

The Newcastle upon Tyne Hospitals NHS Trust
Department of Clinical Biochemistry
Freeman Hospital, Newcastle General Hospital
and
Royal Victoria Infirmary

Standard Operating Procedure for
AU 2700 / AU 640 / AU 400 HDL CHOLESTEROL



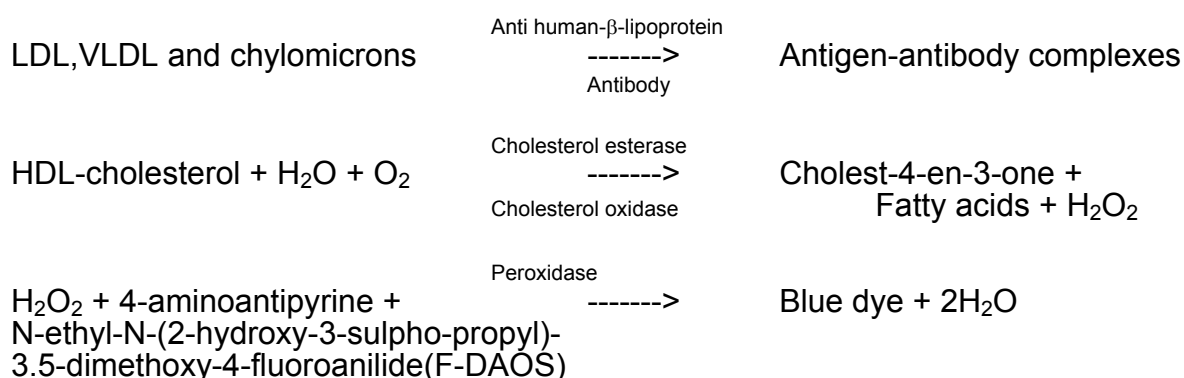
AU 2700 / AU 640 / AU 400 HDL CHOLESTEROL

PERSONNEL

All appropriately trained Scientific/Technical staff and trainees under supervision.

PRINCIPLE

Anti human- β -lipoprotein antibody in Reagent 1 binds to lipoproteins other than HDL (LDL, VLDL and chylomicrons). The antigen-antibody complexes formed block enzyme reactions when reagent 2 is added. HDL-cholesterol is quantified by the presence of an enzyme chromogen system.



SAMPLE

A. Sample Type - Serum is recommended although plasma (EDTA) is acceptable.

B. Sample Stability - After separation, 3 days at room temperature, 7 days at 4°C.

C. Sample Volume - 2 μ L (+25 μ L for dead volume).

D. Interferences - There are no reported interferences.
Haemolysed, icteric and lipaemic samples are not known to interfere.

IMPRECISION

Within batch

	N	MEAN Mmol/L	SD	CV %
LOW	30	0.73	0.05	6.5
MEDIUM	30	1.00	0.02	1.8
HIGH	30	1.82	0.02	1.0

Data obtained during audit June 01



Between batch

	N	MEAN Mmol/L	SD	CV %
LOW	622	0.7	0.04	5.6
MEDIUM	836	1.0	0.05	5.0
HIGH	461	1.7	0.07	4.4

Data obtained during QC audit. 20.10.01

HEALTH AND SAFETY

Refer to the Departmental Safety Manual Index Code : **SAFETY1.DOC** at the RVI and NGH sites and **FSAFETY1.DOC** at the FH site.

Risk Assessment

COSHH document **88** identifies the following compound as a hazard;

Sodium Azide	Irritant to the eyes No significant risk associated in prepared reagent
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ALL REAGENTS SHOULD BE CONSIDERED HARMFUL BY INGESTION

The instrument, sample and waste handling risks are covered by the Olympus Risk Assessment in the instrument SOP.

PREPARATION OF STANDARD

Reconstitute one bottle of Olympus HDL calibrator (Cat no ODC0011) with 3mls of de-ionised water using a volumetric pipette. Mix on rotor mixer for 20 minutes. Prepare fresh each day.

PREPARATION OF REAGENTS

Reagent supplied in Olympus kit, Product Number OSR 6187. Store in the fridge at 4°C on the reagent shelf or in a cold room as available. Use reagent as supplied. Stable until date on box.

Each pack contains	Working Reagent	R1	4 x 27ml
	Working Reagent	R2	4 x 9ml

The reagents are ready for use and can be placed directly on board the analyser.

