERGENS	INHALANT ALLERGENS	INSECT ALLERGENS	DRUG ALLERGENS
<b>GRASS</b>	<b>WEED POLLENS</b>	<b>INHALANT ALLERGENS</b>	<b>INSECT ALLERGENS</b>	<b>DRUG ALLERGENS</b>
<ul style="list-style-type: none"> <li>Grass mix</li> <li>Bahia Grass</li> <li>Bermuda Grass</li> <li>Brome Grass</li> <li>Cocksfoot Grass</li> <li>Golden Rod Grass – Johnson Grass</li> <li>Maize Pollen</li> <li>Cultivated wheat</li> </ul> <p>Grass Allergen components</p> <ul style="list-style-type: none"> <li>Grass group 1 Bermuda</li> <li>Grass group 1 Timothy</li> <li>Grass group 5 Timothy</li> </ul>	<ul style="list-style-type: none"> <li>Weed Mix</li> <li>Common Pigweed</li> <li>Dandelion</li> <li>Lambs Quarters</li> <li>Marguerite</li> <li>Mugwort</li> </ul> <p><b>MITES</b></p> <ul style="list-style-type: none"> <li>House dust mix</li> <li>A.siro</li> <li>B. tropicalis</li> <li>B. spicifera</li> <li>D. farinae</li> <li>D pteronyssinus</li> <li>E. maynei</li> <li>Glycyphagus domesticus</li> <li>Lepidoglyphus destructor</li> <li>Tyrophagus putrescentiae</li> </ul> <p><b>House dust mite components</b></p> <ul style="list-style-type: none"> <li>Der p 1 cystein protease</li> <li>Der p 2 NPC2 protein</li> <li>Der p 10 tropomyosin</li> </ul>	<ul style="list-style-type: none"> <li>Horse serum protein</li> <li>Mouse epithelium</li> <li>Mouse serum protein</li> <li>Mouse urine protein</li> <li>Parrot droppings</li> <li>Parrot feathers</li> <li>Pigeon droppings</li> <li>Pigeon feathers</li> <li>Pigeon serum protein</li> <li>Rabbit epithelium</li> <li>Rabbit serum protein</li> <li>Rabbit urine protein</li> <li>Rat epithelium</li> <li>Rat serum protein</li> <li>Rat urine protein</li> <li>Sheep epithelium</li> <li>Swine epithelium</li> <li>Turkey feathers</li> </ul> <p><b>Animal Allergen components</b></p> <ul style="list-style-type: none"> <li>Can f 1 dog lipocalin</li> <li>Can f 2 dog lipocalin</li> <li>Can f 3 dog serum albumin</li> <li>Can f 5 dog arginine kinase</li> <li>Cat fel d 1 1teroglobin</li> <li>Cat fel d 2 serum albumin</li> <li>Cat fel d 4 lipocalin</li> <li>Equ c1 Horse lipocalin</li> <li>Bos d 6 Cow serum albumin</li> </ul> <p><b>MOULDS</b></p> <ul style="list-style-type: none"> <li>Mould mix</li> <li>Alternaria tenuis</li> <li>Aspergillus fumigatus</li> <li>Botrytis cinerea</li> <li>Candida albicans</li> <li>C herbarum</li> <li>Epicoccum</li> <li>Helminthos haloides</li> <li>Penicillium notatum</li> <li>Phoma betae</li> </ul> <p><b>Mould components:</b></p> <ul style="list-style-type: none"> <li>Alt a 1 Alternaria</li> <li>Asp f 6 Aspergillus (ABPA)</li> </ul>	<ul style="list-style-type: none"> <li>American Cockroach</li> <li>German Cockroach</li> <li>Oriental Cockroach</li> <li>Mosquito</li> <li>Honey Bee</li> <li>Common Wasp</li> <li>European Hornet</li> <li>Hornet (White faced)</li> <li>Hornet (Yellow)</li> <li>Paper Wasp</li> </ul> <p><b>Bee and Wasp components</b></p> <ul style="list-style-type: none"> <li>Api m 1, bee Phospholipase A2,</li> <li>Ves v 1, Common wasp Phospholipase A1;</li> <li>Ves v 5, Common Wasp Allergen 5</li> <li>Pol d 5, European Paper Wasp Allergen 5</li> <li>CCD Marker</li> </ul> <p><b>OCCUPATIONAL</b></p> <ul style="list-style-type: none"> <li>Ethylene Oxide</li> <li>Formaldehyde</li> <li>Isocyanate HDI</li> <li>Isocyanate MDI</li> <li>Isocyanate TDI</li> <li>Latex</li> <li>Maleic Anhydride</li> <li>Phthalic Anhydride</li> <li>Trimellitic Anhydride</li> <li>Silk Natural</li> <li>Untreated cotton</li> </ul>	<ul style="list-style-type: none"> <li>Amoxycillin</li> <li>Ampicillin</li> <li>Penicillin G IgE</li> <li>Penicillin V IgE</li> <li>Cefaclor</li> <li>Insulin Bovine</li> <li>Insulin Human</li> <li>Insulin Porcine</li> <li>Tetanus Toxoid</li> </ul>
<b>CROSS - REACTIVE COMPONENTS</b>				
<ul style="list-style-type: none"> <li>Profilin</li> <li>CCD</li> <li>PR-10</li> <li>LTP</li> </ul> <p><b>TREE POLLENS</b></p> <ul style="list-style-type: none"> <li>Tree mix 1 (Olive, Willow, pine, Acacia, Eucalyptus, Cajeput)</li> <li>Tree mix 2 (Oak, Elm, Plane, Willow, Cottonwood)</li> <li>Acacia</li> <li>Birch</li> <li>Cajeput</li> <li>Cypress</li> <li>Elm</li> <li>Eucalyptus Tree</li> <li>Italian Cypress</li> <li>Japanese Cedar</li> <li>London Plane Tree</li> <li>Oak</li> <li>Olive Tree</li> <li>Pecan Tree</li> <li>Pepper Tree</li> <li>Pine Tree</li> <li>Walnut Tree</li> <li>Willow Tree</li> </ul> <p>Tree mix 1 consists of: Tree mix 2 consists of:</p>	<b>EPIDERMALS AND ANIMAL PROTEINS</b>			

