

**SAS Centres for  
Cardiovascular Biomarkers  
Handbook**



## Index

Preface	2
Contact details	3
Laboratory profiles	5
Specimen requirements and assay availability	9
A brief introduction to lipoprotein metabolism	10
Characteristics of major lipoproteins	11
Physiological functions of the major lipoproteins	11
Characteristics of major apolipoproteins	12
Function of major enzymes of lipoprotein metabolism	12
Lipoprotein metabolism - Exogenous pathway	13
- Endogenous pathway	14
Primary causes of dyslipidaemia	15
Secondary causes of dyslipidaemia	16
Investigation protocol for dyslipidaemia	17
Brief details of available assays:	
Apolipoprotein A1	18
Apolipoprotein B	20
Apolipoprotein E polymorphisms	22
Beta - Quantification (Ultracentrifugation)	23
Homocysteine	25
LDL (Calculated and Direct)	27
Lipoprotein (a)	28
Lipoprotein Lipase	30
Appendix - non-core assays	

## **Preface**

This handbook has been produced as a guide for clinicians and laboratory staff involved in the investigation of dyslipidaemias and in the assessment of cardiovascular risk. The network of SAS centres for cardiovascular biomarkers was set up in response to an increasing demand for tests other than the routine lipid profile to aid in the assessment and management of patients.

The aim of this handbook is not only to list available tests and the laboratories which offer them, but also to give a brief description of the tests, the circumstances in which they are indicated and interpretation of the results.

More information is available in specialist textbooks of clinical chemistry and lipoprotein metabolism

1. Betteridge DJ, Illingworth DR, Shepherd J. Lipoproteins in Health and Disease. Arnold 1999
2. Rifai N, Warnick GR, Dominiczak M. Handbook of Lipoprotein 2nd edition ; AACC Press 2000
3. Ordovas JM. Lipoprotein Protocols ; Methods in Molecular Biology Vol 110. Humana Press 1998

Updated information on this new SAS service is available on the SAS website:

**[www.sas-centre.org](http://www.sas-centre.org)**

## Contact Details for Supra-regional Assay Service Laboratories

### Glasgow

Department of Vascular Biochemistry, Glasgow Royal Infirmary

Postage Address:

Department of Clinical Biochemistry

PO Box 508, Glasgow G31 2ER

Samples on dry ice:

Department of Vascular Biochemistry

4th floor QEB

Glasgow Royal Infirmary, G31 2ER

Director Dr Muriel Caslake

0141 2114596

[m.caslake@clinmed.gla.ac.uk](mailto:m.caslake@clinmed.gla.ac.uk)

Deputy Director Prof Chris Packard

0141 2114979

[chris.packard@clinmed.gla.ac.uk](mailto:chris.packard@clinmed.gla.ac.uk)

Website:

<http://www.gla.ac.uk/departments/pathologicalbiochemistry/index.htm>

### Guildford

Centre for Clinical Science & Measurement

School of Biomedical & Molecular Science

University of Surrey

Guildford, Surrey GU2 7XH

Director: Prof Gordon Ferns

01483 686419

[g.ferns@surrey.ac.uk](mailto:g.ferns@surrey.ac.uk)

Deputy Director: Dr Bryan Starkey

01483 464121

[bstarkey@royalsurrey.nhs.uk](mailto:bstarkey@royalsurrey.nhs.uk)

Deputy Director: Dr Bruce Griffin

01483 879724

[b.griffin@surrey.ac.uk](mailto:b.griffin@surrey.ac.uk)

**Guys, King's, St Thomas's Hospitals Trusts**

Department of Chemical Pathology  
St Thomas' Hospital  
London SE1 7EH

Director: Dr Anthony Wierzbicki

020 7188 1256

[anthony.wierzbicki@kcl.ac.uk](mailto:anthony.wierzbicki@kcl.ac.uk)

Deputy Director: Dr Martin Crook

020 7188 7188

[martin.crook@uhl.nhs.uk](mailto:martin.crook@uhl.nhs.uk)

**Newcastle-upon-Tyne**

Department of Clinical Biochemistry  
Newcastle upon Tyne Hospitals  
Royal Victoria Infirmary  
Newcastle upon Tyne NE1 4LP

Director: Dr Dermot Neely

0191 282 4554

[dermot.neely@nuth.northy.nhs.uk](mailto:dermot.neely@nuth.northy.nhs.uk)

Deputy Director: Dr Michael Laker

0191 282 4566

[mike.laker@nuth.northy.nhs.uk](mailto:mike.laker@nuth.northy.nhs.uk)

**Royal Free and University College Hospitals Trusts**

Department of Clinical Biochemistry  
The Royal Free Hospital  
Pond Street  
London NW3 2QG

Director: Dr Devaki Nair

0207 472 6694

[devaki.nair@royalfree.nhs.uk](mailto:devaki.nair@royalfree.nhs.uk)

Deputy Director: Mr Jahm Persaud

0207 794 0500 x 8849

[jahm.persaud@royalfree.nhs.uk](mailto:jahm.persaud@royalfree.nhs.uk)

## Laboratory profiles

### **Glasgow Royal Infirmary:**

**Website:** <http://www.gla.ac.uk/departments/pathologicalbiochemistry/index.htm>

The Department of Clinical Biochemistry at Glasgow Royal Infirmary encompasses the NHS Institute of Biochemistry and the University of Glasgow Department of Vascular Biochemistry. It is CPA accredited and is the Center for Disease Control (CDC, Atlanta, USA) UK laboratory for the Lipid Standardisation Programme (cholesterol, triglyceride, HDL-C) and the CDC UK Reference Laboratory for cholesterol and HDL-C. The lipid laboratory participates UKNEQAS for lipid investigation (cholesterol, triglyceride, HDL-C) and in the RIQAS lipid programme (apoAI, apoB, HDL-C). Both QA schemes use CDC reference values.

For the past thirty years, the department has had a broad ranging interest in all aspects of the association between lipids and coronary disease and more recently has extended this to novel risk factors such as hsCRP

Much of the research interest has focussed on the separation of lipoprotein subfractions, investigations of lipoprotein metabolism using isotope kinetic multicompartmental modelling and on the influence of diet and drugs on lipoprotein metabolism. The laboratory has also conducted large clinical trials, including the West Of Scotland Coronary Prevention Study (WOSCOPS) and the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER).

The director and deputy director, Dr Muriel Caslake and Professor Chris Packard have international reputations in the field of lipid research and are supported by clinical associates, Dr Denis O'Reilly and Dr Marek Dominiczak, the technical laboratory manager, Ms Anne Bell and the lipid research laboratory supervisor, Mrs Dorothy Bedford. Their extensive publications can be viewed on the website above.

### **Guildford**

The SAS Cardiovascular biomarkers Laboratory at Guildford is based at the University of Surrey and the Royal Surrey County Hospital NHS Trust.

