Brown Clinic Northridge
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BROWN CLINIC
LABORATORY PROCEDURE MANUAL

PROCEDURE: Triglycerides

PURPOSE:
The TGL Flex® reagent cartridge used on the Dimension EXL200 Integrated chemistry system is an *in vitro* diagnostic test intended for the quantitative determination of triglycerides in **serum** and **plasma**. Measurements obtained are used in the diagnosis and treatment of patients with diabetes mellitus, nephrosis, liver obstruction, other diseases involving lipid metabolism, or various endocrine disorders.

PRINCIPLE:
Triglycerides are water-insoluble lipids consisting of three fatty acids linked to one glycerol molecule. Triglycerides are transported in the blood as core constituents of all lipoproteins, but the greatest concentration of these molecules is carried in the triglycerides-rich chylomicrons and very low density lipoproteins (VLDL).[1] Through the action of lipases and bile acids, triglycerides are hydrolyzed into glycerol and fatty acids which are absorbed by adipose tissue for storage or by other tissues requiring a source of energy. A peak concentration of chylomicron-associated triglycerides occurs within 3-6 hours after ingestion of a fat-rich meal; however, the rate of absorption of fats is highly variable, depending on the individual and dietary composition of the fat. After absorption, triglycerides are resynthesized in the epithelial cells and combined with cholesterol and a number of apolipoproteins to form chylomicrons.[2]

The triglycerides method is based on an enzymatic procedure in which a combination of enzymes are employed for the measurement of serum or plasma triglycerides. The sample is incubated with lipoprotein lipase (LPL) enzyme reagent that converts triglycerides into free glycerol and fatty acids. Glycerol kinase (GK) catalyzes the phosphorylation of glycerol by adenosine-5-triphosphate (ATP) to glycerol-3-phosphate. Glycerol-3-phosphate-oxidase oxidizes glycerol-3-phosphate to dihydroxyacetone phosphate and hydrogen peroxide(H_2O_2). The catalytic action of peroxidase (POD) forms quinoneimine from H_2O_2, aminoantipyrine and 4-chlorophenol. The change in absorbance due to the formation of quinoneimine is directly proportional to the total amount of glycerol and its precursors in the sample and is measured using a bichromatic (510, 700 nm) endpoint technique.

$$\text{Triglycerides} \xrightarrow{\text{LPL}} \text{Glycerol + Fatty Acids}$$

$$\text{Glycerol + ATP} \xrightarrow{\text{GK}} \text{Glycerol-3-Phosphate + ADP}$$

$$\text{Glycerol-3-Phosphate} + O_2 \xrightarrow{\text{GPO}} \text{Dihydroxyacetone phosphate} + H_2O_2$$

$$2H_2O_2 + \text{Aminoantipyrine} + \text{4-Chlorophenol} \xrightarrow{\text{POD}} \text{Quinoneimine} + HCL + 4H_2O$$

INSTRUMENT, REAGENTS & SUPPLIES:

Wells[a]	Form	Ingredient	Concentration[b,c]
1–6	Liquid	Lipoprotein Lipase	7.5 KU/L
		ATP	3 mmol/L
		Glycerol kinase	0.5 KU/L
		Glycerol-3-Phosphate-oxidase	2.2 KU/L
		4-aminoantipyrine	0.75 mmol/L
		4-chlorophenol	6 mmol/L
		Peroxidase	5 KU/L
		Mg^{2+}	22.5 mmol/L
		Buffer pH 7.2	50 mmol/L

a. Wells are numbered consecutively from the wide end of the cartridge.

b. This represents the nominal value in final reaction mixture.

c. Contains bovine serum albumin.

Precautions: Sodium azide is present as a microbial inhibitor (< 0.1%). Sodium azide can react with copper or lead pipes in drain lines to form explosive compounds. Proper disposal of this product as a biohazard will minimize this possibility. Used cuvettes contain human body fluids. Handle with appropriate care to avoid skin contact and ingestion.

For *in vitro* diagnostic use

Safety Data Sheets (SDS/MSDS) can be found at www.siemens.com/healthcare

STORAGE & STABILITY:

Store at 2 - 8°C.

Expiration: Refer to carton for expiration date of individual unopened reagent cartridges. Sealed cartridge wells on the instrument are stable for 30 days. Once wells 1 through 6 have been entered by the instrument, they are stable for 10 days.

SPECIMEN REQUIREMENTS:

Normal procedures for collecting and storing serum and plasma may be used for samples to be analyzed by this method.[3]

Blood collection tubes containing glycerol lubricated stoppers should be avoided since they will cause erroneously elevated result.

