Brown Clinic Northridge

511 14th Ave. NE

Watertown, SD

Brown Clinic
506 First Ave. SE
Watertown, SD

BROWN CLINIC
LABORATORY PROCEDURE MANUAL

PROCEDURE: Lipase

PURPOSE:
The LIPL Flex® reagent cartridge used on the Dimension EXL200 Integrated chemistry system is an *in vitro* diagnostic test intended for the quantitative determination of lipase activity in **serum** and **heparinized plasma**.

SUMMARY:
Pancreatic lipase degrades dietary triglycerides to glycerol and free fatty acids in the presence of bile salts. Lipase measurements are used in the diagnosis of diseases of the pancreas, such as acute pancreatitis and obstruction of the pancreatic duct.[1,2] The LIPL method is an adaption of the colorimetric method described by Neumann et al.[3]

PRINCIPLES OF PROCEDURE:

The LIPL method uses as a substrate 1,2-0-dilauryl-rac-glycero-3-glutaric acid-(6′methylresorufin)ester. Lipase catalyzes the hydrolysis of this substrate in the presence of colipase, bile salt, and $CaCL_2$ at alkaline pH. The hydrolysis produces 1,2-0-dilauryl-rac-glycerol and glutaric acid-6'methylresorufin ester. Glutaric acid-6' methylresorufin ester is an unstable reaction intermediate and breaks down to yield chromogenic free methylresorufin in proportion to the activity of lipase in the sample. The rate of production of methylresorufin is measured by a bichromatic rate reaction at 577 and 700 nm.

1,2-0- dilauryl-rac-glycero-3-glutaric $\longrightarrow$ 1,2-o-dilauryl-rac-glycerol + H_2O

glutaric acid 6'-methylresorufin-ester (not stable) $\longrightarrow$ 1,2-o-dilauryl-rac-glycerol + H_2O

glutaric acid 6'-methylresorufin-ester (not stable) $\longrightarrow$ glutaric acid + methylresorufin (absorbs at 577 nm)

INSTRUMENT, REAGENTS & SUPPLIES:

Wells[a]	Form	Ingredient	Concentration[b]	Source

1,2	Liquid	N,N-bis (2-hydroxyethyl)Glycine	50 mmol/L	
		Colipase	≥1.0mg/L	Porcine
		Na desoxycholate	1.6mmol/L	pancreas
		$CaCL_2$	10mmol/L	
3,4	Liquid	Tatrate buffer	10mmol/L	
		1,2-0-diauryl-rac-glycero-3-	0.27 mmol/L	
		Glutaric acid-(6- methylresorufin)		
		Ester		
		Taurodesoxycholate	8.8mmol/L	
5,6	Liquid	NaOH[c]	1.00mol/L	

a. Wells are numbered consecutively from the wide end of the cartridge.
b. Nominal value per well in a cartridge.
c. Sodium hydroxide is used as a probe cleaning solution and is not used in the reaction.

RISK AND SAFETY:

H290, H6314

P280, P301+P310+P331

P303+P361+P353+P310+P305+P310+P501

Danger

May be corrosive to metals. Causes severe skins burns and eye damage.

Wear protective gloves/protective clothing/eye protection/face protection. IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician. Do Not induce vomiting. IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower. Immediately call a POISON CENTER or doctor/physician. IF IN EYES: Immediately call a POISON CENTER or doctor/physician. Dispose of contents and container in accordance with all local, regional, and national regulations.

Contains: Sodium hydroxide

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

REAGENT PREPARATION: All reagents are liquid and ready to use.

REAGENT STORAGE & STABILITY:

Store at 2 – 8° C. Protect from light after opening.

Expiration: Refer to carton for expiration date of individual unopened reagent cartridges. Sealed wells on the instrument are stable for 30 days. Open well stability: 7 days for wells 1-6.

SPECIMEN COLLECTION AND HANDLING:

Recommended specimen types: Serum and plasma (lithium heparin, sodium heparin).