Professor Gordon Ferns is Director of the Laboratory and is a Consultant in Clinical Biochemistry within Partnership Pathology Services (PPS) and Professor of Metabolic & Molecular Medicine within the School of Biomedical & Molecular Sciences. He has more than 250 publications in the area of cardiovascular science, and is particularly interested in the mechanisms of atherogenesis. He is also Deputy Director of the Guildford SAS Laboratory for Trace Elements.

Dr Bruce Griffin is Reader in Nutritional Biochemistry at the University of Surrey,. He undertook his postdoctoral research in pathological biochemistry at Glasgow Royal Infirmary where he developed his interest in lipoprotein metabolism and the metabolic syndrome. He has over 20 years research experience in these areas supported by the MRC, BHF, BBSRC and Food Standards Agency and has expertise in the analysis of serum lipids and lipoproteins.

Dr Bryan Starkey is a Principal Grade Biochemist within PPS. He obtained his PhD at the University of Newcastle upon Tyne. He has a particular interest in analytical biochemistry and has developed several new specialist assays for drugs and for cardiovascular risk factors. He has responsibility for clinical trials within PPS, and is closely involved with the external quality assessment schemes operated from Guildford

### **Guy's, & St Thomas' Hospitals Foundation Trust**

The Lipid laboratory at St.Thomas' provides specialist assay support for the long-established clinics at Guy's and St.Thomas' Hospitals. The laboratory has been involved in clinical research for 40 years measuring lipid fractions by ultracentrifugation, apolipoproteins, enzyme and transfer factor activities as well as markers of inflammation. Landmark studies conducted by the laboratory include the St.Thomas' atherosclerosis regression trial (STARS), the Whitehall, Oxford and EuroDiab studies and numerous investigations in lipoprotein turnover in a variety of diseases. The lipid laboratory is associated with the Guy's, St. Thomas', Lewisham and Conquest Hospital lipid clinics which comprise one of the largest secondary and tertiary referral lipid units in Europe.

The director, Dr Anthony Wierzbicki has published 180 papers on atherosclerosis and is interested in the genetics of hyperlipidaemia, the relationship between dyslipidaemia and hypertension, diseases of peroxisome metabolism (Refsum disease) and how drugs affect lipids and apolipoproteins. Dr Martin Crook, the deputy director, has published 120 papers and has long-standing interests in lipids in diabetes and obesity, the role played by apolipoprotein H as a cardiovascular and coagulation risk factor and the use of sialic acid as a marker of inflammation.

### **Newcastle**

The SAS Lipids and Cardiovascular Biomarkers Laboratory is part of the Department of Clinical Biochemistry, Newcastle upon Tyne Hospitals NHS Trust. Located in the Leazes Wing, Royal Victoria Infirmary, within the Medical School complex, the SAS Laboratories are closely associated with the research laboratories in University of Newcastle Schools of Clinical and Laboratory Sciences and Clinical Medical Sciences.

The Director of the Laboratory is Dr Dermot Neely who is a Consultant Chemical

Pathologist, Head of the Clinical Biochemistry Department and Honorary Clinical Lecturer in the University of Newcastle School of Clinical and Laboratory Sciences. He is also Lead Consultant for the Lipid and Metabolic Outpatient Clinic, Royal Victoria Infirmary, which provides a secondary and tertiary dyslipidaemia referral service for the North-East. His major research interests are familial hypercholesterolaemia and dyslipidaemia associated with insulin resistance and the metabolic syndrome.

Deputy Director Dr Michael Laker is Medical Director of Newcastle upon Tyne Hospitals NHS Trust, Reader in Clinical Biochemistry, University of Newcastle School of Clinical and Laboratory Sciences. His main research interests have been cholesterol metabolism, dyslipidaemia in diabetes mellitus and adapting analytical techniques to biomedical research. He has many years experience in the investigation and management of lipid disorders, having been actively involved in the Lipid and Metabolic Outpatient Clinic, Royal Victoria Infirmary since his appointment as a Consultant in Newcastle in 1980.

Dr David Cook is a Principal Clinical Scientist with responsibility for research and development for the SAS Lipids and Cardiovascular Biomarkers service and has a particular interest in immunoassay, immunoaffinity, and ultracentrifugation techniques. Dr Morteza Pourfarzam, Consultant Clinical Scientist and Head of the Sir James Spence Paediatric Metabolic Laboratory, has a specialist interest in the measurement of plasma sterols and precursors. Expert technical assistance is provided by Mr David Genner, Senior Biomedical Scientist.

### **Royal Free and University College Hospitals Trusts**

The SAS centre for Cardiovascular Biomarkers is based in the Lipid Laboratory, Department of Clinical Biochemistry, Royal Free Hampstead NHS Trust. The Department has a long-standing interest in the management of lipid disorders including familial hypercholesterolaemia.

The Director, Dr Devaki Nair, is the clinical lead for lipids and cardiovascular risk prevention. Her special interests include antiretroviral drug-induced hyperlipidaemia, familial hypercholesterolaemia and cardiovascular risk factors in South Asians.

The Deputy Director, Mr Jahm Persaud, is a Principal Grade Biochemist in charge of the Lipid and Renal specialist section of the Department of Clinical Biochemistry. He has a long-standing interest in lipoprotein metabolism in renal disease. His current interests include familial hypercholesterolaemia, lipoprotein(a) and HDL subclasses.

Dr Dimtri P Mikhailidis, Reader and Hon. Consultant with research interests in lipids, platelets and thrombosis has more than 235 publications in this area.

Dr Michael Thomas, Clinical Director of Pathology and Head of Clinical Biochemistry collaborated in the Exercise Evaluation Randomised Trial, an NHS R & D sponsored study. He was also involved in the British Regional Heart Study and Ten Towns study both sponsored by the British Heart Foundation.

The Department, in collaboration with the Genetics Knowledge Park, is also participating in a Department of Health funded project comparing DNA testing for FH with traditional diagnostic methods.

The lipid laboratory is supported by 2 Clinical associates, Dr David Wheeler, Senior Lecturer and Hon.Consultant in Nephrology with an interest in cardiovascular risk factors and lipoprotein metabolism in renal failure and Dr Gerry Coghlan, Consultant Cardiologist with interest in Cardiac biomarkers.