Please note that this is not a comprehensive list of tests but only represents the most common allergens. Please phone the laboratory at (012) 678 08614 to enquire about the availability of other allergens.

## ImmunoCap® IgE

## LIST OF ALLERGY TESTS



SCREENING TESTS	LEGUMES	FISH ALLERGENS CONT	FRUIT	VEG ALLERGENS CONT	DIVERSE
<ul style="list-style-type: none"> <li>Fx5 food mix</li> <li>Phadiatop inhalant mix</li> <li>IgE</li> </ul>	<ul style="list-style-type: none"> <li>Chick pea</li> <li>Green beans</li> <li>Lentils</li> <li>Lupin seed</li> <li>Peas</li> <li>Red kidney bean</li> <li>Soya bean</li> <li>White beans</li> </ul> <p><b>Soya Components:</b></p> <ul style="list-style-type: none"> <li>Gly m 4 PR-10</li> <li>Gly m 5 storage protein</li> <li>Gly m 6 storage protein</li> </ul>	<ul style="list-style-type: none"> <li>Mackerel</li> <li>Salmon</li> <li>Sardine</li> <li>Snoek</li> <li>Sole</li> <li>Swordfish</li> <li>Tuna</li> <li>Trout</li> </ul> <p><b>Fish Components:</b></p> <ul style="list-style-type: none"> <li>Parvalbumin carp</li> <li>Parvalbumin cod</li> </ul>	<ul style="list-style-type: none"> <li>Tropical fruit mix</li> <li>Apple</li> <li>Apricot</li> <li>Banana</li> <li>Blackberry</li> <li>Blueberry</li> <li>Cherry</li> <li>Cranberry</li> <li>Dates</li> <li>Grape</li> <li>Grapefruit</li> <li>Guava</li> <li>Jackfruit</li> <li>Kiwi</li> <li>Lemon</li> <li>Litchi</li> <li>Mango</li> <li>Melon</li> <li>Naartjie</li> <li>Orange</li> <li>Passion fruit</li> <li>Pawpaw</li> <li>Peach</li> <li>Pear</li> <li>Pineapple</li> <li>Plum</li> <li>Strawberry</li> </ul>	<ul style="list-style-type: none"> <li>Onion</li> <li>Potato</li> <li>Pumpkin &amp; Squash</li> <li>Spinach</li> <li>Sweet potato</li> <li>Tomato</li> </ul> <p><b>HERBS AND SPICES</b></p> <ul style="list-style-type: none"> <li>Spice mix</li> <li>Anise</li> <li>Basil</li> <li>Black pepper</li> <li>Cardamom</li> <li>Caraway</li> <li>Chilli pepper</li> <li>Cinnamon</li> <li>Clove</li> <li>Coriander</li> <li>Curry</li> <li>Dill</li> <li>Garlic</li> <li>Ginger</li> <li>Mint</li> <li>Mustard</li> <li>Nutmeg</li> <li>Oregano</li> <li>Paprika</li> <li>Parsley</li> <li>Saffron</li> <li>Tarragon</li> <li>Thyme</li> </ul>	<ul style="list-style-type: none"> <li>Ascaris</li> <li>Bakers yeast</li> <li>Bilharzia</li> <li>Canola oil</li> <li>Cocoa (Chocolate)</li> <li>Coffee</li> <li>Cotton</li> <li>Gelatin</li> <li>Formaldehyde</li> <li>Honey</li> <li>Hops</li> <li>P. notatum</li> <li>Seminal (semen) fluid</li> <li>Tea</li> <li>Tobacco leaf</li> </ul>
<b>FOOD ALLERGENS</b> <p><b>GRAIN</b></p> <ul style="list-style-type: none"> <li>Grain mix</li> <li>Barley</li> <li>Buckwheat</li> <li>Maize</li> <li>Malt</li> <li>Oats</li> <li>Rice</li> <li>Rye</li> <li>Wheat</li> </ul> <p><b>Wheat allergen components:</b></p> <ul style="list-style-type: none"> <li>Omega 5 Gliadin</li> <li>Gluten IgE</li> <li>Tri a 14 LTP, Wheat</li> </ul> <p><b>NUTS AND SEEDS</b></p> <ul style="list-style-type: none"> <li>Nut mix</li> <li>Almond</li> <li>Brazil nut</li> <li>Cashew