

Intended Use

For the quantitative determination of Total Cholesterol in serum.

Method History

A Cholesterol method developed in the late 1800's by Lieberman¹ and Burchard² is still in use today despite its corrosive nature and its susceptibility to many interfering substances.

Work on an enzymatic procedure was begun by Flegg³ and Richmond⁴ in the early 70's. Allain⁵ and Roeschlau⁶ began using cholesterol esterase and oxidase, in a single reagent to determine total cholesterol in serum.

Trinder's⁷ color system of peroxidase/phenol/4-aminoantipyrine has been used successfully for some time now. The system's only drawback was the corrosive properties of phenol. The present method utilizes a phenol substitute that performs like phenol but without being corrosive.

Principle

Cholesterol Esters $\xrightarrow{\text{C. Esterase}}$ Cholesterol + Fatty Acids

Cholesterol + O₂ $\xrightarrow{\text{C. Oxidase}}$ Cholesterol-3-one + H₂O₂

2H₂O₂ + 4-Aminoantipyrine + p-HBS $\xrightarrow{\text{Peroxidase}}$ Quinoneimine + 2 H₂O
(red dye)

The intensity of the red color produced is directly proportional to the total cholesterol in the sample when read at 520nm.

Reagents

Concentrations refer to reconstituted reagent.

4-Aminoantipyrine 0.6mM, Sodium Cholate 8.0mM, Cholesterol Esterase ≥ 150 u/L, Cholesterol Oxidase ≥ 200 u/L, Horseradish Peroxidase 1000u/L, p-Hydroxybenzene Sulfonate 20mM, Surfactant, Buffer, pH 7.2, non-reactive stabilizers and fillers with Sodium Azide (.01%) as a preservative.

Reagent Preparation

Reconstitute powdered reagent with the volume of distilled water stated on the vial label. Swirl to dissolve.

Reagent Storage

1. Store reagent at 2-8°C.
2. Reconstituted reagent is stable for sixty days when stored in an amber bottle at 2-8°C.

Reagent Deterioration

Do not use if:

1. Moisture has penetrated the vial and caking has occurred.
2. The working reagent does not meet stated performance parameters.

Precautions

1. This reagent is for *in vitro* diagnostic use only.
2. Reagent contains sodium azide. Poison. Do not ingest. May react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with a large volume of water to prevent azide build up.

Specimen Collection and Storage

Nonhemolyzed serum is recommended. Cholesterol in serum is reported stable for seven days at room temperature (18-25°C) and six months when frozen and properly protected against evaporation.^{8,9}

Interferences

A number of drugs and substances affect concentrations of cholesterol. See Young, et al.¹⁰

Materials Provided

Cholesterol Reagent.

Materials Required but not Provided

1. Accurate pipetting devices.
2. Timer/Test tubes/rack
3. Spectrophotometer with ability to read at 520 nm.
4. Heating Block (37°C).

Procedure (Automated)

Refer to specific instrument application instructions.

Procedure (Manual)

1. Reconstitute reagent according to instructions.
2. Label test tubes: "Blank", "Standard", "Control", "Patient", etc.
3. Pipette 1.0 ml of reagent into each tube and pre-warm at 37°C for at least five minutes.
4. Add 0.01 ml (10ul) of sample to respective tubes. Mix and return to 37°C.
5. Incubate all tubes at 37°C for five minutes.
6. Zero spectrophotometer with reagent blank at 520nm.
7. Read and record absorbances of all test tubes.

Procedure Notes

1. If the spectrophotometer being used requires a final volume greater than 1.0ml for accurate reading, use 0.025ml (25ul) of sample to 3.0ml of reagent. Perform the test as described above.
2. Grossly lipemic serums require a "sample blank". Add 0.01ml (10ul) of sample to 1.0ml saline, mix and read the absorbance against water. Subtract this value from the patient absorbance to obtain the corrected reading.

Limitations

Samples with values exceeding 500 mg/dl should be diluted 1:1 with saline and re-run. The final answer should be multiplied by two.

Calibration

Aqueous standards can be used to calibrate the procedure or an appropriate serum calibrator.

Calculation

Abs. = Absorbance

$$\frac{\text{Abs. (Patient)}}{\text{Abs. (Standard)}} \times \text{Concentration of Std. (mg/dl)} = \text{Cholesterol (mg/dl)}$$

Cholesterol Reagent Set

Example: Abs. (Patient) = 0.40, Abs. (Standard) = 0.32, Concentration of Standard = 200 mg/dl.

$$\frac{0.40}{0.32} \times 200 = 250 \text{ mg/dl}$$

Quality Control

Serum controls with known normal and elevated values should be run routinely to monitor the validity of the reaction.

Expected Values¹¹

A Recommended Range of the NCEP is:

Desirable Cholesterol:	<200mg/dl
Borderline-High Cholesterol:	200-239mg/dl
High Cholesterol:	>240mg/dl

Performance

1. Linearity: 500 mg/dl
2. Comparison: A comparison between this procedure and one utilizing phenol produced a regression equation of $y = 0.99x + 4.0$ with a correlation coefficient of 0.998.
3. Precision:

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Within Run			Run to Run		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
153	1.0	0.7	151	1.6	1.1
410	2.8	0.7	410	4.1	1.0

4. Specificity: Cholesterol Oxidase is not totally specific for cholesterol. Other analogs of cholesterol. Other analogs of cholesterol (dihydrocholesterol, 7-dehydrocholesterol, 20-hydroxycholesterol, etc.) are also oxidized. These analogs do not normally occur in any appreciable amounts in serum.

References

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