## Specimen Requirements and Assay Availability

### - Core assays

G = Glasgow; Gu = Guildford; GT = Guy's & St Thomas's, London; N = Newcastle;  
 R = Royal Free/ University College, London  
 E = EDTA plasma; EWB = EDTA Whole blood; S = serum; L = Lithium-heparin plasma  
 FCP = First class post; F = Transport frozen by courier

Assay	Sample Volume	Specimen	Transport	Availability
<b>Lipid profile - fasting</b> TChol, HDL, Tgs TC:HDL ratio, calculated LDL	5ml	S (fasting)	FCP	G, GU, GT N, R
<b>Direct LDL cholesterol</b>	1ml	S	FCP	R, GT
<b>B-Quantification (VLDL, LDL, HDL by ultracentrifugation)</b>	2ml	E or S (Fasting)	FCP	G, Gu, GT, N
<b>Apolipoproteins</b>				
Apolipoprotein A1	1ml	E or S	FCP	G, Gu, GT, R, N
Apolipoprotein B	1ml	E or S	FCP	G, Gu, GT, R, N
Lipoprotein (a)	1ml	E or S	FCP	G, Gu, GT, R, N
<b>Genetic Tests</b>				
Apolipoprotein E Genotype	1ml	EWB	FCP	G, Gu, GT, N
<b>Lipoprotein-related enzymes</b>				
Lipoprotein lipase	5ml	E	F	G, GT, Gu
				(Pre and post iv heparin - Contact laboratory for details)
<b>Non-Lipid risk factors</b>				
Homocysteine	1ml	E	FCP	Gu, GT, R
				(Separate plasma within 30min of collection)
High-sensitivity C-Reactive Protein	1ml	L or S	FCP	G, Gu, GT, R, N

## A Brief Introduction to Lipoprotein Metabolism

Lipoprotein particles are macromolecular complexes of cholesterol, cholesterol ester, triglycerides and phospholipids with apolipoproteins which render them water soluble. There are several classes of lipoprotein particles characterised by their flotation density, particle size and apolipoprotein content (table 1) and differing in their physiological functions (table 2). The apolipoproteins also possess intrinsic functional properties (table 3).

The different lipoprotein complexes are linked in two major metabolic pathways; the exogenous and endogenous pathways (figures 1 and 2). The functions of the enzymes involved in these pathways are summarised in table 4.

Abnormalities of lipoprotein metabolism are often associated with increased risk of atherosclerosis, the major underlying cause of coronary heart disease, stroke and peripheral vascular disease. Disorders of lipoprotein metabolism may also be associated with pancreatitis, hepatic steatosis and extravascular lipid deposition (xanthomas). These abnormalities are genetically determined (primary), or secondary to other conditions (tables 5 and 6). It is important to screen for possible secondary causes of dyslipidaemia prior to treatment with specific lipid lowering agents.

The following tests are sufficient to exclude the major secondary causes:

<b>Secondary cause</b>	<b>Recommended Tests (minimum)</b>
Liver dysfunction	T.Bili, Albumin, ALP, ALT, Gamma GT
Hypothyroidism	TSH, FT <sub>4</sub>
Renal disease	Urea & Electrolytes, Dipstick for protein
Diabetes mellitus	Fasting Glucose
Also recommended	CK (baseline), FBC (anaemia), Urate (risk of gout)

A scheme for investigation of lipid related disorders is outlined in figure 3.

**Table 1 - Characteristics of the Major Lipoproteins**

Lipoprotein Class	Diameter (nm)	Flotation density (g/ml)	Major apoproteins
Chylomicron (CM)	80 -1000	< 0.95	B48, E, CII
Chylomicron remnant (CMR)	60 -100	1.006 -1.019	B48, E, CIII
Very low density lipoprotein (VLDL)	40 - 80	0.95 -1.006	B100, E, CII
Intermediate density Lipoprotein (IDL)	25 - 35	1.006 - 1.019	B100, E, CIII
Low density lipoprotein (LDL)	20 - 22.5	1.019 -1.063	B100, E
High density lipoprotein (HDL)	7.5 -15	1.063 -1.21	AI, AII, E

**Table 2 - Physiological functions of the Major Lipoproteins**

Particle	Chol	Trig	Function
Chylomicrons	+	++++	Transport of dietary (exogenous) triglycerides from gut to peripheral tissues.
VLDL	+	++++	Transport of triglycerides synthesised in the liver (endogenous) to peripheral tissues.
Chylomicron Remnant (CR)	+++	+++	Derived from chylomicrons. Usually rapidly cleared by the liver.
IDL	+++	+++	Derived from VLDL. Usually rapidly cleared by the liver or converted to LDL
LDL	+++++	+	Derived from IDL. Transport of cholesterol and fat soluble vitamins to peripheral tissues.
HDL	++++	+	Reverse transport of cholesterol from peripheral tissues to the liver; antioxidant

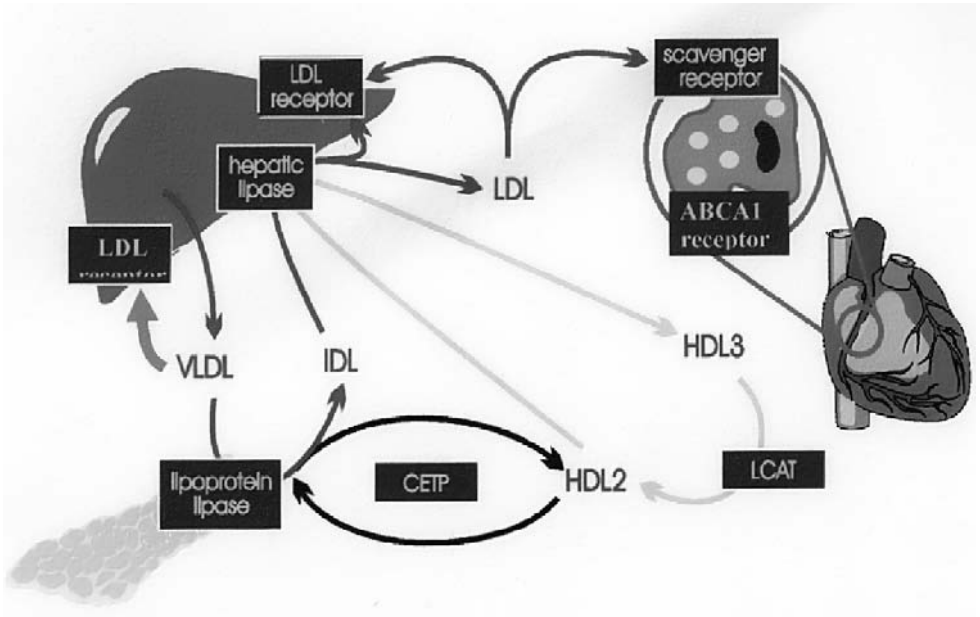
**Table 3 - Characteristics of the Major Apolipoproteins**

<b>Apolipoprotein</b>	<b>Molecular Weight (Da)</b>	<b>Function</b>
AI	28,000	Structural component of HDL Lecithin: cholesterol acyl transferase (LCAT) activation
AII	17,400	Structural component of HDL
B100	550,000	Structural component of VLDL, LDL, IDL. Ligand for LDL receptor
B48	264,000	Structural component of CM and CMR
CII	8,900	Lipoprotein lipase (LPL) activation cofactor
CIII	8,800	Inhibition of LPL
E	33,000	Receptor ligand for LDL VLDL and CM remnant