nut</li> <li>Coconut</li> <li>Hazelnut</li> <li>Linseed</li> <li>Macadamia nut</li> <li>Pecan nut</li> <li>Pistachio nut</li> <li>Peanuts</li> <li>Pine Nut</li> <li>Poppy seed</li> <li>Sesame seed</li> <li>Sunflower seed</li> <li>Walnut</li> </ul> <p><b>Peanut components</b></p> <ul style="list-style-type: none"> <li>Ara h 1 storage protein</li> <li>Ara h 2 storage protein</li> <li>Ara h 3 storage protein</li> <li>Ara h 8 profilin</li> <li>Ara h 9 LTP</li> </ul>	<ul style="list-style-type: none"> <li>Abalone</li> <li>Blue mussel</li> <li>Clam</li> <li>Crab</li> <li>Crayfish</li> <li>Langoustine</li> <li>Lobster</li> <li>Octopus</li> <li>Oyster</li> <li>Scallop</li> <li>Shrimp</li> <li>Snail</li> <li>Squid</li> </ul> <p><b>DAIRY</b></p> <ul style="list-style-type: none"> <li>Diary mix</li> <li>Milk (Cow)</li> <li>Milk (Goats)</li> <li>Cheddar cheese</li> <li>Mould cheese</li> </ul> <p><b>Milk components</b></p> <ul style="list-style-type: none"> <li>α Lactalbumin</li> <li>β Lactoglobulin</li> <li>Casein</li> <li>Lactoferrin</li> <li>Bovine serum albumin</li> </ul> <p><b>EGG AND POULTRY</b></p> <ul style="list-style-type: none"> <li>Chicken</li> <li>Turkey</li> <li>Egg yolk</li> </ul> <p><b>Egg white components</b></p> <ul style="list-style-type: none"> <li>Ovomucoid</li> <li>Ovalbumin</li> <li>Conalbumin</li> <li>Lysozyme</li> </ul> <p><b>FISH ALLERGENS</b></p> <ul style="list-style-type: none"> <li>Fish mixture</li> <li>Anchovy</li> <li>Anisakis (fish parasite)</li> <li>Cod fish</li> <li>Hake</li> <li>Herring</li> </ul>	<p><b>SHELLFISH</b></p> <ul style="list-style-type: none"> <li>Shellfish mix</li> <li>Abalone</li> <li>Blue mussel</li> <li>Clam</li> <li>Crab</li> <li>Crayfish</li> <li>Langoustine</li> <li>Lobster</li> <li>Octopus</li> <li>Oyster</li> <li>Scallop</li> <li>Shrimp</li> <li>Snail</li> <li>Squid</li> </ul> <p><b>Shellfish components</b></p> <ul style="list-style-type: none"> <li>Tropomyosin Shrimp</li> </ul> <p><b>MEAT</b></p> <ul style="list-style-type: none"> <li>Meat mix</li> <li>Beef</li> <li>Gelatin</li> <li>Mutton/Lamb</li> <li>Pork</li> </ul> <p><b>Beef components:</b></p> <ul style="list-style-type: none"> <li>Alpha 1,3-Gal</li> <li>Bovine serum albumin</li> </ul>	<ul style="list-style-type: none"> <li>Tropical fruit mix</li> <li>Apple</li> <li>Apricot</li> <li>Banana</li> <li>Blackberry</li> <li>Blueberry</li> <li>Cherry</li> <li>Cranberry</li> <li>Dates</li> <li>Grape</li> <li>Grapefruit</li> <li>Guava</li> <li>Jackfruit</li> <li>Kiwi</li> <li>Lemon</li> <li>Litchi</li> <li>Mango</li> <li>Melon</li> <li>Naartjie</li> <li>Orange</li> <li>Passion fruit</li> <li>Pawpaw</li> <li>Peach</li> <li>Pear</li> <li>Pineapple</li> <li>Plum</li> <li>Strawberry</li> </ul> <p><b>VEGETABLES</b></p> <ul style="list-style-type: none"> <li>Asparagus</li> <li>Avocado</li> <li>Bamboo shoot</li> <li>Beetroot</li> <li>Brinjal</li> <li>Brussel sprouts</li> <li>Cabbage</li> <li>Carrot</li> <li>Celery</li> <li>Cucumber</li> <li>Green pepper</li> <li>Lettuce</li> <li>Mushrooms</li> <li>Olive</li> </ul>		

