Bialex®

CHOLESTEROL ENZYMATIC OXIDASE

CLINICAL SIGNIFICANCE

Physiological Distribution and Specificity. Cholesterol is a fundamental lipid found in all body tissues, serving as a structural component of cell membranes and a precursor for steroid hormones and bile acids. It is transported in the blood by lipoproteins (HDL, LDL, and VLDL). In individuals, high levels of circulating cholesterol—specifically LDL—are recognized as a primary contributory factor in the formation of atherosclerotic plaques within the arterial walls

Diagnostic Utility. While the determination of total cholesterol has limited diagnostic utility when used in isolation, it is a critical parameter in assessing cardiovascular risk. The prevalence of atherosclerotic complications is significantly higher in individuals with elevated total cholesterol levels. Monitoring these levels is essential for the diagnosis and management of hyperlipidemias and for evaluating the effectiveness of dietary or pharmacological treatments

METHOD PRINCIPLE

The Bialex cholesterol determination is based on a specific enzymatic colorimetric method (CHOD-PAP). The process involves the following synchronized reactions: Trace amounts of **Lipoprotein Lipase (LPL)** act as a clearing agent, hydrolyzing the triglycerides in VLDL and chylomicrons. This "opens" the lipoprotein structure and reduces initial sample turbidity, ensuring the enzymes have full access to the cholesterol core Cholesterol esters are hydrolyzed by **Cholesterol Esterase (CHE)** to release free cholesterol and fatty acids. The free cholesterol is then oxidized by **Cholesterol Oxidase (CHOD)**. This reaction produces Cholest-4-en-3-one and **Hydrogen Peroxide (H_2O_2)**. In the presence of **Peroxidase (POD)**, the hydrogen peroxide reacts with 4-Aminoantipyrine (4-AAP) and Phenol (HBA) to form a red **Quinoneimine** dye.

Reagents Composition

Name	Qty	Characteristics
CHE	1000 U/L	pH 7.2-7.7
Cholesterol Oxidase	400 U/L	pH 6.5-7.2
4-Aminoantipyrine	0.25 mMol	NRD
Lipoprotein Lipase (LPL)	300 U/L	NRD
Peroxidase	1000 U/L	NRD
Buffer/Surfactant System	NRD	ph 6.7
Preservant	0.01%	NRD
Cholesterol Standard	200 mg/dL	ACS

Reagents Provided: Bottles reagents and Vial of standard **(Ref: GQ-CC-100, GQ-CC-125, GQ-CC-2X125,GQ-CC-250, GQ-CC-4X125)**

Materials Required but not provided

- Clinical Chemistry Analyzer (Full, Semi-automated) with 37°C temperature control and filter of 500 to 520nm
- Deionized water and Class A glassware.
- Automatic Pipettes
- Assayed Human Control serum (Optional, for validation)
- PassCheck Calibrator (GQ-CAL-5)

Sample Collection	Interference	Storage and Stability of Reagent	ASSAY PRECAUTIONS
Serum: Use standard collection tubes. **Plasma:** Use only anticoagulants that do not interfere with the enzymatic reaction (e.g., Heparin or EDTA). **Pre-analytical Handling** • **Hemolysis: DO NOT USE hemolyzed samples.** Hemoglobin can interfere with the colorimetric reading at 500 nm, leading to false results. • **Centrifugation:** Samples containing visible precipitates or turbidity must be centrifuged before testing to ensure a clear supernatant. • **Patient Condition:** It is recommended that the patient fasts for 12 hours prior to sample collection for a complete lipid profile. • Refrigerated Samples up 7 to days • Freezer Samples up to 6 Months	The performance of this enzymatic method is highly robust against common endogenous substances. Thanks to the integrated **Clearing System (LPL + specialized surfactant)**, the reagent effectively minimizes interference from turbidity and lipids. Lipemia, no significant interference up to **1020 mg/dL**. The trace amounts of LPL ensure sample clearing during the initial incubation phase. **Hemoglobin:** No significant interference up to **400 mg/dL**. (However, grossly hemolyzed samples are still not recommended) **Bilirubin (Total):** No significant interference up to **4.5 mg/dL**	• Store at **2-8°C** protected from light. • The reagent is stable until the expiration date indicated if uncontaminated. • **On-Board Stability: 30 days**, provided the reagents are kept in the refrigerated compartment (2 to 8°C) • **Signs of Deterioration** • The reagent should be clear and slightly yellow or pinkish. Discard the reagent if: • The **Reagent Blank absorbance** (at 500 nm) is greater than **0.150** against distilled water. • The solution shows visible **turbidity** or signs of microbial growth (particles or cloudiness). • Control serum values fall outside the established ranges.	1. For *in vitro* diagnostic use only. 2. Reagent contains Sodium Azide (0.09%) as a preservative. 3. **Calibration Failure:** Inability to recover the expected values for commercial control serums.

