

LIPASE FL

LP F060 CH

6 x 10 ml

SUMMARY OF TEST

Human lipase is a glycoprotein with a molecular weight of 48000 and an isoelectric point of about 5.8. For full catalytic activity and greatest specificity, the presence of bile salts and a cofactor, called colipase, is required. Lipases are defined as enzymes that hydrolyze glycerol esters of long-chain fatty acids. Both lipase and colipase are synthesized in the pancreatic acinar cells and secreted by the pancreas in roughly equimolar quantities. Lipase measurements on serum, plasma, and ascitic and pleural fluid are used exclusively to investigate pancreatic disorders, usually pancreatitis. The complete absence of lipase and colipase has been reported. Such congenital absence results in severe steatorrhea. After an attack of acute pancreatitis, the serum lipase activity increases within 4 to 8 h, peaks at about 24 h, and decreases within 8 to 14 days; levels remain elevated longer than those of amylase. Lipase elevations usually parallel those of amylase, but increases in lipase activity may occur sooner or later than increases in amylase activity, and lipase may rise to a greater extent. In acute pancreatitis, normoamylasemia may occur in up to 20% of such patients. Likewise, the existence of hyperlipemia may cause a spurious normoamylasemia. For this reason, it is suggested that the two assays complement and not exclude each other and that both enzymes be assayed. The increase in serum lipase activity is not necessarily proportional to the severity of the attack. Acute pancreatitis is sometimes difficult to diagnose because it must be differentiated from other acute intra-abdominal disorders with similar clinical findings, such as perforated gastric or duodenal ulcer, intestinal obstruction, mesenteric vascular obstruction, and many others. Elevation of serum lipase activity is probably a more specific diagnostic finding in these cases than serum amylase activity, because many of these conditions are less likely to cause increases in lipase activity than in amylase activity. It must be emphasized, however, that these foregoing comments apply to the results obtained with a specific lipase assay. Serum lipase assays may also be of value in the diagnosis of chronic pancreatitis, but severe destruction of the acinar tissue in the later stages of the disease results in a reduction of the amount of enzyme that can enter the circulation and in subnormal serum activities. Marginal or no increase of serum lipase activity is therefore not unusual in this disease. Obstruction of the pancreatic duct by a calculus or by carcinoma of the pancreas may cause an increase in serum lipase activity, depending on the location of the obstruction and the amount of remaining functioning tissue. In acute and chronic renal disease, serum lipase activity is increased, although this elevation is neither as frequent nor as pronounced as that with serum amylase activity. Thus, care should be exercised in the interpretation of elevated serum lipase values in the presence of renal disease. In contrast to amylase, which is present in both the pancreas and the parotid glands, lipase is not present in the parotid gland. Therefore, in mumps (acute parotitis) without pancreatic involvement, serum lipase activity is usually not elevated but serum amylase activity is.

In 1932, Cherry and Crandall first recognized the clinical value of blood lipase measurement in monitoring pancreatic injury. The method bearing their names used a buffered, stabilized 50% emulsion of olive oil as substrate; lipase activity was defined by the amount of free fatty acids released in a 24-h incubation at 37°C and estimated by titration to neutrality with dilute alkali. Since 1932, many lipase methods have been described; they have used both triglyceride and nontriglyceride substrates in titrimetric, turbidimetric, spectrophotometric, fluorometric, and immunological techniques. In the present method, the new colorimetric substrate 1,2-O-Dilauryl-rac-glycero-3-glutaric acid-(6'-methyl-resorufin)-ester is used. The method is rapid and very sensitive, highly specific for pancreatic lipase and compares well with turbidimetric method, at the same time avoiding some of its drawbacks.

PRINCIPLE OF THE METHOD

The colorimetric substrate 1,2-O-Dilauryl-rac-glycero-3-glutaric acid-(6'-methyl-resorufin)-ester is cleaved by pancreatic lipase and the resulting dicarboxylic acid ester is hydrolysed under the alkaline test conditions to yield the chromophore methylresorufine. The kinetic of colour formation at 580 nm is monitored and it is proportional to lipase activity in sample.

KIT COMPONENTS

For in vitro diagnostic use only.

The components of the kit are stable until expiration date on the label.
Keep away from direct light sources.

Reagent A 5 x 10 ml (liquid) blue cap

Composition: Tris buffer 40 mM pH 8.30, colipase ≥ 1 mg/l, desoxycholate ≥ 1.8 mM, taurodesoxycholate ≥ 7.0 mM.

Reagent B 1 x 10 ml (liquid) red cap

Composition: Tartrate buffer 15 mM pH 4.00, lipase substrate ≥ 0.70 mM, calcium ions ≥ 1 mM.

Calibrator: lyophilized (value on label) - 3 ml

Store all components at 2-8°C.

