

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

**Standard Operating Procedure for**  
**AU2700 / AU 640 / AU 400 CHOLESTEROL**



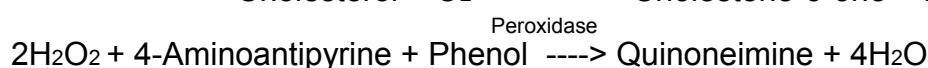
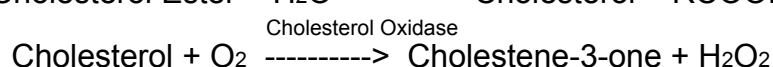
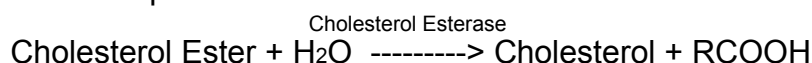
## AU2700 / AU 640 / AU 400 CHOLESTEROL

### PERSONNEL

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

### PRINCIPLE

The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase.



### SAMPLE

**A. Sample Type** - Serum is recommended although plasma (Lithium heparin, EDTA) is acceptable.

**B. Sample Stability** - After separation, 7 days at room temperature, 30 days at 4°C.  
- Before separation see data from stability study.

**C. Sample Volume** - 3µL (+25µL for dead volume).

**D. Interferences** - There are no reported interferences. Haemolysed (up to 2g/L), icteric and lipaemic samples are not known to interfere.

### IMPRECISION

#### Within batch

|        | N  | MEAN<br>mmol/<br>L | SD   | CV<br>% |
|--------|----|--------------------|------|---------|
| LOW    | 30 | 3.30               | 0.02 | 0.6     |
| MEDIUM | 30 | 4.41               | 0.05 | 1.1     |
| HIGH   | 30 | 7.70               | 0.07 | 0.9     |

Data obtained during QC audit. 20.10.01



## Between batch

|        | N   | MEAN<br>g/L | SD   | CV<br>% |
|--------|-----|-------------|------|---------|
| LOW    | 721 | 3.3         | 0.07 | 2.2     |
| MEDIUM | 717 | 4.4         | 0.09 | 2.1     |
| HIGH   | 445 | 7.6         | 0.14 | 1.9     |

Data obtained during QC audit. June 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 assessment **99** identifies the following compound as a hazard;

|                     |  |
|---------------------|--|
| Sodium Azide        | Irritant to the eyes<br>No significant risk associated in prepared reagent |
| Potassium Hydroxide | Irritant   |

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 system calibrator with 5mls 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 6116. Store at 4C on the reagent shelf in fridge or in a cold room as available. Use reagent as supplied. Stable until date on box.

Each pack contains Working Reagent R1 4 x 22.5ml

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



## Reagents Concentration in test

|                           |       |        |
|---------------------------|-------|--------|
| Phosphate buffer (pH 6.5) | 103   | mmol/L |
| 4-Aminoantipyrine         | 0.31  | mmol/L |
| Phenol                    | 5.2   | mmol/L |
| Cholesterol Esterase      | >0.2  | kU/L   |
| Cholesterol Oxidase       | >0.2  | kU/L   |
| Peroxidase                | >10.0 | kU/L   |
| Preservative              |       |        |

## 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 VERIFICATION 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

0.64 – 18.0 mmol/L Results >18.0 – automatic re-run (RVI and FH sites only) will dilute sample in accordance with instrument specific parameters (see appendix) or (NGH site only) dilute 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

See FATS guide lines in Reference range document.  
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.

## REFERENCES

Olympus kit insert.

Richmond, W., Clin. Chem. 19 (1973), 1350

Roeschlau, P., Bernt, E. and Gruber, W., Z. Klin. Chem. Klin. Biochem. 12 (1974), 403.

Trinder, P., Ann. Clin. Biochem. 6 (1969), 24.

Zilva and Pannall Second Edition Chapter 9 Pages 218-233.

