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
TRIGLYCERIDES

AU2700 / AU 640 / AU 400 TRIGLYCERIDES

PERSONNEL

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

PRINCIPLE

The triglycerides are determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxide, 4-aminoantipyrine and 4-chlorophenol under the catalytic influence of peroxidase.

Triglycerides	Lipases ------>	Glycerol + Fatty acids
Glycerol + ATP	GK ------>	Glycerol-3-phosphate + ADP
Glycerol-3-phosphate + O_2	GPO ------>	Dihydroxyacetonephosphate + H_2O_2
$2H_2O_2$ + 4-aminoantipyrine + 4-chlorophenol	POD ------>	Quinoneimine + HCl + $4H_2O$

SAMPLE

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

B. Sample Stability - After separation, 4 days at room temperature, 7 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, icteric and lipaemic samples are not known to interfere.

IMPRECISION

Within batch

	N	MEAN g/L	SD	CV %
LOW	30	1.17	0.05	4.1
MEDIUM	30	1.61	0.03	1.6
HIGH	30	2.19	0.04	2.0

Data obtained during QC audit. 20.10.01

Between batch

	N	MEAN g/L	SD	CV %
LOW	659	1.18	0.04	3.4
MEDIUM	666	1.62	0.05	3.1
HIGH	418	2.20	0.06	2.5

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

Sodium Azide	Toxic by ingestion. Irritant to eyes and skin

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, or as required at the FH site.

PREPARATION OF REAGENTS

Reagent supplied in Olympus kit, Product Number OSR 6133. Store at 4°C 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 50ml
	Working Reagent	R2	4 x12.5ml

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

Reagents Concentration in test

PIPES buffer (pH 7.5)	50	mmol/L	Lipases	1.5	kU/L
4-Aminoantipyrine	0.25	mmol/L	Glycerol kinase	0.5	kU/L
Mg^{2+}	4.6	mmol/L	ATP	1.4	mmol/L
Peroxidase	0.95	kU/L	4-chlorophenol	5.4	mmol/L
Glycerol-3-phoshate Oxidase	1.23	kU/L			
Ascorbate oxidase	>2	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

0.12 – 11.64 mmol/L Results >11.64 – 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

Male and female 0.8 - 1.90 mmol/l (fasting)

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

CLINICAL SIGNIFICANCE

Measurement of plasma triglyceride is important in the characterisation of hyperlipidaemia and the assessment of cardiovascular risk. Triglyceride circulates mainly in the VLDL lipoproteins in the fasting state (12-14 hours), but IDL and chylomicron remnants may also make a contribution when triglyceride is moderately elevated (1.9-5.0mmol/l). Triglyceride concentrations increase 2-4 fold after a meal and due to the appearance of chylomicrons and an increase in VLDL. Moderate hypertriglyceridaemia is frequently associated with low HDL cholesterol, atherogenic small dense LDL and other features of insulin resistance including obesity, glucose intolerance and diabetes mellitus, hyperuricaemia and hypertension. Other causes of hypertriglyceridaemia include alcohol abuse, renal disease, beta blocker drugs, oestrogens, pregnancy and hypothyroidism, all of which may result in severe hypertriglyceridaemia (>5.0mmol/l). Rarely, inherited lipoprotein lipase deficiency may be the cause of severe hypertriglyceridaemia. Chylomicrons are usually present when triglyceride is >10 mmol/l. There is an increasing risk of pancreatitis with worsening chylomicronaemia, particularly when triglyceride concentration rises to >20 mmol/l and urgent intervention is then required to control triglyceride levels if pancreatitis is to be prevented. Note that when the serum is usually grossly lipaemic, normal amylase does not exclude pancreatitis.

REFERENCES

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Koditschek, L.K., Umbreit, W.W., J. Bacteriol. 98 (1969), 1063 – 1068.
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