**Table 4 - Function of Major Enzymes of Lipoprotein Metabolism**

<b>Enzyme</b>	<b>Function</b>
Cholesteryl ester transfer protein (CETP)	Exchange of cholesteryl ester for triglycerides in apoB-containing lipoproteins (CM, VLDL, IDL, LDL)
Lecithin cholesterol acyl transferase (LCAT)	Esterification of cholesterol
Lipoprotein lipase (LPL)	Hydrolysis of triglycerides in CM, VLDL and IDL to glycerol and free fatty acids
Hepatic lipase (HL)	Hydrolysis of triglycerides and phospholipids in VLDL remnant, IDL, LDL and HDL to glycerol and free fatty acids; conversion of IDL to LDL



**Figure 2 - Lipoprotein Metabolism - Endogenous Pathway**

HDL = high density lipoprotein; LDL = low density lipoprotein;  
 IDL = intermediate density Lipoprotein; VLDL = very low density lipoprotein;  
 CETP = cholesterol ester transfer protein; LCAT = lecithin cholesterol acyl transferase;  
 ABCA1 = ATP binding cassette A1

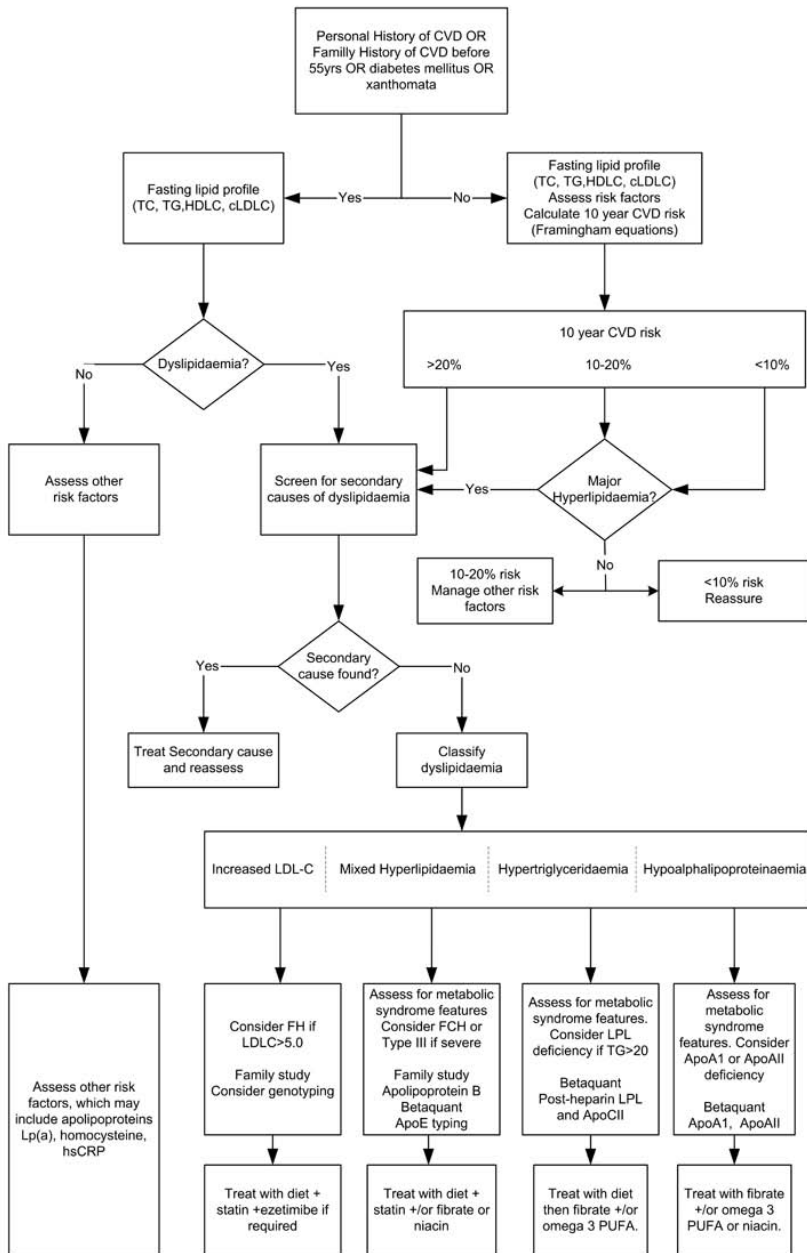
**Table 5 - Primary causes of dyslipidaemia**

Condition	Lipoprotein elevated	Typical serum lipid (mmol/L)		Risk	Population frequency
		Cholesterol	Triglyceride		
Polygenic hypercholesterolaemia	LDL	6.0	2.3	CHD	1:3
Familial hypercholesterolaemia	LDL	>7.5	<2.3	CHD	1:500
Familial defective Apo-B100	LDL	7.5	<2.3	CHD	1:600
Familial combined dyslipidaemia	LDL/VLDL	-12	3-12	CHD	1:100
Remnant particle disease	IDL	8-14	8 -14	CHD, PVD	1:5000
Familial hypertriglyceridaemia	VLDL/CM	3.5-12	4.5-30	Pancreatitis	1:100
LPL deficiency	CM	<6.6	10-100+	Pancreatitis	1:10 <sup>5</sup>

**Table 6 - Secondary causes of dyslipidaemia**

Condition	Lipoprotein elevated	Lipid change		HDL
		Chol	TG	
Diabetes mellitus	VLDL		++/+	-
Hypothyroidism	LDL	++		
Alcohol excess	VLDL		++/+	+
Obesity	VLDL		+	-
Chronic renal Failure	VLDL/LDL	+	++	-
Nephrotic Syndrome	VLDL/LDL	++	+	
Cholestasis	LDL/ LpX	++		
Gout	VLDL		+	
Pregnancy	VLDL	+/-	+/++	
Anorexia nervosa	LDL	+		
Hypopituitarism	VLDL/LDL/IDL	+/++	++/+	-
<b>Drug treatment:</b>				
Anticonvulsants	HDL			+/++
Androgens	HDL			-/--
Atypical antipsychotics	VLDL/LDL	+/++	+/+++	-
β-blockers	VLDL		+	
Corticosteroids	VLDL/ LDL	+	+	+
Cyclosporin	LDL/ VLDL	+	+	-
HIV/Anti-retroviral therapy	VLDL		+/++	
Oral oestrogens	VLDL		+	+
Retinoids	VLDL		++/+	-

Figure 3. Investigation Protocol for Dyslipidaemia



## Brief Details of Available Assays

### Apolipoprotein AI (Apo AI)

Apo AI comprises 65% of the apolipoprotein of high density lipoprotein (HDL), providing the structural scaffold for its formation. It is also a co-factor for lecithin cholesterol acyl transferase (LCAT), required for esterification of cholesterol to cholesteryl esters.