Instrument Assay Procedure

Semi Automatic	Automatic
• **Method:** End Point • **Calibration Type:** One Point Linear • **Calibration Point 1:** 1 • **Calibration 1 Conc:** 200 • **Wavelength:** 505 nm. • **Temperature:** 37°C. • **Blank Setting:** Reagent • **Reagent Volume:** 500 µL • **Sample Volume:** 5 µL. • **Lag Time:** 5 seconds. • **Read Time:** 5 seconds • Range: 10 -100 • Linear: 1-600 • Run reagent and samples 5 minutes at 37°C and read on the instrument	• **Reaction Type:** Increasing • **Calibration Mode:** Linear / Calibrator • **Wavelength:** 505 nm. • **Temperature:** 37°C. • **Blank Setting:** Reagent • **Reagent Volume:** 300 µL. • **Sample Volume:** 3 µL. • **Read Time:** 5 minutes • **Reagent Onbord:** 4 Weeks - Refrigerated

OTHER REFERENCES

Performance	Reference Values	Common errors	BIBLIOGRAPHY
• **Limit of Detection:** 3 mg/dL (Instrument dependent). • **Linearity:** Up to 1000 U/L (Dilute 1:5 for higher samples than 1000U/L). • **Precision:** Intra-assay C.V. < 0.99%. • Correlation coefficient (r): 0.998 • Regretion equation: y = 0.995x + 1.2	**Serum or Plasma:** <200 mg/dL >240 mg/dL (High Risk) It is strongly recommended that each laboratory establishes its own reference range based on its specific population and local clinical requirements.	**Check List:** • **1. High Reagent Blank Absorbance (>0.150 Abs)** • **Cause:** Contamination of the reagent or exposure to direct light/high temperatures for extended periods. • **Solution:** Check the storage temperature (2°C-8°C). Ensure the reagent bottle is always capped. If the blank remains high, discard and use a new vial. • **Cuvettes Opaque or dirty :** Residues from other reagents containing detergents will inhibit coenzyme activity. • **Abnormally Low Results** • **Cause (Interference):** Presence of high levels of **Ascorbic Acid (Vitamin C)** in the sample, which consumes the hydrogen peroxide before the color reaction. • **Cause (Calibration):** Failure to use a serum-based **Calibrator**. Aqueous standards may not react properly with the LPL/surfactant clearing system. • **Cause (Sample):** Grossly icteric or hemolyzed samples. • **Sample Blank Issues (Turbidity)**	1. **Allain, C.C., Poon, L.S., Chan, C.S., Richmond, W., and Fu, P.C.** (1974). Enzymatic determination of total serum cholesterol. *Clinical Chemistry*, 20(4), 470–475. (Referencia fundamental del método enzimático). 2. **Roeschlau, P., Bernt, E., and Gruber, W.** (1974). Enzymatic determination of total cholesterol in serum. *Zeitschrift für Klinische Chemie und Klinische Biochemie*, 12(5), 226. (Fundamento CHOD-PAP). 3. **National Cholesterol Education Program (NCEP).** Third Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *NIH Publication No. 02-5215*, 2002. (Valores de Referencia). 4. **Young, D.S.** (2000). *Effects of Drugs on Clinical Laboratory Tests*. 5th Edition. AACC Press. (Referencia para interferencias medicamentosas). 5. **Tietz, N.W.** (2006). *Clinical Guide to Laboratory Tests*. 4th Edition. W.B. Saunders Co., Philadelphia, PA. (Guía general de laboratorio clínico).

		• **Cause:** In manual techniques, reading the absorbance too early (before 5 minutes at 37°C). • **Solution:** Allow the full incubation time for the **LPL and Surfactant** to completely clear the triglyceride-rich particles.	

QUALITY CONTROL

In-House Performance Validation:

The performance of Enzymatic Cholesterol reagent has been validated internally using an automated analyzer and certified commercial control materials. The following data represent the results of our internal quality control (QC) assessment. **On-Board Stability and Method Comparison**

A study was conducted to evaluate the reagent's consistency during routine laboratory operation. A total of **50 clinical samples** were

System Validation and Calibration:

Frequency:
Recalibrate the system under the following conditions:

- Whenever a new **lot of reagent** is used.
- When **Quality Control** results fall outside the acceptable limits.
- After significant **maintenance** or replacement of critical parts in the analyzer (e.g., lamp or probe).

Intra-Laboratory Quality Program:

To ensure the long-term reliability of results, each laboratory must implement an internal quality control program. This program should include:

- **Daily Monitoring:** Analysis of at least two levels of control materials (Normal and Pathological) every 24 hours or with each batch of samples.
- **Trend Analysis:** Results should be plotted on **Levey-**

analyzed over a continuous period.

- **Observed Variation:** The assay demonstrated a cumulative variation of **±1%** across the clinical range.
- **Correlation:** Despite the observed deviation, the method maintains a strong correlation with the initial calibration values and clinical expectations.
- **Conclusion:** The reagent remains functional and reliable for routine use. However, for maximum clinical precision, **daily calibration** or the use of daily control serums is recommended to compensate for the ± 2% operational drift.

Jennings charts to monitor the precision of the assay. The application of **Westgard Rules** is recommended to identify systematic errors (shifts) or random errors (imprecision).

- **Reagent Blank Tracking:** The initial absorbance of the Reagent Blank should be recorded daily. A sudden drop in absorbance indicates reagent degradation or light exposure.
- **Acceptability Criteria:** The laboratory should define its own "Total Allowable Error" (TEa) based on clinical requirements. If results exceed the established standard deviation (SD), a full system review (calibration, temperature, and flow cell cleanliness) must be performed.

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