EDTA, potassium oxalate, sodium fluoride and citrate have been shown to inhibit lipase results and should not be used. [4]

Serum and plasma should be collected using recommended procedures for collection of diagnostic blood specimens by venipuncture.[5,6] Follow the instructions provided with your specimen collection device for use and processing.[7] Complete clot formation should take place before centrifugation. Serum or plasma should be physically separated from cells as soon as possible with a maximum limit of 2 hours from the time of collection.[6] Specimens should be free of particulate matter. Bacterial contamination of the specimen may cause increased lipase values.[8]

Specimen Stability:

Room Temperature: 24 hours
Refrigerated at 2-6°C: 7 days
Frozen at -20°C or colder: up to 12 months

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 LIPL Flex® reagent cartridge, Cat. No. DF56, is required to perform the LIPL test. This test is performed on the Dimension® clinical chemistry system after the method is calibrated (see Reference Material in Calibration section).LIPL Calibrator, Cat. No. DC56.

Test Steps

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

d. The sample container (if not a primary tube) must contain sufficient quantity to accomodate the sample volume plus the dead volume; "precise" container filling is not required.

Test Conditions

- Sample Size 3 µL
- Reagent 1 Volume: 186 µL
- Reagent 2 Volume: 115 µL
- Test Temperature 37°C ±0.1°C
- Reaction Time 5.5 minutes
- Wavelength 577 nm read/700 nm blank
- Type of Measurement Bichromatic Kinetic

Calibration

The general calibration procedure is described in your Dimension® system manual.

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

Assay Range:	10 –1500 U/L
Calibration Material:	LIPL Calibrator (Cat. No. DC56).
Units	U/L
Typical Calibration Levels:	0, 550, 1550 U/L
Calibration Scheme:	Three levels in triplicate
Calibration Frequency:	Every new reagent cartridge lot.
	Every 45 days for any one lot.
Anew calibration is Required:	For each new lot Flex© reagent cartridges.
	After major maintenance or service, if indicted by quality control Results.
	As indicted in laboratory quality control procedures.
	When required by government regulations
Assigned Coefficients:	C_0 0.6103
	C_1 0.0529

e. This is the range of analyte values that can be measured directly from the specimen without any dilution or pretreatment that is not part of the usual analytical process and is equivalent to the assay range.

Backup Process:
Refer to Brown Clinic Back-up Policy

RESULTS:

The instrument calculates and prints the concentration of lipase in U/L using the calculation scheme described in your Dimension® Operator's Guide.

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

LIMITATIONS OF PROCEDURE:

The instrument reporting system contains flags and comments to provide the user with the information regarding instrument processing errors, instrument status information and potential errors in LIPL results. Refer to your Dimension® Operator's Guide for the meaning of report flag and comments. Any report containing flags and/pr comments should be addressed according to your Laboratory's procedure manual and not reported.

EXPECTED VALUES:
100-259 U/L

The reference interval was calculated parametrically and represents the central 95% of the population tested, which consisted of 169 healthy adults. Each laboratory should establish its own reference interval for the lipase method as determined on the Dimension® system.

REPORTING:
report format: U/L

LIMITATIONS:

Results: Samples with results in excess of 1500U/L should be repeated on dilution.

Manual dilution: Make appropriate dilution with reagent grade water to obtain result within assay range. Enter dilution factor on the instrument. Reassay. Resulting readout is corrected for dilution.

Autodilution (AD): (serum/plasma): the recommended autodilute sample volume is 2µL. Refer to your Dimension® system manual.

Samples with results less than 10 U/L should be reported as "less that 10 U/L".

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

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: LIP Flex® reagent cartridge insert sheet PN 717055.101 Issue Date 2003-08 Rev. F

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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
6/4/15	Amy Harms	Updated to package insert information dated 7/24/2013
6/13/16	Amy Harms	Updated risk and safety package information date 1/30/15
10/18/17	Heather Hall	Modified Backup method