HDL-cholesterol is involved in the reverse transport of cholesterol from peripheral tissues to the liver, from where it can be excreted. Hence apo A1 deficiency confers increased risk of coronary artery and peripheral vascular disease, even in the absence of other coronary risk factors. Patients with significant arteriosclerosis generally have lower plasma Apo A1 concentrations than a normal population.

Specific genetic abnormalities of the apo A1 gene may be associated with reduced levels of apo A1 and HDL. These are not all associated with increased coronary risk.

Reduced apo AI values are also associated with smoking, diets rich in carbohydrates and/or polyunsaturated fats, dyslipoproteinaemias (eg familial hypoalphalipoproteinaemia), uncontrolled diabetes, liver disease, chronic renal failure, and some therapies (beta blockers, diuretics, progestins, androgens).

Raised apo A1 concentrations are associated with pregnancy, familial hyperalphalipoproteinaemia, and with drugs such as carbamazepine, phenytoin, phenobarbitone, oestrogens, oral contraceptives, ethanol, niacin, fibrates and statins.

Most genetic hypoalphalipoproteinaemias are caused by mutations in enzymes, and transporters involved in reverse cholesterol transport. Mutations in apoA-I are rare and associated with amyloidosis, peripheral neuropathy and both increased and decreased risks of atherosclerosis.

Serum apo A1 and apo B levels are increasingly recognised as better indicators of atherosclerotic risk than cholesterol and triglycerides alone. Atherosclerotic patients are better distinguished from normal individuals by the finding of increased plasma apo B or decreased plasma apo A1 than by a raised LDL- and low HDL-cholesterol. The ratio of apo A1 to apo B may provide a better index of cardiovascular risk than the individual values.

**Clinical Indications:**

Apo A1 measurements can be used to characterise patients with genetic disorders which lead to low HDL-Cholesterol levels. There are few prospective studies of apo A1 in cardiovascular risk prediction and in some, HDL-Cholesterol was superior.

Apo A1 assays are theoretically more accurate and precise than HDL-cholesterol methods.

**Approximate reference range:**

0.8 -1.9g/L

(See individual laboratory report)

**Patient preparation:**

Patients should follow their normal diet for 3 weeks prior to sampling. A fasting sample is preferred, but non-fasting is acceptable. Standardise posture to reduce effect of change in plasma volume - seat the patient for 5 minutes before sampling. Avoid venous stasis - apply tourniquet briefly before inserting needle and release before drawing the sample.

**Sample details:**

EDTA plasma or serum (min. vol. 0.5ml).

Stable 4 days at 4°C, 2months at -20°C

Transport - First Class Post (avoid weekends)

**Information required:**

Age, sex, NHS/Hospital No.

Medication

HDL-cholesterol (if available)

**References:**

1. Wald NJ, Law M, Watt HC et al. Apolipoproteins and ischaemic heart disease; implications for screening. *Lancet* 1994; **343**: 75-79
2. Sniderman AD, Cianflone K. Measurement of apoproteins; time to improve the diagnosis of atherogenic dyslipidaemias *Clin Chem* 1996; **42**: 489-91

## Apolipoprotein B (Apo B)

Apo B is central to lipoprotein transport, being essential for the secretion of triglyceride-rich lipoproteins from the liver and gut. One molecule of apo B is present in each chylomicron, VLDL or LDL particle. It exists in two forms; apoB100 and apoB48 encoded by the same gene on human chromosome 2. ApoB48 is secreted by enterocytes and is the major protein constituent of chylomicrons (CM). ApoB100 is the major component of all lipoproteins except CM and HDL and 90% of circulating apoB100 is found in LDL. It is essential to the formation of VLDL particles and their release into the circulation. ApoB100 is the ligand for the LDL receptor on hepatocytes and in peripheral tissues. Mutations of the apo B gene terminus at or close to codon 3500 is associated with abnormal ligand binding and a familial form of hypercholesterolaemia (familial defective apo B)

Elevated plasma apo B100 is a marker of increased numbers of LDL particles and is a risk factor for coronary artery disease even in the presence of a relatively normal LDL cholesterol concentration.

Apo B100 is raised in hyperlipoproteinaemia types IIa, IIb, IV and V, hyperapobetalipoproteinaemia (normal LDL, elevated apo B) hepatic obstruction, renal disease, diabetes, hypothyroidism, Cushing's syndrome, anorexia and pregnancy. Drugs which cause an increase include cyclosporin, diuretics, corticosteroids, beta blockers, alcohol, androgens, progestins and catecholamines. Diets rich in saturated fats and cholesterol also increase plasma apo B concentrations.

Decreased apo B levels are found in abetalipoproteinaemia (Tangier disease), heterozygous hypobetalipoproteinaemia, LCAT deficiency, hyperlipoproteinaemia type I, lipoprotein lipase cofactor (apo CII) deficiency, hyperthyroidism, malnutrition, malabsorption, severe hepatocellular dysfunction, Reye's syndrome, inflammatory joint disease, pulmonary disease, myeloma and weight reduction.

### **Clinical Indications:**

Apo B determination is useful in estimating the adequacy of endogenous pathway inhibition by drug therapy as assessed by LDL particle numbers; the diagnosis of certain primary disorders of lipoprotein metabolism (eg. abetalipoproteinaemia, homozygous hypobetalipoproteinaemia - see above) and as a research tool in the investigation of lipoprotein metabolism.

### **Approximate reference range:**

0.5 - 1.0g/L;

(See individual laboratory report)

**Ranges related to CHD risk:**

< 0.9g/l	Target for secondary prevention
<1.05 g/l	desirable
1.05-1.245g/l	borderline risk
1.25-1.39 g/l	high risk
>1.40 g/l	very high risk

**Patient preparation:**

Patients should follow their normal diet for 3 weeks prior to sampling. A fasting sample is preferred, but non-fasting is acceptable. Standardise posture to reduce effect of change in plasma volume - seat the patient for 5 minutes before sampling. Avoid venous stasis - apply tourniquet briefly before inserting needle and release before drawing sample.

**Sample details:**

EDTA plasma or serum (min. vol. 0.5ml).

Stable 4 days at 4°C, 2months at -20°C

Transport - First Class Post (avoid weekends)

**Information required:**

Age, sex, NHS/Hospital No.

