ALTA CALIDAD EN PRUEBAS PARA SU LABORATORIO

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LIPASE REAGENT SET

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Lipase reagent is used for the quantitative turbidimetric determination of pancreatic lipase in human serum.

INTRODUCTION

Lipase is defined as that group of enzymes, which hydrolyze the glycerol esters of long-chain fatty acids. The measurement of lipase activity in serum and other fluid is to evaluate conditions associated with pancreas.[1] Voget et al. proposed an olive oil emulsion in measuring the rate of change in turbidity over a specific unit of time.[2] Later, Shihabi et al. modified the previous method and eliminated some of interference.[3] Our method is based on the above modifications.

PRINCIPLE

Triglyceride + H_20 $\longrightarrow$ mono. + di-glycerides + fatty acids

Serum lipase hydrolyzes the olive oil emulsion. The decrease in turbidity at 400 nm (after incubation) is proportional to lipase activity in the specimen.

REAGENT (MATERIALS PROVIDED)

1. Substrate: 0.8% (w/v) olive oil in alcohol.
2. Buffer: Tris Buffer 69 mM, Sodium Deoxycholate 10 mM, pH 9.0 (37°C).

WARNINGS AND PRECAUTIONS

1. This reagent is for “in vitro” diagnostic use only.
2. Exercise the normal precautions required for the handling of all laboratory reagents. Pipetting by mouth is not recommended for any laboratory reagent.

REAGENT PREPARATION

1. Add lipase buffer to a 50 ml Erlenmeyer flask. Add 25 m1 distilled water and swirl to dissolve.
2. Pipette 1 ml of well-mixed lipase substrate into buffer solution. Note: The absorbance of the emulsion prior to use must be greater than 1.0. Due to variations in regional temperatures, the absorbance may be less than 1.0. If this occurs, add 0.5 – 1.0 ml more substrate until absorbance is greater than 1.0.

REAGENT STORAGE

1. Unreconstituted reagent should be stored at room temperature (25 - 30°C).
2. Reconstituted reagent is stable for seven days refrigerated (2 - 8°C) and tightly capped.

REAGENT DETERIORATION

Do not use the emulsion if the absorbance is more than 0.3 below the absorbance of the emulsion when fresh. Prepare a fresh emulsion.

SPECIMEN COLLECTION STORAGE

1. Use fresh serum specimens.
2. Lipase activity in serum is stable at room temperature for one week; sera may be stored for three weeks in the refrigerator (4-8°C) and for several months if frozen. Bacterial contamination of the specimens may result in an increase in lipase activity.[4]

INTERFERING SUBSTANCES

1. Hemolyzed specimens should not be used.
2. A number of drugs and substances affect lipase activity. For a comprehensive list, see Young, et al.[5]

MATERIALS REQUIRED BUT NOT PROVIDED

1. Accurate pipetting devices (3.0 ml and 0.1 ml).
2. 25 ml measuring vessel.
3. 50 ml Erlenmeyer flask.
4. Timer.
5. Test tubes/rack.
6. Spectrophotometer with temperature controlled cuvette.
7. Heating bath (37°C).
8. Serum controls

PROCEDURE (MANUAL)

1. Reconstitute lipase reagent according to instructions.
2. Label test tubes "blank", "control", "patient", etc.
3. Pipette 3.0 ml of reagent into appropriate test tubes and pre-warm at 37°C for at least five (5) minutes.
4. Zero spectrophotometer with distilled water at 400 nm. (Wavelength range: 390-420 nm).
5. Read and record absorbance of blank and place back in heating bath.
6. Using timed intervals, add 0.1ml (l00 ul) of sample to each tube, mix, and read initial absorbance. Return each tube to heating bath after initial reading.
7. Exactly five (5) minutes after the initial absorbance reading, using the same timed intervals, remove each tube from the heating bath and mix each tube. Read the absorbance of the blank and each sample tube against distilled water.

