A Complete 3 Minute Whole Blood Cholesterol Test

FOR PROFESSIONAL USE ONLY

INTENDED USE

This test can be used to identify elevated blood cholesterol levels associated with increased risk of coronary artery disease.

The CHEMCARD™ Cholesterol Test provides a preliminary semi-quantitative analytical test result. All results indicating elevated blood cholesterol levels should be verified by a quantitative cholesterol method. Clinical considerations and professional judgment should be applied to the interpretation of results by this test.

SUMMARY

Studies have been conducted that show the incidence of Coronary Artery Disease (CAD) rises linearly with blood cholesterol.[1] Individuals with elevated blood cholesterol levels of 200 mg/dL or greater are considered at increased risk for the development of Coronary Artery Disease.[2] The CHEMCARD™ Cholesterol Test is intended to identify those individuals with elevated blood cholesterol levels associated with increased risk of CAD by providing the physician with fast, accurate results and the opportunity for further testing.

PRINCIPLE

The CHEMCARD™ Cholesterol Test is a solid phase chemistry which employs the same principle as the enzymatic wet chemistries which are presently being used in many hospital laboratories.[3] This enzymatic reaction makes use of a highly sensitive chromogen, tetramethylbenzidine, which allows for a visual determination of blood cholesterol. The CHEMCARD™ Cholesterol Test incorporates a unique cell separator as an integral part of the device. This separation device allows the use of whole blood as a specimen.

Cholesterol ester $\xrightarrow{\text{ESTERASE}}$ Cholesterol + Fatty Acid

Cholesterol + Oxygen $\xrightarrow{\text{CHOLESTEROL OXIDASE}}$ 4-Cholesterone + Hydrogen peroxide

Hydrogen peroxide + Chromogen $\xrightarrow{\text{PEROXIDASE}}$ Dye + Water

COMPOSITION

Buffer
Tetramethylbenzidine
Cholesterol esterase EC 3.1.1.13
Cholesterol oxidase EC 1.1.3.6
Sodium cholate
Peroxidase ES 1.11.1.7
Other materials required for stability and system compatibility.

FOR IN-VITRO DIAGNOSTIC USE ONLY

STORAGE and STABILITY

CHEMCARD™ Cholesterol Tests are to be stored at room temperature, not to exceed 86°F (30°C). Refrigeration is not required. The test cards may be used until the expiration date stamped on package. Any foil packages that appear to have a broken seal should not be used for testing. Return the package to Chematics, Inc. for replacement.

SPECIMEN

The CHEMCARD™ is intended for use with fresh whole capillary blood collected from a fingerstick.

Fingerstick samples must be obtained from free flowing capillary blood. Excessive squeezing or milking can produce somewhat lower results.

MATERIALS PROVIDED

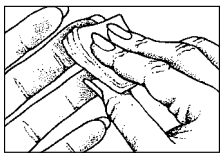
Test Card

MATERIALS NEEDED BUT NOT PROVIDED

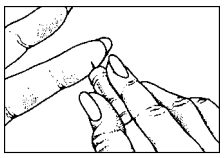
Alcohol or Alcohol Swab
Clean Dry Tissue
Watch or Other Timing System
Sterile Lancet

PROCEDURE

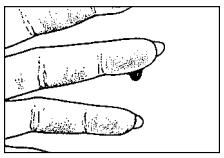
1 Clean fingertip with alcohol and let dry.



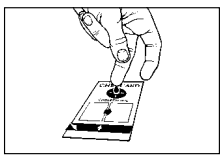
2 Prick finger with lancet. Discard first drop of blood.



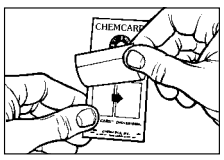
3 Squeeze finger to obtain a large hanging drop of blood.



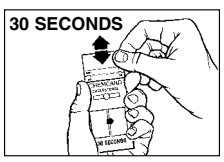
4 Gently bring the hanging drop into contact with the test area.



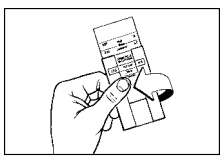
5 After 3 minutes, remove entire TAB area from CHEMCARD™ and discard.



6 Compare overall color of the Test Area with color in window on either side by sliding inner card up and down. Find closest matching color in 30 SECONDS. Note: the reactive pad will begin to fade within 3–5 minutes.