Medication

Lipid profile results including LDL-cholesterol (if available)

**References:**

1. Bhatnagar D, Durrington PN. Does measurement of apolipoproteins add to the clinical diagnosis and management of dyslipidemias?. *Curr Opin Lipidol* 1993; **4**: 299-304.
2. Wald NJ, Law M, Watt HC et al. Apolipoproteins and ischaemic heart disease; implications for screening. *Lancet* 1994; **343**: 75-79
3. Sniderman AD, Cianflone K. Measurement of apoproteins; time to improve the diagnosis of atherogenic dyslipidaemias *Clin Chem* 1996; **42**: 489-91
4. Miremadi S, Sniderman A, Frohlich J. Can measurement of serum apolipoprotein B replace the lipid profile monitoring of patients with lipoprotein disorders? *Clin Chem* 2002;**48**: 484-488

## Apolipoprotein E Polymorphisms

Apolipoprotein E contains 299 amino acids and an arginine-rich glycoprotein. It is associated with VLDL, chylomicrons, chylomicron remnants, intermediate density lipoproteins and with some cholesterol-rich subclasses of HDL.

The structural gene for apo E is polymorphic, with 3 common alleles ( $\epsilon 2$ ,  $\epsilon 3$ ,  $\epsilon 4$ ) coding for three isoforms of Apo E protein: E2, E3 and E4. These differ in their amino acid sequence at positions 112 and 158. Apo E3, the prevalent allele, has cysteine at position 112 and arginine at position 158. In Apo E2 a cysteine residue replaces arginine at position 158, whilst Apo E4 has arginine at both positions.

Individuals inherit one Apo E allele from each parent and the six genotypes  $\epsilon 2/2$ ,  $\epsilon 2/3$ ,  $\epsilon 3/3$ ,  $\epsilon 2/4$ ,  $\epsilon 3/4$ ,  $\epsilon 4/4$ , encode 6 phenotypes.

Apo E isoforms differ in charge and can be phenotyped by isoelectric focusing and immunoblotting. Genotyping is performed using PCR based methods.

### Clinical indications:

Homozygosity at the  $\epsilon 2$  locus is associated with the development of type III dyslipidaemia or 'remnant disease'. More than 90% of patients with type III hyperlipidaemia express  $\epsilon 2/\epsilon 2$ . Approximately 1% of the population are homozygous for  $\epsilon 2/\epsilon 2$  but only 1 in 50 of homozygotes develop type III hyperlipidaemia. Additional factors are required and may include the development of hypothyroidism, obesity, insulin resistance or diabetes.

Variation in apo E geno / phenotype is also believed to account for approximately 8% variation in serum cholesterol concentration at the population level, carriage of the  $\epsilon 2$  and  $\epsilon 4$  alleles being associated with lower and higher serum cholesterol respectively. The apo E geno / phenotype is also a marker of responsiveness to cholesterol-lowering dietary and drug therapies that target either cholesterol synthesis or absorption. This is largely due to an association between apo E isoforms and cholesterol synthesis and absorption;  $\epsilon 2$  carriers are high cholesterol synthesisers;  $\epsilon 4$  carriers are high cholesterol absorbers.

### Interpretation:

Apo E bands are visualised by UV transillumination compared to reference primers. Patients with remnant (Type III) hyperlipidaemia are usually homozygous for the Apo E2 allele which may be demonstrated by either phenotyping or genotyping. Different degrees of protein post-translational modification may give rise to misleading results, particularly in pathological states such as diabetes mellitus, in which case genotyping is more reliable. Rare alleles may not be detected by allele specific genotyping methods. Mutations which do not alter electrophoretic mobility will not be detected by phenotyping.

**Patient preparation:**

A non-fasting sample is adequate

**Sample preparation and transport:**

EDTA plasma is preferred for phenotyping. Genotyping requires EDTA whole blood.  
Transport by first class post

**Information required:**

Age, sex, NHS/Hospital No.  
Medication

**References:**

1. Utermann G, Hees M, Steinmetz A. Polymorphism of apolipoprotein E and occurrence of dysbetalipoproteinaemia in man. *Nature*. 1977;**269**: 604-7
2. Havekes LM, de Knijff P, Beisiegel U, Havinga J, Smit M, Klassen E. A rapid micro method for Apolipoprotein E phenotyping directly in serum. *J Lipid Res* 1987; **28**: 455-63
3. Song Y, Stampfer MJ, Liu S. Meta-analysis: apolipoprotein E genotypes and risk for coronary heart disease. *Ann Intern Med*. 2004; **141**: 137-47

## Beta Quantification (lipid ultracentrifugation)

Risk of cardiovascular disease is assessed, in part, by laboratory measurements of the lipoproteins, VLDL, LDL and HDL.  $\beta$  quantification uses ultracentrifugation to partially separate lipoprotein classes and is the basis for the reference methods for LDL- and HDL- cholesterol as practised by the CDC. This 'gold standard' method has been used to establish the concentrations of the major lipoprotein classes in most of the epidemiological and clinical trials that have been used as guidelines for risk assessment.

The most useful aspect of  $\beta$  quantification is the efficient removal of triglyceride-rich VLDL which often interferes with the measurement of LDL- and HDL- cholesterol by other methods. Estimation of LDL-cholesterol by the Friedwald equation is invalid for non-fasting samples containing chylomicrons and when triglyceride concentrations exceed 4.5 mmol/L. It is used in the diagnosis of type III hyperlipidaemia when the ratio of VLDL-C to total triglyceride exceeds 0.65.

It is important to note that the LDL fraction will also contain IDL and Lp(a).

Chylomicrons are removed by preliminary low speed centrifugation. Plasma or serum is centrifuged overnight at  $d=1.006\text{g/ml}$  to spin up VLDL. The apolipoprotein B containing lipoproteins in the bottom fraction (mainly LDL) are precipitated using heparin and manganese chloride, leaving HDL in solution.

LDL cholesterol is calculated by subtracting VLDL-C and HDL-C from total cholesterol. Alternatively, multiple fractions can be isolated by gradient ultracentrifugation.

### Target Values:

Triglyceride	<2.3 mmol/L
Cholesterol	<5.0 mmol/L
LDL-C	<3.0 mmol/L
HDL-C males	>1.0 mmol/l
HDL-C females	>1.2 mmol/l

### Sample Details:

Fasting (12-14h) EDTA plasma or serum, minimum volume 2ml.

Stable for one week at 4°C and one year at -70°C.

Transport - first class post.

### Information required:

Age, sex, NHS/Hospital No.

Medication

### Reference:

Lipid Research Clinics Manual of Laboratory Operations - DHEW Publication no. (NIH) 75 -628, 1974.

## Homocyst(e)ine

Elevated plasma homocyst(e)ine concentrations are associated with atherosclerotic cardiovascular disease. Increased concentration may result from decreased renal function and from some drug therapies (eg phenytoin). Hyperhomocysteinaemia may also result from folate and/or vitamin B12 deficiency, which leads to decreased remethylation of homocysteine to methionine; and from deficiency of vitamin B6 which is an essential cofactor for cystathione beta-synthase in the trans-sulphuration pathway.

Genetic defects of homocysteine metabolism also increase homocysteine concentrations. The C677T mutation in the methylenetetrahydrofolate reductase (MTHFR) gene, has a prevalence of 20% in Asians, 12% in Caucasians and less than 2% in Africans. TT homozygotes have plasma homocyst(e)ine concentrations approximately 25% higher than the wild CC genotype.