PROCEDURE NOTES

1. If the Abs. of the "blank" is a negative value, consider it zero.
2. Elevated blank rates i.e. (0.005) and above may be caused by olive oil coating on cuvette surface. Periodically rinse with acetone followed by water flush.
3. Turbid samples should be diluted with distilled water (1:5). Multiply final answer by dilution factor.
4. Use fresh sera, when possible, for greatest accuracy.

CALIBRATION

The lipase activity in the sample is calculated based on the millimolar absorptivity of olive oil (3.15 in working solution).

QUALITY CONTROL

1. Fresh control sera with known normal and abnormal values should be run for each assay to monitor the validity of the reaction.
2. Serum lipase activity should be validity by titrimetric method.

CALCULATIONS

The enzyme activity is expressed in International Units. One International Unit (IU) is defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute under decreed conditions.

$$\text{Units/Liter} = \frac{\text{Corrected } \Delta\text{Abs./5min}}{\text{Initial blank absorbance}} \times 1953$$

Corrected ΔAbs. /5min = ΔAbs test- ΔAbs blank

Example:

(blank) Initial Abs.	= 0.970
(blank) 5 min. Abs.	= 0.967 (if negative result, treat as zero)
ΔAbs blank	= 0.970 – 0.967 = 0.003
(test) Initial Abs.	= 1.300
(test) 5 min. Abs.	= 1.271
ΔAbs test	= 1.300 – 1.271 = 0.029
Corrected ΔAbs./5 min	= 0.029 – 0.003 = 0.026

Therefore: $\frac{0.026}{0.970} \times 1953 = 52$ IU/L

DERIVATION OF FACTOR (1953)

$$\frac{\text{Corrected } \Delta\text{Abs./5min} \times 315 \times 3.1}{\text{Initial Abs/blank} \times 0.1} = \frac{315 \times 3.1}{0.1} = \frac{9765}{\Delta\text{Abs/min} \times 5} = 1953$$

315 = concentration of olive oil (micromoles/liter) in the working solution.
3.1 = volume of reaction mixture.
0.1 = volume of sample in ml.
(ΔAbs./min) × 5 = conversion to ΔAbs./min

Note:

To convert IU/L to Cherry-Crandall units, divide IU/L by 70.

Example:

$$\frac{52 \text{ IU/L}}{70} = 0.74 \text{ Cherry-Crandall units}$$

LIMITATIONS

1. Samples with values above 280 IU/L should be diluted 1:1 with distilled water, re-assayed and final results multiplied by two.
2. Bacterial contamination of the specimens may result in an increase in lipase activity.[4]

EXPECTED VALUES[4]

Adults: 10-150 U/L (age more than 60 years old 18-180 IU/L)

It is strongly recommended that each laboratory establish its own normal range.

PERFORMANCE

1. Linearity: 280 IU/L
2. Sensitivity: Based on an instrument resolution of A = 0.001, this procedure has a sensitivity of 5.4 IU/L.
3. Comparison: Studies done manually between this procedure and a similar procedure yielded a correlation coefficient of 0.98 with a regression equation of Y = 0.94X + 9.13 (N = 59).
4. Precision:

Within Run

Mean (mg/dl)	S.D.	C.V.(%)
36	3.9	10.9
380	35	9.3

Run-to-Run

Mean (mg/dl)	S.D.	C.V.(%)
37	5.4	14
298	40	13

REFERENCES

1. Tietz, N.W., *Fundamentals of Clinical Chemistry*, W.B. Saunders, Philadelphia. p.633 (1976).
2. Voget, W.C. et al. , *Clin. Chem*. 9:168 (1963).
3. Shihabi, Z.K. et al., *Clin. Chem*. 17:11556 (1971).
4. Tietz, N.W., *Text Book in Clinical Chemistry*, W.B. Saunders, Philadelphia, p 735 (1986).
5. Young D.S. et al., *Clin. Chem*. 21:1D (1975).