## Cast Tests (CAST)

## LIST OF ALLERGY TESTS



SCREENING TESTS:	FOOD ADDITIVES CONT:	FOOD ALLERGENS CONT:	DRUGS	GENERAL ANAESTHETICS CONT:
Food mix Consists of: <ul style="list-style-type: none"><li>• Egg White</li><li>• Egg yolk</li><li>• Milk</li><li>• Codfish</li><li>• Wheat</li><li>• Soya</li><li>• Peanut</li><li>• Hazelnut</li><li>• Shrimp</li></ul> Inhalant mix Consists of: <ul style="list-style-type: none"><li>• Grass mix</li><li>• Cultivated rye grass</li><li>• Common birch</li><li>• Hazel tree</li><li>• Mugwort</li><li>• Ribwort/ plantain</li><li>• Altenaria tenuis</li><li>• D. pteronyssinus</li><li>• D. farinae</li><li>• Cat</li><li>• Dog</li></ul>	<b>CAST FOODS</b> <b>MILK AND DAIRY</b> Egg White Egg Yolk Milk Milk components: <ul style="list-style-type: none"><li>• <math>\alpha</math> - Lactalbumin</li><li>• <math>\beta</math> - Lactoglobulin</li><li>• Casein</li></ul> <b>CEREALS AND GRAINS</b> Baker's yeast Barley flour Maize Oats Rice Rye flour Soya Wheat Allergen components: <ul style="list-style-type: none"><li>• Maize profilin</li><li>• Wheat profilin</li><li>• CCD</li></ul> <b>SEAFOOD</b> Anisakis Cod Crab Oyster Shrimp Squid <b>MEAT</b> Pork Beef <b>NUTS AND SEEDS</b> Hazelnut Almond Cashew nut Sesame seed Peanut Peanut components <ul style="list-style-type: none"><li>• Arah 1 storage protein</li></ul>	<b>FRUITS AND VEGETABLES</b> Carrot Celery Orange Peach Tomato <b>INHALANTS</b> <b>ANIMAL DANDER</b> Cat epithelium Dog epithelium <b>POLLENS</b> 6 grass mix Bermuda grass Cultivated rye grass Common birch Hazel tree Mugwort Ribwort P officinalis <b>MOULDS</b> Penicillium notatum Aspergillus fumigatus Candida albicans Alternaria tenuis Cladosporium herbarum <b>MITES</b> Storage mite mix D pteronyssinus D farinae Acarus siro <b>OCCUPATIONAL</b> Carboxymethylcellulose Latex Formaldehyde Chlorhexidine Alpha amylase <b>INSECT VENOMS</b> Honey Bee Culicoides Antigen Yellow jacket hornet Paper wasp European hornet	<b>ANTIBIOTICS</b> Amoxycillin Ampicillin Penicillin G Penicillin V Benzylpenicilloyl (PPL) Minor determinant Mix Clavulanic acid + Amoxycillin Cephalosporin C Cefazolin Ceftriaxone Cefuroxime Clarithromycin Tetracycline Trimethoprim Sulfamethoxazole Levofloxacin Ciprofloxacin Cefaclor Cefamandole Rifampicin <b>ANALGESIC/ ANTI-INFLAMMATORIES</b> Paracetamol Indomethacin Mefenamic acid Naproxen Diclofenac Ibuprofen Aspirin Dipyrrone/Metamizole Phenylbutazone <b>GENERAL ANESTHETICS</b> General anaesthetic mix, (breakdown if positive) Muscle Relaxants <ul style="list-style-type: none"><li>• Vecuronium</li><li>• Suxamethonium</li><li>• Rocuronium</li><li>• Mivacurium</li><li>• Atracurium</li><li>• Pancuronium</li><li>• Cisatracurium</li></ul>	Propofol <b>LOCAL ANAESTHETICS</b> Lidocaine / Lignocaine (Xylocaine) Articaine (Ubistesin) Mepivacaine (Carbocaine) Bupivacaine (Marcaine) <b>RADIO CONTRAST MEDIA</b> Iobitridol Iohexol (Omnipaque) Iomeprol Iopamidol Iopromide Ioxaglate Ixitalamate <b>ANTISEPTICS</b> Chlorhexidine <b>DIVERSE DRUG ALLERGY TESTING</b> Diverse CAST  Please contact the laboratory for details (012) 678 0627  Send tablets/ medication with patient Heparin specimens, clearly marked.  Collect specimens Mondays to Thursdays, and not on the day before a Public Holiday.  Discontinue parenteral corticosteroids two weeks prior to testing. Ideally wait two weeks after an acute allergic reaction or illness before testing.