The rarer cystathione beta-synthase deficiency, a genetic defect affecting the trans-sulphuration pathway, has an incidence of less than 1 in 25000 in the general population. It is associated with homocystinuria, hyperhomocysteinaemia (homocysteine > 100µmol/L), adolescent age strokes and transient ischemic attacks.

Vitamin B6 deficiency, causes only a minimal increase in plasma homocysteine, but can be unmasked by an oral methionine load.

Homocysteine is thought to exert its toxicity by damaging vascular endothelial cells, preventing normal endothelium-mediated vasodilatation. Auto-oxidation of homocysteine by trace metal ions produces reactive oxygen species (superoxide anion, hydrogen peroxide, hydroxyl and thiol free radicals) which oxidise LDL, potentiating its deleterious effects. Homocysteine may also interact with growth factors and cytokines in atherosclerotic lesions to induce proliferation of smooth muscle cells during atherogenesis.

Elevated homocysteine appears to be an independent risk factor, conferring an additive effect on other risk factors. A rise of 5µmol/L in homocysteine is considered equivalent in terms of cardiovascular risk to an increase of 0.5mmol/L cholesterol.

The finding of a high homocysteine should prompt investigation of renal function and of folate and vitamin B12 status. Supplementation with folate reduces plasma homocysteine concentration, and prospective trials are underway to evaluate its benefits. Further investigation of a high plasma homocysteine concentration may include demonstration of the presence or absence of the thermo-labile MTHFR gene mutation.

In addition to being a risk factor for cardiovascular disease and thrombosis, an elevated plasma homocysteine concentration has also been implicated as a risk factor for neural tube defects and psychogeriatric illness (eg Alzheimers disease).

**Clinical Indications:**

Premature cardiovascular disease

Risk assessment should be based on major risk determinants. A high plasma homocysteine concentration may prompt more aggressive treatment of other risk factors.

**Approximate Reference range:**

5 - 15 $\mu$ mol/L	(Adult)
5 - 20 $\mu$ mol/L	(Adult >70y)
<10 $\mu$ mol/L	(Children)

Mild elevation:	16-30 $\mu$ mol/L
Moderate elevation:	31-100 $\mu$ mol/L
Severe elevation:	>100 $\mu$ mol/L

**Patient preparation:**

An overnight fast is required. Blood samples for B12 and folate and for renal function should be taken at the same time. Due to circadian rhythm, values are lowest in the morning. Values are increased by a high protein diet, during pregnancy, the follicular phase and post-menopause, and by some drugs (eg phenytoin).

**Sample details:**

EDTA plasma - separated within 30 minutes (10% increase per hour). min. vol. 0.5ml.  
Stable 4 days at 4°C, >1year at -20°C  
Transport - First Class Post (avoid weekends)

**Information required:**

Age, sex, NHS/Hospital No.  
Medication

**Reference:**

1. Rasmussen K, Moller J. Total homocysteine measurement in clinical practice. *Ann Clin Biochem* 2000; **37**: 627-648

## LDL-Cholesterol - Calculated and Direct

LDL-cholesterol plays a causal role in the development of atherosclerosis. Its measurement is therefore important for the diagnosis and treatment of hyperlipidaemia. Most laboratories estimate LDL Cholesterol by the Friedewald equation:

$$\text{LDLC} = \text{TC} - \text{HDLC} - \text{Trig}/2.2$$

where Trig/2.2 approximates to VLDL Cholesterol.

This approximation assumes that total cholesterol is normally distributed across the three major lipoprotein classes. It is not valid at high triglyceride concentrations (>4.5mmol/L) or for non-fasting samples containing chylomicrons, when it under-estimates LDLC; or in Type III Hyperlipoproteinaemia, when it over-estimates LDLC.

Direct LDL measurements are indicated when the triglyceride concentration exceeds 4mmol/L. Beta-quantitation, based on ultracentrifugation, is the reference method, but automated methods evaluated against this procedure are now available.

### Clinical indications:

LDLC concentration is a primary criterion which, together with other risk factors, is used in the treatment recommendations for hyperlipidaemia.

### Reference Range:

LDL cholesterol (Target value) Less than 2.0mmol/L  
(derived from patients with a >20% risk of CVD over 10 years)

### Patient preparation:

Patients should follow their normal diet for 3 weeks prior to sampling. A fasting sample is required. Standardise posture to reduce effect of change in plasma volume - seat the patient for 5 minutes before sampling. Avoid venous stasis - apply tourniquet briefly before inserting the needle and release before drawing the sample.

### Sample details:

EDTA plasma or serum (min. vol. 2mL).  
Stable 4 days at 4°C, 2 months at -20°C  
Transport - First Class Post (avoid weekends)

### Information required:

Age, sex, NHS/hospital No.  
Medication

### Reference:

1. Friedewald WT, Levy RI, Fredrickson DS ;Estimation of the concentration of low-density lipoprotein cholesterol in plasma without the use of the preparative ultracentrifuge. Clin Chem 1972;**18**; 499-502

## Lipoprotein (a) (Lp(a))

Lp(a) particles are heterogeneous with a molecular weight of 280-700 kDa. They consist of ApoB100 linked by a single disulphide bridge to Apo(a), a highly glycosylated hydrophilic protein with structural homology to plasminogen. This homology suggests that Lp(a) may be associated with thrombotic and atherosclerotic processes.

The particles have a kringle and a protease domain. The kringle domain has 11 kringle types, ten (K4 type 1 and 3) of which are similar to each other and to kringle 4 of plasminogen. Kringle K4 type 2 exists as 3-40 multiple repeats, which accounts for the size heterogeneity; Apo (a) isoforms vary from 187 kDa when 12 K4 domains are present to 662kDa when there are 50 K4 domains.

The serum concentration of Lp(a) is principally genetically determined and may be an independent risk factor for development of atherosclerosis. Concentrations vary widely. Plasma Lp(a) concentrations above 0.3g/L, despite the presence of a normal cholesterol, confer a two fold increased risk for development of coronary heart disease. The risk is 8-fold if LDLC and Lp (a) are both elevated.

Since plasma Lp(a) concentrations are principally genetically determined, lifestyle changes, or treatment with statins or fibrates are ineffective, although niacin or nicotinic acid may effect a 20% reduction. The finding of a high Lp(a) should stimulate aggressive treatment of more easily modifiable risk factors.

Since no international standard exists, values are method/instrument dependent and results from different laboratories may not be comparable.

### **Clinical indications:**

General population screening is not recommended.

Measurement may be useful in patients with coronary heart disease, a family history of premature coronary disease, or familial hypercholesterolaemia.