7 Turn the card over. The cholesterol level appears in the clear windows in both mg/dL and mmol/L.



RESULTS

Results are obtained by visually comparing the developed color of the Test Area with those of the sliding color standard appearing in the windows at the side of the Test Area. After a visual match is made, the card is turned over and the total cholesterol concentration value appears in the window on the back side of the card. A Test Area that appears lighter than the 150 color block should be interpreted as a cholesterol result of less than 150 mg/dL. A Test Area that appears darker than the 300+ color block should be interpreted as greater than 300 mg/dL.

CALIBRATION

Calibration of the CHEMCARD™ Cholesterol Test is not required. The color development for each lot of test cards is calibrated by use of precise standards during manufacturing.

QUALITY CONTROL

It is recommended that quality control material be analyzed at regular intervals. Upon request, a control is available from Chematics, Inc. to verify the integrity of the test cards in each new shipment and for use in routine testing. It is also recommended for use in training new test operators.

Other manufacturer control materials are not recommended for use with CHEMCARD™ Cholesterol. As an alternate control material, a fresh capillary blood sample from an individual with a known cholesterol level may be used to verify the integrity of the test.

For technical assistance, Chematics, Inc. Technical Service may be contacted by dialing **1-574-834-2406.**

LIMITATIONS OF PROCEDURE

The results of this test should not be used for instituting drug treatment in individuals with elevated cholesterol levels or altering drug treatment in individuals whose cholesterol levels are being monitored.

CHEMCARD™ Cholesterol allows the specific, enzymatic determination of cholesterol and cholesterol esters. Hematocrit will cause a bias in an inverse relationship.[4] For this reason plasma and serum will read high and are not recommended for use with this test.

Cholesterol oxidase may exhibit some activity for certain steroids such as epiandrosterone, dehydroepiandrosterone, campesterol and sitosterol.[5] However, the plasma concentration of these substances are negligible compared to plasma total cholesterol.[6] Hemoglobin, uric acid, glucose, ascorbic acid, and creatinine do not interfere when fresh capillary blood is used; however if elevated, bilirubin may be responsible for low results.[7] No interference was found in lipemic samples.[8]

When stored in the original package and in the temperature range specified, the cards are stable up to the expiration date shown on the package label.

If the values obtained are unusually high or low, repeat the test using a fresh sample on a new unused test card.

EXPECTED RESULTS

The National Heart, Lung and Blood Institute's National Cholesterol Education Program at the National Institutes of Health released in October, 1987, a report on the detection, evaluation and treatment of high blood cholesterol in adults.[9] The panel simplified the previous guidelines published in 1985.[2] The new report eliminates the age and sex categories for risk levels and classifies total cholesterol levels as follows;

Total cholesterol levels:

Less than 200 mg/dL — Desirable Blood Cholesterol
200 to 239 mg/dL — Borderline High Blood Cholesterol
240 mg/dL and above — High Blood Cholesterol

All results of 200 mg/dL or greater should be repeated by a quantitative laboratory method before a diagnosis of hypercholesterolemia is made.

In addition, if an individual's blood cholesterol level does fall into a borderline high or high cholesterol category, it is recommended that a lipid profile be completed to determine the extent of the risk for CAD; taking into account other risk factors such as hypertension, cigarette smoking and family history.[9,10,11]

PERFORMANCE CHARACTERISTICS

MEASURING RANGE

150–300 mg/dL or 3.9–7.8 mmol/L

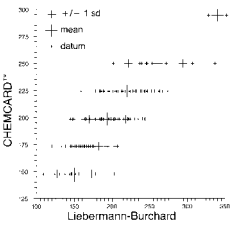
ACCURACY

The following data were calculated from the testing of a total of 158 patients at three different sites.[12]

Results by CHEMCARD™ Cholesterol were compared to results by the Liebermann-Burchard method[13] and by an enzymatic method.[14,15] The comparison data are presented in the following graphs:

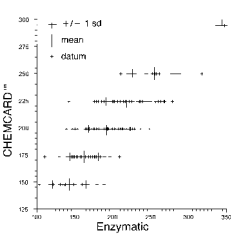
N = 158
Range X = 109–348 mg/dL
Range Y = 150–300 mg/dL

CHEMCARD™ RESULT (mg/dL)	L-B MEAN (mg/dL)	L-B S.D.- (mg/dL)	N
150	149.2	22.9	13
175	165.2	16.9	40
200	192.1	24.8	51
225	218.7	25.4	43
250	257.0	37.5	9
300+	336.5	**	2



N = 158
Range X = 99–339 mg/dL
Range Y = 150–300 mg/dL

CHEMCARD™ RESULT (mg/dL)	ENZ MEAN (mg/dL)	ENZ S.D. (mg/dL)	N
150	137.5	21.9	13
175	157.0	18.3	40
200	186.2	23.3	51
225	212.8	27.5	43
250	247.9	38.8	9
300+	335.0	**	2



In the following table **only,** the Liebermann-Burchard and enzymatic laboratory assay results were rounded to the nearest expected color block. Each column in the following table represents the distribution of observed CHEMCARD™ Cholesterol results with respect to the expected results. The percent of CHEMCARD™ Cholesterol results within ± 1 color block of the laboratory assay result is represented in the last row in the following table.