## MELISA, Skin Prick Test and Patch Test

## LIST OF ALLERGY TESTS



### HEAVY METALS

- Aluminium
- Arsenic
- Barium
- Beryllium
- Chromium
- Cobalt
- Copper
- Ethyl Mercury
- Gallium
- Gold
- Iridium
- Indium
- Iron
- Inorganic Mercury
- Lanthanum
- Lead
- Manganese
- Methyl mercury
- Molybdenum
- Nickel
- Platinum
- Phenyl Mercury
- Palladium
- Platinum
- Ruthenium
- Silver
- Tin
- Titanium
- Thimerosal mercury
- Vanadium
- Zinc
- Zirconium

### ANTISEPTICS

- Mercurochrome
- Iodine
- Chlorhexidine

See list of CAST allergens:  
all allergens available on CAST  
is also available for MELISA  
testing, including diverse drug  
allergy testing.

### SKIN PRICK TEST ALLERGENS

#### INHALANTS

#### ANIMAL ALLERGENS



#### Cat

#### Dog

#### MOULDS



#### Alternaria alternata

#### Cladosporium

#### Aspergillus



#### FEATHERS



#### GRASSES



#### Bermuda Grass

#### 6-grass mix

#### POLLEN



#### Maize pollen

#### Weed mix

#### Plane tree pollen

#### Oak pollen

#### Olive pollen

#### Cypress pollen

#### Acacia pollen

#### Eucalyptus

### MITES



House dust mite mix  
B.tropicalis  
Cockroach  
(A shortened profile is also available)

### FOODS

#### Cow's milk

#### Egg

#### Cod fish

#### Shellfish

#### Soya

#### Wheat

#### Peanut

#### Maize/Corn

#### Potato

#### Tomato

#### Orange

#### Apple

#### Banana

#### Bakers yeast

(A shortened profile is available)

### PATCH TESTS:

European baseline, sunscreen,  
cosmetic and hairdressing series.

## SPECIMEN REQUIREMENTS:

TEST	SPECIMEN	SPECIAL INSTRUCTIONS
IgE	SST/Clotted Tube/1ml	
RAST	SST/Clotted Tube/5ml - depending on the number of allergens requested	
ECP	SST/Clotted Tube/1ml	Blood must be taken in a SST tube and serum must be separated within 1 hour.
Mast Cell Tryptase	SST/Clotted Tube/1ml	
ISAC	SST/Clotted Tube/1ml or less	
CAST	Heparin Tube/5ml	Must reach the lab within 24 hours on week days and before 12:00 on Fridays.
MELISA	Citrate Tube/5ml (4-6 depending on the number of allergens requested)	Must reach the lab within 24 hours on week days and before 12:00 on Fridays.

**ISAC Tests****LIST OF ALLERGY TESTS**

ONE COLLECTIVE TEST THAT INCLUDES 112 FOOD AND INHALANT ALLERGEN COMPONENTS. ISAC ALLERGEN COMPONENTS

ALLERGEN COMPONENT	ALLERGEN SOURCE COMMON NAME	PROTEIN GROUP
<b>GRASS POLLEN</b>		
Cyn d 1	Bermuda Grass	Grass group 1
Phl p 1	Timothy Grass	Grass group 1
Phl p 2	Timothy Grass	Grass group 2
Phl p 4	Timothy Grass	
Phl p 5	Timothy Grass	Grass group 5
Phl p 6	Timothy Grass	
Phl p 7	Timothy Grass	Polcalcin
Phl p 11	Timothy Grass	
Phl p 12	Timothy Grass	Profilin
<b>TREE POLLEN</b>		
Aln g 1	Alde Tree	PR-10 protein
Bet v 1	Birch Tree	PR-10 protein
Bet v 2	Birch Tree	Profilin
Bet v 4	Birch Tree	Polcalcin
Cor a 1.0101	Hazel pollen	PR-10 protein
Cry j 1	Japanese cedar	
Cup a 1	Cypress Tree	
Ole e 1	Olive Tree	
Ole e 7	Olive Tree	Lipid transfer protein (LTP)
Ole e 9	Olive Tree	
Pla a 1	Plane Tree	
Pla a 2	Plane Tree	
Pla a 3	Plane Tree	Lipid transfer protein (LTP)
<b>WEED POLLEN</b>		
Che a 1	Goosefoot	
Amb a 1	Ragweed	
Pla l 1	Plantain (English)	
Art v 1	Mugwort	
Art v 3	Mugwort	Lipid transfer protein (LTP)
Mer a 1	Annual mercury	Profilin
Par j 2	Wall pellitory	Lipid transfer protein (LTP)
Sal k 1	Saltwort	
<b>FRUIT</b>		
Act d 1	Kiwi	Thaumatin-like protein
Act d 2	Kiwi	
Act d 5	Kiwi	
Act d 8	Kiwi	PR-10 protein
Api g 1	Celery	PR-10 protein
Mal d 1	Apple	PR-10 protein
Pru p 1	Peach	PR-10 protein
Pru p 3	Peach	Lipid transfer protein (LTP)