It is essential to complete stock control sheets when a new kit is opened.



Reagents Concentration in test

Anti human- β -lipoprotein antibody		
Cholesterol esterase	0.8	IU/ml
Cholesterol oxidase	4.4	IU/ml
Peroxidase	1.7	IU/ml
Ascorbate oxidase	2.0	IU/ml
Good's buffer (pH 7.0)	30	mmol/L
N-ethyl-N-(2-hydroxy-3-sulpho-propyl)- 3,5-dimethoxy-4-fluoroaniline(F-DAOS)	0.20	mmol/L
4-aminoantipyrine	0.67	mmol/L
Preservative		
Detergent		

QUALITY CONTROL

Internal QC and External QA must be performed as defined for this chemistry in the Quality Control Policy, **QC1.DOC**.

FREQUENCY OF CALIBRATION

Reagent blanks and a single point calibration weekly or change of reagent lot.

PROCEDURE

Refer to Part 1 of the Standard Operating Procedure (see **OLYMOP.DOC**).

See Appendix for instrument specific parameters.

CALCULATION AND VALIDATION OF DATA

Refer to the QC Policy, Index Code : **QC1.DOC** for current ranges and Westgard rules, as appropriate, to determine acceptability of quality control results.

Linearity

0.05 – 4.65 mmol/L Results >4.65 – automatic re-run (RVI and FH sites only) will dilute sample in accordance with instrument specific parameters (see appendix) or dilute (NGH site only) sample 1/5, with deionised water.

If in any doubt refer to the Senior MLSO or the Duty Biochemist.



REPORTING OF RESULTS

- a.) Report to one decimal place..
- b.) Computer :- Results are passed from the Olympus via the PGP(for verification) to Apex.

Results processing

If a request is notified to the laboratory as urgent then the results must be telephoned to the appropriate ward. Mark the computer record as telephoned. Refer to the "Protocol for the verbal transmission of results", Index code **TELPRO.DOC**.

ADULT REFERENCE RANGE

MALE: 1.0 – 1.5 mmol/L
FEMALE: 1.2 – 1.8 mmol/L

Ranges obtained from Reference Range document, Index Code : **REF.DOC**

CLINICAL SIGNIFICANCE

Cholesterol is synthesised and utilised by most tissues of the body and is a component of cell membranes. It is catabolised only by the liver and consequently any excess of cholesterol, or cholesterol derived from cell breakdown, must be transported to the liver. Part of the cholesterol is degraded by the liver to bile acids and bile salts while the remainder is excreted as cholesterol. Many hormones particularly the thyroid hormones affect cholesterol metabolism. Certain types of hyperlipidaemia are associated with increased risk of cardiovascular disease. In affluent societies there is a high incidence of ischaemic heart disease. Primary causes may be familial. Secondary causes are diabetes mellitus, hypothyroidism, nephrotic syndrome, SLE and paraproteinaemia, and alcohol abuse.

Hyperlipidaemia's

- Type I - Large increase in triglycerides, normal cholesterol. It presents clinically as eruptive xanthomata, lipaemia retalis, hepatomegaly and attacks of abdominal pain.
- Type II a,b - Increased cholesterol level. Clinical signs are xanthomata and cardio-vascular disease.
- Type III - Inherited. Both cholesterol and triglycerides levels are increased.
- Type IV and V - Increased triglyceride, normal or slight increase in cholesterol levels. Clinical signs are obesity, impaired glucose tolerance, hyperuricaemia and often abdominal pain and pancreatitis.



The HDLs and LDLs are the cholesterol-rich lipoprotein fractions; therefore, high or low levels of cholesterol in the plasma may reflect high or low levels of those lipoproteins. Unfortunately, the factors that regulate the levels of LDL and HDL in the plasma have not been identified. Therefore, the specific causes of high levels of cholesterol in the plasma remain unknown. Generally, high plasma levels of cholesterol that reflect high levels of LDLs may be caused by an inherited defect in lipoprotein metabolism, by disease of the endocrine system, by liver disease, or by renal disease. Low levels of cholesterol in the plasma may reflect an inherited deficiency of either LDL or HDL, or they may reflect impairment of liver function.

REFERENCES

Zilva and Pannall Second Edition Chapter 9 Pages 218-233.
Tietz. Second Edition Chapter 10 Pages 474-541.