MATERIALS REQUIRED BUT NOT SUPPLIED

Current laboratory instrumentation. Spectrophotometer UV/VIS with thermostatic cuvette holder. Automatic micropipettes. Glass or high quality polystyrene cuvettes. Saline solution. Calibrator.

REAGENT PREPARATION

Use separate reagents ready to use.

Stability: up to expiration date on labels at 2-8°C;

Stability since first opening of vials: ≥ 90 days at 2-8°C.

Caution: reagent B is a micro-emulsion. Therefore, a slight apparent precipitation could occur, showing a light red deposit on the bottom of vial. It is a normal behaviour and it is recommended to resuspend solution before analysis, with a mild shaking.

PRECAUTIONS

Reagent may contain some non-reactive and preservative components. It is suggested to handle carefully it, avoiding contact with skin and swallow.

Some commercial reagents for triglycerides determination could contain microbial lipases, whose could stick on surface of instrument plastic cuvettes. It is recommended to program a "wash" procedure before lipase determination, if a contamination is suspected.

Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

SPECIMEN

Serum, plasma heparinate.

Samples are stable 7 days at 2-8°C.

TEST PROCEDURE

Wavelength:	580 nm (allowed 570 ÷ 590 nm)		
Lightpath:	1 cm		
Temperature:	37°C		
dispense:	blank	calibrator	sample
reagent A	1 ml	1 ml	1 ml
reagent B	200 µl	200 µl	200 µl
water	10 µl	-	-
calibrator	-	10 µl	-
sample	-	-	10 µl

Mix, execute a first reading of absorbance after 1 minute, incubating at 37°C. Perform other 2 readings at 60 seconds intervals. Calculate the $\Delta A/\text{min}$.

RESULTS CALCULATION

$$\Delta A/\text{min}_{(\text{calibrator-net})} = \Delta A/\text{min}_{(\text{calibrator})} - \Delta A/\text{min}_{(\text{blank})}$$

$$\Delta A/\text{min}_{(\text{sample-net})} = \Delta A/\text{min}_{(\text{sample})} - \Delta A/\text{min}_{(\text{blank})}$$

serum/plasma sample:

$$U/l \text{ (methylresorufine } 37^\circ\text{C)} = \frac{\Delta A/\text{min}_{(\text{sample-net})}}{\Delta A/\text{min}_{(\text{calibrator-net})}} \times \text{Calibrator value}$$

EXPECTED VALUES

normal subjects: ≤ 63 U/l (methylresorufine 37°C)

Each laboratory should establish appropriate reference intervals related to its population.

QUALITY CONTROL AND CALIBRATION

It is suggested to perform an internal quality control. For this purpose the following human based control sera are available:

QN 0050 CH QUANTINORM CHEMA 10 x 5 ml
with normal or close to normal control values

QP 0050 CH QUANTIPATH CHEMA 10 x 5 ml
with pathological control values.

If required, a multiparametric, human based calibrator is available:

AT 0030 CH AUTOCAL H 10 x 3 ml

Please contact Customer Care for further information.

TEST PERFORMANCE

Linearity

the method is linear up to 250 U/l.

If the limit value is exceeded, it is suggested to dilute sample 1+9 with distilled water and to repeat the test, multiplying the result by 10.

Sensitivity/limit of detection (LOD)

the limit of detection is 5 U/l.

Interferences

no interference was observed by the presence of:

hemoglobin ≤ 150 mg/dl

bilirubin ≤ 20 mg/dl

lipids ≤ 300 mg/dl

(lipids at concentration more elevated than 300 mg/dl give a -6% negative interference)

Precision

intra-assay (n=20)	mean (U/l)	SD (U/l)	CV%
sample 1	11.80	2.63	22.27
sample 2	119.20	4.14	3.47
sample 3	215.35	6.11	2.84

inter-assay	mean (U/l)	SD (U/l)	CV%
sample 1	11.65	2.80	24.06
sample 2	119.55	6.82	5.71
sample 3	215.03	12.33	5.73

Methods comparison

a comparison between Chema and a commercially available product gave the following results:

Lipase Chema = y
Lipase competitor = x
n = 101

$$y = 0.50054x + 3.9443 \quad r = 0.997$$

WASTE DISPOSAL

This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.

S57: use appropriate container to avoid environmental contamination.

S61: avoid release in environment. Refer to special instructions/safety data sheets.

REFERENCES

Kaplan, L.A., Pesce, A.J.: "Clinical Chemistry", Mosby Ed. (1996).








Jakobs, D.S., Kasten, Jr., BL., Demmott, W.R., Wolfson, W.L.: "Laboratory Test Handbook", Lexi-Comp and Williams & Wilkins Ed. (2nd Edition - 1990).

Neumann, U. et al.: "New substrates for the optical determination of lipase". EP 207252 (1987).

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SYMBOLS

	for in vitro diagnostic use only
	lot of manufacturing
	code number
	storage at temperature interval
	expiration date (year/month)
	warning, read enclosed documents
	read the directions