POINTE SCIENTIFIC, INC.
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Cholesterol (Liquid) Reagent Set

Intended Use
For the quantitative determination of Total Cholesterol in serum.

Method History
A Cholesterol method developed in the late 1800's by Lieberman[1] and Burchard[2] is still in use today despite its corrosive nature and its susceptibility to many interfering substances.
Work on an enzymatic procedure was begun by Flegg[3] and Richmond[4] in the early 70's. Allain[5] and Roeschlau[6] began using cholesterol esterase and oxidase, in a single reagent to determine total cholesterol in serum.
Trinder's[7] color system of peroxidase/phenol/4-aminoantipyrine has been used successfully for some time now. With appropriate calibrator value assignment, this method has been shown to provide excellent accuracy in relation to the reference methodology.

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

$$\text{Cholesterol} + O_2 \xrightarrow{\text{C. Oxidase}} \text{Cholesterol-3-one} + H_2O_2$$

$$2H_2O_2 + \text{4-AAP} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine (red dye)} + 4\,H_2O$$

The intensity of the red color produced is directly proportional to the total cholesterol in the sample when read at 500nm.

Reagents
4-Aminoantipyrine 0.25mM, Cholesterol Esterase >150u/L, Cholesterol Oxidase >150u/L, Peroxidase >1500u/L, Phenol >15mM, Phosphate Buffer, pH 6.8, non-reactive stabilizers and preservatives.

Reagent Preparation
The reagent is ready to use.

Reagent Storage
1. Store reagent at 2-8°C.
2. The reagent is stable until the expiration date when stored at 2-8°C.

Reagent Deterioration
Do not use if:
1. The reagent is turbid.
2. The reagent does not meet stated performance parameters.

Precautions
1. This reagent is for *in vitro* diagnostic use only.
2. Not to be used internally in humans or animals. Normal precautions for handling laboratory reagents should be followed.
3. Additional safety information concerning storage and handling of this product is in the Material Safety Data Sheet for this product.

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.
3. Test tubes/rack
4. Spectrophotometer with ability to read at 500 nm.
5. Heating Block (37°C).

Procedure (Automated)
Refer to specific instrument application instructions.

Procedure (Manual)
1. Label test tubes: "Blank", "Standard", "Control", "Patient", etc.
2. Pipette 1.0 ml of reagent into each tube and pre-warm at 37°C for at least five minutes.
3. Add 0.01 ml (10ul) of sample to respective tubes. Mix and return to 37°C.
4. Incubate all tubes at 37°C for five minutes.
5. Zero spectrophotometer with blank at 500nm.
6. 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 700 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. The procedure should be calibrated according to the instrument manufacturer's instructions. If control results are found to be out of range, the procedure should be re-calibrated.

Calculation
Abs. = Absorbance

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

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. These controls should be run at least with every working shift in which Cholesterol assays are performed. It is recommended that each laboratory establish their own frequency of control determination.

Expected Values[11]

Recommended Range:

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

Performance

1. Linearity: 700 mg/dl
2. Comparison: A comparison between this procedure and a similar dry powder reagent produced a regression equation of y = 1.02x + 0.8 with a correlation coefficient of 0.999.
3. Precision:

Within Run			Run to Run		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
157	1.1	0.7	155	1.3	0.8
224	1.7	0.8	227	2.4	1.1

4. Specificity: Cholesterol oxidase is not totally specific for 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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