Brown Clinic Northridge
511 14th Ave. NE
Watertown, SD

Brown Clinic
506 First Ave. SE
Watertown, SD

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 Stadtman[1] 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.

$$\text{Cholesterol esters} \xrightarrow{\text{CE}} \text{Cholesterol + Fatty Acids}$$

$$\text{Cholesterol + } O_2 \xrightarrow{\text{CO}} \text{Cholest-4-ene-3-one + } H_2O_2$$

$$2\ H_2O_2 + \text{DEA•HCl/AAP} \xrightarrow{\text{HPO}} 4\ H_2O + \text{Oxidized DEA•HCl/AAP}$$

INSTRUMENT, REAGENTS & SUPPLIES:

Reagents

Wells [a]	Form	Ingredient	Concentration [b]	Source
1–3	Tablet[c]	CE CO HPO	0.7 U/mL 0.1 U/mL 2.4 U/mL	Fungal Microbial 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 precessing.[8]

Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products. Variations in these products may exist between manufacturers and, at times, from lot to lot.

Specimen Stability:
Specimens are stable for 8 hours at room temperature
2 days at 2-8 °C
For longer storage, specimens may be frozen at -20 °C or colder. Repeated freezing and thawing should be avoided.

CONTROLS:
At least once daily run solutions at two levels of a quality control material with known concentrations.

For further details, refer to your Dimension® system manual. The result obtained should fall within limits defined by the day-to-day variability of the system as measured in the user's laboratory. If the results fall outside the laboratory's acceptable limits, follow the procedure in the quality control policy.

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

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

Analytical measurement range (AMR): 50-600 mg/dL
[1.3-15.5 mmol/L]

This is the range of analyte values that can be directly measured on the specimen without any dilution or pretreatment that is not part of the usual analytical process and is equivalent to the assay range.

-Samples with results in excess of 600 mg/dL [15.5 mmol/L] should be repeated on dilution.

Manual Dilution: Make appropriate dilutions with Reagent grade water to obtain result within assay range
Enter dilution factor. Reassay. Resulting readout is corrected for dilution.

Autodilution (AD): Refer to you Dimension® Operators guide.

CHOL results less than 50 mg/dL [1.3mmol/L] should be reported as "less than 50 mg/dL [1.3 mmol/L]" instead of the numerical value.

PROCEDURE:
Material provided:

CHOL flex® reagent cartridge, Cat No. DF27

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.

EXPECTED VALUES:

<200 mg/dL [5.2 mmol/L]	Desirable
200–239 mg/dL [5.2–6.2 mmol/L]	Borderline High
≥240 mg/dL [6.2 mmol/L]	High

Each laboratory should establish its own reference interval for cholesterol as performed on the Dimension® system.

REPORTING:
report format: mg/dl

LIMITATIONS:

Results:	> 600 mg/dL [15.5 mmol/L]
Manual dilutions:	Make appropriate dilutions with Purified Water to obtain result within the assay range. Enter dilution factor. Reassay. Resulitng readout is corrected for dilution.
Autodilution (AD):	Refer to your Dimension® system literature.

CHOL results less than 50 mg/dL [1.3 mmol/L] should be reported as "less than 50 mg/dL [1.3 mmol/L]" instead of the numerical value.

The instrument reporting system contains error messages to warn the operator of specific malfunctions. Any report slip containing such error messages should be held for follow-up. Refer to your Dimension® system manual.

Certification
This method has been evaluated by and met the certification acceptance criteria of the Cholesterol Reference Method Laboratory Network (CRMLN).

Analytical Specificity

For Known Interfering Substances section refer to package insert.

For Known Non-Interfering Substance refer to package insert.

For Additional Technical Information refer to package insert.

Reference: CHOL Flex® reagent cartridge insert sheet PN 717027.001 Issue Date 5/5/2015

Origination Date: 9-10-07
Date of Implementation: 11-10-10
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Written By: ____Lori Murray MT(ASCP)_____ Date: _8-8-12

Approved By: _____Aaron Shives, MD_____ Date: ___10/21/2017_____
Laboratory Director

REVIEW - REVISION SUMMARY DOCUMENTATION

Date	By	Revision Summary
07/10	Lori Murray	New format

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 Kasparek	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