### **Reference Range:**

Less than 0.3g/L

### **Patient preparation:**

Patients should follow their normal diet for 3 weeks prior to sampling. A fasting sample is preferred, but non-fasting is acceptable. Standardise posture to reduce effect of change in plasma volume - seat the patient for 5 minutes before sampling. Avoid venous stasis - apply tourniquet briefly before inserting the needle and release before drawing the sample.

**Sample details:**

EDTA plasma or serum (min. vol. 0.5ml).

Stable 4 days at 4°C, 2months at -20°C

Transport - First Class Post (avoid weekends)

**Information required:**

Age, sex, NHS/Hospital No.

Medication

## Lipoprotein Lipase (LPL) Activity (Total post-heparin)

Total plasma lipase activity comprises the activities of three endothelial lipases, lipoprotein lipase (LPL) from the peripheral circulation, hepatic lipase (HL) from the microcirculation of the liver and the newly discovered endothelial lipase (EL) of unknown significance. LPL hydrolyses chylomicron- and VLDL- triglycerides to di and mono-glycerides and free fatty acids in the capillaries of skeletal muscle and adipose tissue. The free fatty acids are used either for resynthesis of triglycerides subsequently stored in adipose tissue, or as a source of energy. HL has similar actions to LPL on smaller lipoprotein particles and EL has more phospholipid lipase activity than triglyceride lipase activity.

LPL and HL are adsorbed to capillary endothelial surface oligosaccharides and do not normally circulate in the blood, but may be released by heparin and measured in plasma. This forms the basis of the test for post-heparin lipolytic activities of total and hepatic lipase.

Low LPL activity is associated with gross hypertriglyceridaemia. It may be due to decreased activation, to a rare mutation of the LPL gene, or to a deficiency of apo C-II, an activator for LPL. Apo C-III and apo C-1 inhibit LPL activity. Apoprotein C-II and C-III can be measured by immunoturbidimetry or by gel electrophoresis, using isoelectric focusing followed by protein staining and quantitation. It is usual to measure apo C-II at the same time as LPL activity.

### **Clinical indications:**

Measurement is useful when a decrease in LPL activity in plasma is suspected to underlie a gross hypertriglyceridaemia and the E2/2 phenotype has been excluded. Low LPL activity may result from a relative deficiency of LPL or its co-factor apo C-II. Type I hyperlipidaemia, a rare genetic condition, results from a genetic deletion of the LPL or C-II gene and presents as a severe hypertriglyceridaemia (chylomicronaemia).

### **Approximate reference range:**

LPL: 2-12  $\mu\text{mol}$  fatty acid/ml/hr

Type I hyperlipidaemia < 0.2  $\mu\text{mol}$  fatty acid/ml/hr

Apo CII: 1.9 - 4.1 mg/dl

These ranges are method dependent. Please refer to laboratory report.

**Patient preparation:**

The test should not be performed in subjects who have a sensitivity to heparin, are taking aspirin, have a history of bleeding diathesis, proliferative retinopathy, or CVA, or have rheumatic fever or a peptic ulcer. The patient should have fasted overnight, and refrained from alcohol in the previous 24 hours and heavy exercise in the previous 48 hours.

**Sample details:**

Detailed sample collection protocols should be obtained from the appropriate laboratory.

Plasma lipase activities are measured in post-heparin plasma (PHP).

Samples must be transported to laboratory on dry ice as soon as possible - analysis should be within 7 days

**Information required:**

Age, sex, Hospital/NHS No.

Medication

Plasma triglyceride and HDL-cholesterol

**Reference:**

1. Brunzell JD, Deeb SS (2001) Familial lipoprotein lipase deficiency, apo CII deficiency and hepatic lipase deficiency. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) *The Metabolic and Molecular Bases of Inherited Disease*, 8 ed. McGraw-Hill, New York, pp2789-2816.
2. Otarod JK, Goldberg IJ. Lipoprotein lipase and its role in regulation of plasma lipoproteins and cardiac risk. *Curr Atheroscler Rep* 2004; **6(5)**:335-342
3. Jansen H. Hepatic lipase: friend or foe and under what circumstances? *Curr Atheroscler Rep* 2004; **6(5)**:343-347.
4. Henderson AD, Richmond W, Elkeles RS. Hepatic and lipoprotein lipases selectively assayed in postheparin plasma. *Clin Chem* 1993; **39(2)**:218-223
5. Watson TD, Tan CE, McConnell M, Clegg SK, Squires LF, Packard CJ. Measurement and physiological significance of lipoprotein and hepatic lipase activities in preheparin plasma. *Clin Chem* 1995; **41(3)**: 405-412.

**Appendix - Non-core assays**

For information about the following assays, please contact the appropriate Laboratory. Other assays may be available.

Assay	Sample Volume	Specimen	Transport	Availability
<b>Apolipoproteins</b>				
Apolipoprotein A-II (Quant)	1ml	E or S	FCP	GT
Apolipoprotein C-I (Quant)	1ml	E or S	FCP	GT
Apolipoprotein C-II (Quant)	1ml	E or S	FCP	G, Gu, GT
Apolipoprotein C-II (Qualit)	1ml	E or S	FCP	R
Apolipoprotein C-III(Quant)	1ml	E or S	FCP	GT
Apolipoprot. E phenotype	2ml	E or S	FCP	R
Apolipoprotein E (Quant)	1ml	E or S	FCP	R, GT
Apolipoprotein H (Quant)	1ml	E or S	FCP	GT
( $\beta$ 2-glycoprotein I) Lipoprotein(a) isoforms	1ml	E or S	FCP	R
<b>Genetic Tests</b>				
LDL receptor mutation Analysis/ sequencing	2ml	EWB	FCP	R
Thermo-labile MTHFR				
C677T Gene mutation	2ml	EWB	FCP	Gu, GT
Factor V <sub>Leiden</sub> mutation	2ml	EWB	FCP	Gu, GT
Prothrombin <sub>20210</sub> mutation	2ml	EWB	FCP	Gu, GT
Apolipoprotein B 3500 mutation	2ml	EWB	FCP	Gu
<b>Ultracentrifugation analysis</b>				
Lipoprotein subfractions (VLDL1, 2; IDL; LDL)	5ml	E (fasting)	FCP	G, GT, R
HDL subfractions (HDL2, 3)	2ml	E	FCP	G, Gu, R
LDL subfractions	10ml	E	FCP	G, GT, Gu
<b>Lipoprotein-related enzymes</b>				
Hepatic lipase	5ml	E	F	G, GT, Gu
CETP	5ml	E	F	GT
<b>Others</b>				
Sterol Profile	1ml	E or S	FCP	N
Soluble-ICAM-1	1ml	S	FCP	Gu
NT-pro BNP	1ml	S	FCP	R
BNP	1ml	S	FCP	Gu
Sialic acid	1ml	S	FCP	GT



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[www.randox.com](http://www.randox.com)





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