

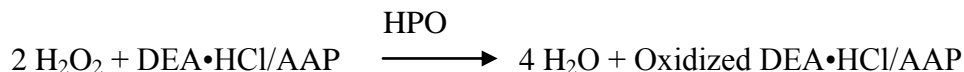
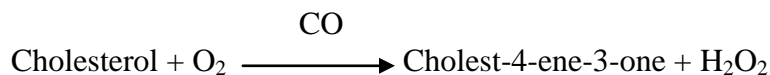
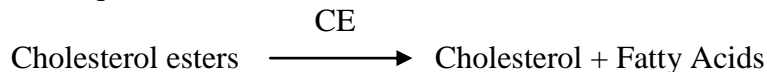
**BROWN CLINIC
LABORATORY PROCEDURE MANUAL****PROCEDURE:** Cholesterol**PURPOSE:**

The CHOL method used on the Dimension EXL200 Integrated chemistry system is an *in vitro* diagnostic test intended for the quantitative determination of total cholesterol in **serum** and **plasma**.

PRINCIPLE:

The CHOL method is based on the principle first described by Stadtman¹ and later adapted by other workers,^{2,3} including Rautela and Liedtke.⁴ Lipids and lipoproteins in circulation have been strongly associated with coronary heart disease (CHD), associated lipid metabolism disorders, and atherosclerosis, a cause of CHD.⁵

Cholesterol esterase (CE) catalyzes the hydrolysis of cholesterol esters to produce free cholesterol which, along with preexisting free cholesterol, is oxidized in a reaction catalyzed by cholesterol oxidase (CO) to form cholest-4-ene-3-one and hydrogen peroxide. In the presence of horseradish peroxidase (HPO), the hydrogen peroxide thus formed is used to oxidize N,N-diethylaniline-HCl/4-aminoantipyrine (DEA-HCl/AAP) to produce a chromophore that absorbs at 540 nm. The absorbance due to oxidized DEA-HCl/AAP is directly proportional to the total cholesterol concentration and is measured using a polychromatic (452, 540, 700 nm) endpoint technique.



INSTRUMENT, REAGENTS & SUPPLIES:

Reagents

Wells ^a	Form	Ingredient	Concentration ^b	Source
1–3	Tablet ^c	CE	0.7 U/mL	Fungal
		CO	0.1 U/mL	Microbial
		HPO	2.4 U/mL	Plant
1–3	Tablet ^c	AAP Buffer Cholate	4.5 µmol	
4–6	Liquid	DEA Surfactant	5.8 µmol	

a. Wells are numbered consecutively from the wide end of the cartridge.

b. Nominal value per test at manufacture.

c. Tablet contains excipients.

Risk and Safety: H412

H412, P273, P501

Harmful to aquatic life with long lasting effects.

Avoid release to the environment. Dispose of contents and container in accordance with local, regional, and national regulations.

Contains: Polyethylene glycol octaphenyl ether

Safety data sheets (MSDS/SDS) available on www.siemens.com/healthcare

Precautions: Used cuvettes contain human body fluids; handle with appropriate care to avoid skin contact and ingestion.⁵

For *in vitro* diagnostic use

Reagent Preparation: Hydrating, Mixing and diluting are automatically performed by the instrument

Expiration: Refer to carton for expiration date of individual unopened reagent cartridges. Sealed or unhydrated cartridge wells on the instrument are stable for 30 days. Once wells have been entered by the instrument, they are stable for 5 days.

REAGENT STORAGE & STABILITY:

Store at 2 – 8 °C.

SPECIMEN COLLECTION AND HANDLING.

Normal procedures for collecting and storing serum and plasma may be used for samples to be analyzed by this method.⁵

Serum and Plasma can be collected using recommended procedures for collection of diagnostic blood specimens by venipuncture.⁶

Complete clot formation should take place before centrifugation.⁷

Follow the instructions provided with your specimen collection device for use and processing.⁸

Materials required but not provided:
CHOL calibrator, Cat. No. DC16
Quality control materials.

Test Steps

Sampling, reagent delivery, mixing, processing, and printing of results are automatically performed by the Dimension® system. For details of this processing, refer to your Dimension® system manual.

The sample container (if not a primary tube) must contain sufficient quantity to accommodate the sample volume plus dead volume. Precise container filling is not required.

Test Conditions

- Sample Size: 3 µL
- Reagent 1 Volume: 88 µL
- Reagent 2 Volume: 26 µL
- Diluent Volume: 241 µL
- Test Temperature: 37 °C
- Wavelength: 452, 540 and 700 nm
- Type of Measurement: polychromatic endpoint

Backup:

Refer to Brown Clinic Backup Policy

Calibration

The general calibration procedure is described in your Dimension® system manual (see Appendix B also).

The following information should be considered when calibrating the cholesterol method:

Assay Range:	50–600 mg/dL [1.3–15.5 mmol/L]
Calibration Material:	CHOL Calibrator (Cat. No. DC16).
Suggested Calibration Levels:	50, 250, 450 mg/dL [1.3, 6.5, 11.6 mmol/L]
Calibration Scheme:	Three levels in triplicate
Calibration Frequency:	Every new reagent cartridge lot
	Every month for any one lot
	For each new lot of Flex reagent cartridges
	After major maintenance or service, if indicated by quality control results
	As indicated in laboratory quality control procedures
	When required by government regulations

Assigned Coefficients:	C ₀	0.3162
	C ₁	0.7886

INTERPRETATION:

The instrument automatically calculates and prints the concentration of cholesterol in mg/dL [mmol/L] using the calculation scheme illustrated in your Dimension® system manual.

Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

8-8-12	Lori Murray	Changed instrumentation to EXL200
4/24/15	Heather Hall	Update information to package insert dated 3/4/2008
6/13/16	Cassie Kasperek	Update Risk and Safety to package insert dated 5/5/2015
2/21/17	lori murray	backup method clarification
10/17/17	Heather Hall	Updated information to package insert 5-5-2015 Modified Backup method