## ISAC Tests Continued...

## LIST OF ALLERGY TESTS



ALLERGEN COMPONENT	ALLERGEN SOURCE COMMON NAME	PROTEIN GROUP
<b>NUTS AND LEGUMES</b>		
Ana o 2	Cashew nut	Storage protein 11 S globulin
Ara h 1	Peanut	Storage protein, 7 S globulin
Ara h 2	Peanut	Storage protein, conglutin
Ara h 3	Peanut	Storage protein, 11 S globulin
Ara h 8	Peanut	PR-10 protein
Ara h 6	Peanut	Storage protein, conglutin
Ara h 9	Peanut	Lipid transfer protein (LTP)
Ber e1	Brazil nut	Storage protein, 2S albumin
Cor a 1.0401	Hazelnut	PR-10 protein
Cor a 8	Hazelnut	Lipid transfer protein (LTP)
Cor a 9	Hazelnut	Storage protein, 11 S globulin
Jug r 1	Walnut	Storage protein, 2S albumin
Jug r 2	Walnut	Storage protein, 7S globulin
Jug r 3	Walnut	Lipid transfer protein (LTP)
Gly m 4	Soybean	Pr-10 protein
Gly m 5	Soybean	Storage protein, B-conglycinin
Gly m 6	Soybean	Storage protein, Glycinin
Ses i 1	Sesame seed	Storage protein, 2S albumin
<b>GRAINS</b>		
Fag e 2	Buckwheat	Storage Protein, 2S albumin
Tri a 14	Wheat	Crude gliadin (LTP)
Tri a 19.0101	Wheat	Omega-5 gliadin
Tri a aA_Ti	Wheat	
<b>OCCUPATIONAL</b>		
Hev b 1	Latex	
Hev b 3	Latex	
Hev b 8	Latex	
Hev b 5	Latex	
Hev b 6.01	Latex	Profilin
Bos d 4	Cow's milk	$\alpha$ -lactalbumin
Bos d 5	Cow's milk	B-lactoglobulin
Bos d 6	Cow's milk and meat	Serum albumin
Bos d 8	Cow's milk	Casein
Bos d lactoferrin	Cow's milk	Transferrin
<b>EGG</b>		
Gal d 1	Egg white	Ovomucoid
Gal d 2	Egg white	Ovalbumin
Gal d 3	Egg white	Conalbumin
Gal d 5	Egg yolk	Serum albumin
<b>FISH</b>		
Gad c 1	Cod	Parvalbumin
Ani s 3	Anisakis (Fish parasite)	Tropomyosin
Ani s 1	Anisakis (Fish parasite)	
Pen m 2	Shrimp	Arginine kinase