CHEMCARD™ LIEBERMANN-BURCHARD (mg/dL) (mg/dL)	150	175	200	225	250	300+	ENZYMATIC (mg/dL) 150	175	200	225	250	300+
150	10	2	1	0	0	0	11	2	0	0	0	0
175	15	20	5	0	0	0	25	12	3	0	0	0
200	6	14	21	7	3	0	7	21	15	7	1	0
225	1	5	13	14	10	0	1	5	17	14	6	0
250	0	0	1	2	4	2	0	0	1	2	5	1
300+	0	0	0	0	0	2	0	0	0	0	0	2
TOTAL	32	41	41	23	17	4	44	40	36	23	12	3
%±1BLOCK	78	88	95	100	82	100	82	88	97	100	92	100

CHEMCARD™ Cholesterol was read at the 150 or 175 mg/dL color block 53 times. Of those, 50 and 52 patients were 199 mg/dL or less by the Liebermann-Burchard and Enzymatic laboratory method respectively. This yields a 94.3% to 98.1% frequency of positively identifying patients with non-elevated levels of cholesterol, below 200 mg/dL, when CHEMCARD™ Cholesterol is read at the 150 or 175 mg/dL color block.

CHEMCARD™ Cholesterol was read at the 225, 250 or 300+ mg/dL color block 54 times. Of those, 46 and 39 patients were greater than 199 mg/dL by the Liebermann-Burchard and Enzymatic laboratory method respectively. This yields an 85.2% to 72.2% frequency of positively identifying patients with borderline or elevated levels of cholesterol, greater than 199 mg/dL, when CHEMCARD™ Cholesterol is read at the 225, 250 or 300+ mg/dL color block.

CHEMCARD™ Cholesterol was read at the 250 or 300+ mg/dL color block 11 times. Of those, 9 patients were greater than 239 mg/dL by both the Liebermann-Burchard and Enzymatic laboratory methods yielding an 81.8% frequency of positively identifying patients with elevated levels of cholesterol, above 239 mg/dL, when CHEMCARD™ Cholesterol is read at the 250 or 300+ mg/dL color block.

Fourteen random samples taken from the 158 specimens above were sent, under the recommendation of the Center for Disease Control, to the University of Wisconsin Reference Laboratory to obtain results for comparison from the modified Abell-Kendall proposed Reference Method.[16] Satisfactory results were obtained.[12]

PRECISION

A study was conducted utilizing six whole blood samples (preserved with sodium citrate) with known cholesterol values at 153, 178, 204, 223, 260 and 325 mg/dL (as determined by Liebermann-Burchard). Each sample was tested with a CHEMCARD™ Cholesterol test card and the results interpreted by 10 observers for a total of 60 determinations.[17] The following table shows the distribution of observed CHEMCARD™ Cholesterol results with respect to the Liebermann-Burchard results.

CHEMCARD™ (mg/dL)	LIEBERMANN-BURCHARD (mg/dL) 153	178	204	223	260	325
150	4	0	0	0	0	0
175	6	8	0	0	0	0
200	0	2	2	2	1	0
225	0	0	6	2	1	1
250	0	0	2	6	8	6
300+	0	0	0	0	0	3

REPEATABILITY

Twelve specimens from duplicates of six samples of whole blood (preserved with sodium citrate) with known cholesterol values at 153, 178, 204, 223, 260 and 325 mg/dL (as determined by the Liebermann-Burchard method), were blindly tested by twenty observers. A total of 240 observations were recorded.[18] The following table shows the distribution of observed CHEMCARD™ Cholesterol results with respect to Liebermann-Burchard results.

CHEMCARD™ (mg/dL)	LIEBERMANN-BURCHARD (mg/dL) 153	178	204	223	260	325
150	26	15	0	0	0	0
175	11	18	0	2	1	0
200	3	3	12	11	1	0
225	0	4	21	24	9	0
250	0	0	7	3	26	16
300+	0	0	0	0	3	24

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CHEMATICS INC.
TM P.O. Box 293 • North Webster, IN 46555

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