Specimen:

patient preparation: fasting specimens recommended
specimen type: serum or heparinized plasma
handling: see comments above
Stability: Separated serum and plasma samples are stable at room temperature for 8 hours, 2 days at 2-8 degrees C, longer storage should be at -20 degrees C.

CONTROLS:
At least once daily run solutions at two levels of a quality control material with known concentrations.

For further details, refer to your Dimension® system manual. The result obtained should fall within limits defined by the day-to-day variability of the system as measured in the user's laboratory. If the results fall outside the laboratory's acceptable limits, follow the procedure in the quality control policy.

PROCEDURE:
The TGL Flex® reagent cartridge, Cat. No DF69A, is required to perform the TGL test. This test is performed on the Dimension® clinical chemistry system after the method is calibrated (Refer to your Dimension® system manual in the Calibration section).

Material Needed: TGL Flex® reagent cartridge, Cat. No. DF69A
CHEM II Calibrator, Cat. No. DC20
Quality control material

Test Steps

Sampling, reagent delivery, mixing, processing, and printing of results are automatically performed by the Dimension® system. For details of this processing, refer to your Dimension® system manual.

The sample container (if not a primary tube) must contain sufficient quantity to accommodate the sample volume plus dead volume.

Test Conditions

- Sample Size: 4 µL
- Reagent 1 Volume: 133 µL
- Test Temperature: 37°C ± 0.1°C
- Wavelengths: 510 and 700 nm
- Type of Measurement: bichromatic endpoint
- Units: mg/dL [mmol/L]

Calibration

The following information should be considered when calibrating the TGL method:

Assay Range:	15-1000 mg/dL [0.17-11.3 mmol/L]
Reference Material[e]:	Primary glycerol standards such as Dimension® CHEM II Calibrator (Cat. No. DC20) or secondary calibrators.
Suggested Calibration Levels:	120, 240, 485 mg/dL [1.37, 2.74, 5.54 mmol/L]

Calibration Scheme:	Three levels in triplicate.
Calibration Frequency:	Every new reagent cartridge lot
	Every month for any one lot
	For each new lot of Flex reagent cartridges
	After major maintenance or service, if indicated by quality control results
	As indicated in laboratory quality control procedures
	When required by government regulations
Assigned Coefficients:	C_0 –2.6
	C_1 1.5

e. Triglycerides-based samples (not glycerol samples) should be used to verify the assay range.

Backup Process:
Refer to Brown Clinic Back-up Policy

INTERPRETATION:
The instrument automatically calculates and prints the concentration of triglycerides in mg/dL [mmol/L] using the calculation scheme illustrated in your Dimension® system manual.

Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

Expected Values:
The National Cholesterol Education Program Adult Treatment Panel III (NCEP- ATP III)[6] provides the following categories of triglycerides concentrations:

	Serum Triglycerides	
Category	**mg/dL**	**[mmol/L]**
Normal	< 150	< 1.70
Borderline high	150 – 199	1.70 – 2.25
High	200 – 499	2.26 – 5.64
Very High	≥ 500	≥ 5.65

Each laboratory should establish its own reference interval for triglycerides as performed on the Dimension® system.

REPORTING:
report format: mg/dl

LIMITATIONS:

Results:	>1000 mg/dL [11.3 mmol/L]
Manual dilution:	Make appropriate dilution with purified water to obtain a result within the assay range. Enter the dilution factor. Reassay. Resulting readout is corrected for dilution.

Autodilution (AD) (for serum, plasma): If using the auto-dilution feature, results above 1000 mg/dL [11.3 mmol/L] will automatically be repeated.

Results < 15 mg/dL [0.17 mmol/L] should be reported as less than 15 mg/dL [0.17 mmol/L].

The instrument reporting system contains error messages to warn the user of specific malfunctions. Any report slip containing such error messages should be held for follow-up. Refer to your Dimension® system manual.

Venipuncture should occur prior to N-Acetyl Cysteine or Metamizole (Subpyrine) administration due to the potential for falsely depressed results.

Analytical Specificity

For Known Interfering Substances section refer to package insert.

For Known Non-Interfering Substance refer to package insert.

For Additional Technical Information refer to package insert.

Reference: TGL Flex® reagent cartridge insert sheet PN 717069.101 Issue Date 2/29/2008

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Written By: ____Lori Murray MT(ASCP)_____ Date: _8-8-12

Approved By: __Aaron Shives, MD___ Date: __10/23/2017___
Laboratory Director

REVIEW - REVISION SUMMARY DOCUMENTATION

Date	By	Revision Summary
07/10	Lori Murray	New format
8-8-12	Lori Murray	Changed instrumentation to EXL200
4/23/15	Heather Hall	Update information to package insert dated 2/29/2008
10/18/17	Heather Hall	Updated information to package insert dated 10/20/16