## ISAC Tests Continued...

## LIST OF ALLERGY TESTS

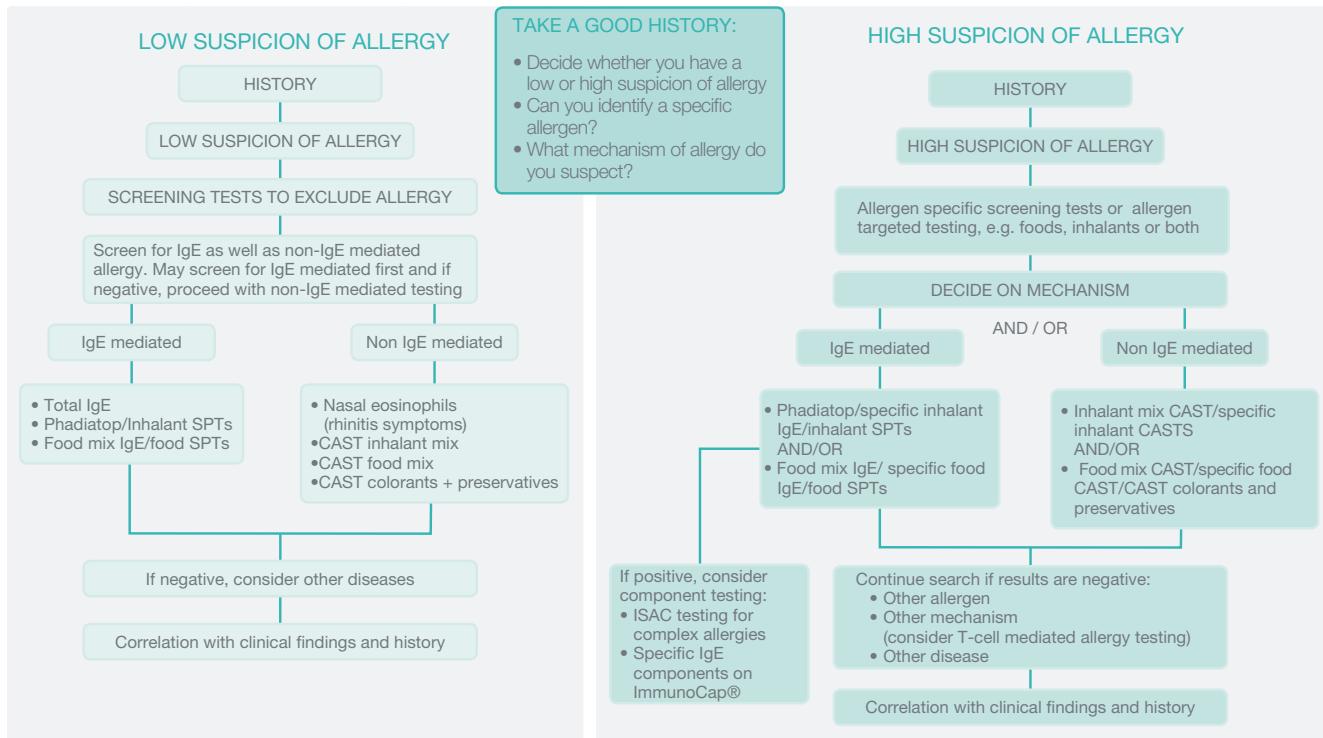


Pen m 4 Pen m 1	Shrimp Shrimp	Sarcoplasmic Ca-binding protein Tropomyosin
<b>MITES</b>		
Der f 1 Der f 2 Der p 1 Der p 2 Der p 10 Blo t 5 Lep d 2	House dust mite ( <i>D.farinae</i> ) House dust mite ( <i>D.farinae</i> ) House dust mite ( <i>D.pteronyssinus</i> ) House dust mite ( <i>D.pteronyssinus</i> ) House dust mite ( <i>D.pteronyssinus</i> ) House dust mite ( <i>B.tropicalis</i> ) Storage mite	Tropomyosin
<b>MOULDS</b>		
Alt a 1 Alt a 6 Asp f 1 Asp f 3 Asp f 6 Cla h 8	Alternaria Alternaria Aspergillus Aspergillus Aspergillus Cladosporium	Enolase  Mn Superoxide dismutase
<b>INSECTS AND PARASITES</b>		
Api m 1 Api m 4 Pol d 5 Ves v 5 Ani s 1 Ani s 3 Bla g 1 Bla g 2 Bla g 7 Bla g 5	Honey bee venom Honey bee venom Paper wasp venom Common wasp venom Anisakis Anisakis Cockroach Cockroach Cockroach Cockroach	Phospholipase A2 Melittin Venom, Antigen 5 Venom, Antigen 5  Tropomyosin  Tropomyosin
<b>ANIMALS</b>		
Can f 1 Can f 2 Can f 3 Can f 5 Equ c 3 Equ c 1 Fel d 1 Fel d 2 Fel d 4 Mus m 1	Dog Dog Dog Dog Horse Horse Cat Cat Cat Mouse	Lipocalin Lipocalin Serum albumin Arginine esterase Serum albumin Lipocalin Uteroglobin Serum albumin Lipocalin Lipocalin
<b>CROSS-REACTIVE PLANTS</b>		
Bet v 4 Phl p 7 Bet v 2 Hev b 8 Mer a 1 Ole e 7 Phl p 12 MUXF 3	Birch Timothy Birch Latex Annual mercury Olive Timothy Sugar epitope from Bromelain	Polcalcin Polcalcin Profilin Profilin Profilin Lipid transfer protein (LTP) Profilin CCD-marker

# AN INTRODUCTION TO ALLERGY DIAGNOSIS



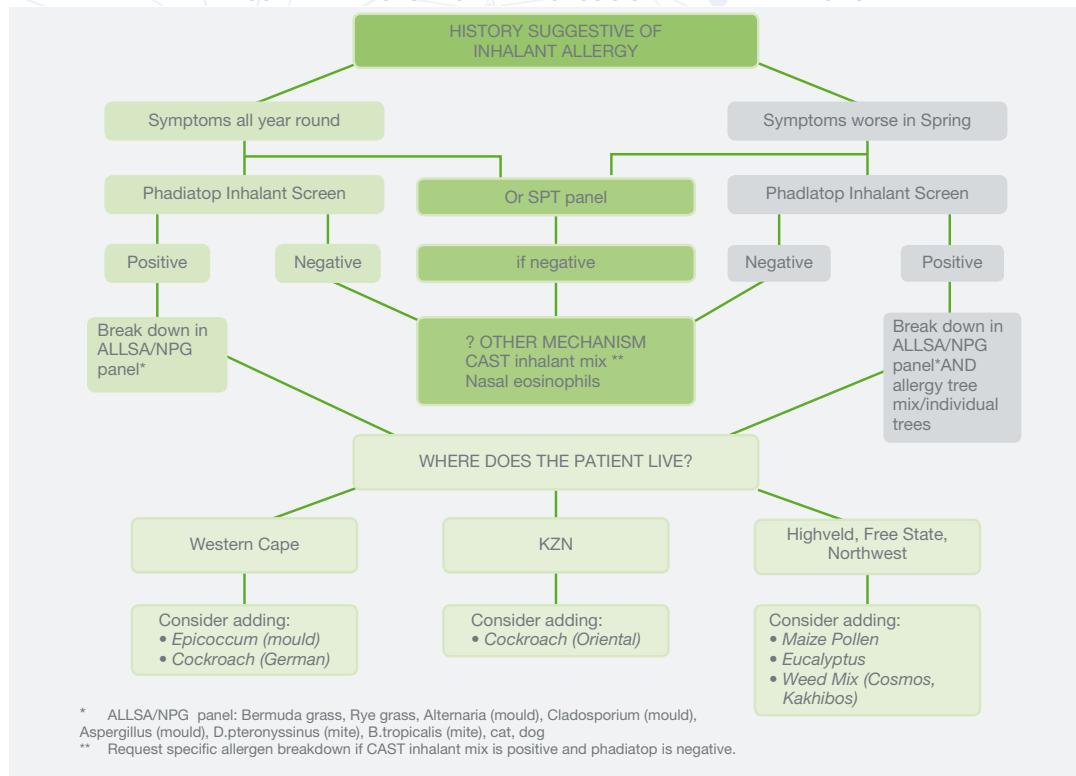
## AN APPROACH TO ALLERGY DIAGNOSIS – MADE EASY WITH FLOW-DIAGRAMS:



## AN APPROACH TO INHALANT ALLERGY



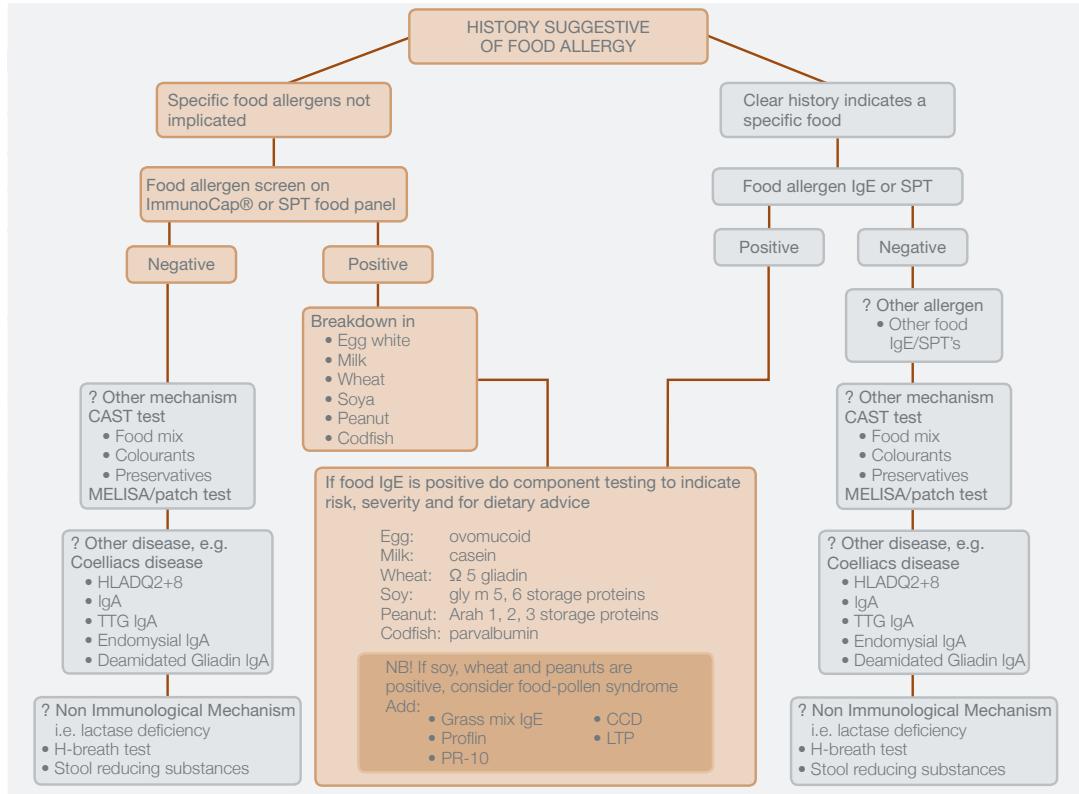
**FIGURE 2: APPROACH TO THE DIAGNOSIS OF INHALANT ALLERGIES.**



# AN APPROACH TO FOOD ALLERGY



THE FOLLOWING DIAGNOSTIC TOOLS ARE KEY IN THE ASSESSMENT OF A POSSIBLE FOOD ALLERGY:



## Notes





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