TECO DIAGNOSTICS
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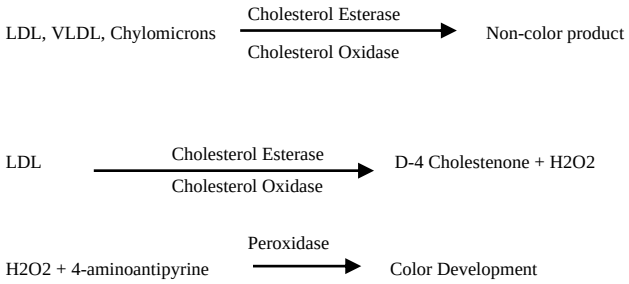
Direct LDL Cholesterol Reagent
TC MATRIX-160

INTENDED USE
For the direct quantitative determination of low density lipoprotein cholesterol (LDL-C) in human serum or plasma on TC Matrix analyzers.

SUMMARY AND EXPLANATION OF THE TEST
Plasma lipoproteins are spherical particles that contain varying amounts of cholesterol, triglycerides, phospholipids, and proteins. The phospholipid, free cholesterol and protein constitute the outer surface of the lipoprotein particle; the inner core contains mostly esterified cholesterol and triglycerides. These particles serve to solubilize and transport cholesterol and triglycerides in the bloodstream.

The relative proportions of protein and lipid determine the density of these plasma lipoproteins and provide a basis for their classification. The classes are: very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoprotein (HDL). Numerous clinical studies have shown that the different lipoprotein classes have varied effects. The studies all point to LDL cholesterol as the key factor in the pathogenesis of artherosclerosis and coronary artery disease (CAD),[2-8] while HDL cholesterol has often been observed to have a protective effect. Even within the normal range of total cholesterol concentrations, an increase in LDL cholesterol can occur with an associated risk for CAD.

Serum is reacted with a detergent which solubilises the non-LDL lipoproteins, ie., HDL, VLDL and Chylomicrons. The cholesterol released is consumed by cholesterol esterase and cholesterol oxidase in a non-color forming reaction. A second detergent solubilizes the remaining LDL particles and a chromogenic coupler allows for a color forming reaction. The color that is produced is proportional to the amount of LDL cholesterol present in the sample.



REAGENT PREPARATION
No preparation is required.

REAGENT COMPOSITION
MES Buffer (pH 6.3): 50 mmol/L
Detergent 1: 0.1%
Detergent 2: 0.1%
Cholesterol Oxidase: >800 u/L
Cholesterol Esterase: >800 u/L
Peroxidase (from Horseradish): >5000 u/L
4-aminoantipyrine: 4 mmol/L
N,N-bis(4-sulfobutyl)-m-toluidine, disodium: 0.7 mmol/L
Perservative: 0.01 mmol/L
Also non-reactive chemicals for optimal system performance.

REAGENT STORAGE AND STABILITY
Direct LDL Cholesterol Reagent stored unopened at 2ºC to 8ºC is stable until the expiration date shown on the bottle label. Once opened, Direct LDL Cholesterol Reagent is stable for 30 days or until the expiration date shown on label, whichever occurs first.
DO NOT FREEZE.

SPECIMEN COLLECTION AND HANDLING
1. The test can be performed on serum or plasma. For serum, blood is drawn into a tube which does not contain anticoagulant and it is allowed to clot. The serum should then be separated from the clot within two hours from the time of collection.
2. Separated serum or plasma should not remain at room temperature longer than 8 hours. If assays are not completed within 8 hours, serum and plasma should be stored at 2ºC to 8ºC. If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at -15ºC to -20ºC. Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.
3. For plasma, add whole blood directly into a tube containing anticoagulant. Acceptable anticoagulants are listed in the "LIMITATIONS" section.

CALIBRATION
1. TECO lipid Calibrator is required but is not provided in the kit.
2. The system must have a valid calibration in memory before controls or patient samples can be run.
3. The TC Matrix system will automatically perform checks on the calibration and produce data at the end of calibration.

Note: Refer to the TC Matrix manual for further instructions on calibrating the instrument

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT
TECO LIPID Calibrator
At least two levels of control material.

LIMITATIONS
1. Anticoagulants containing citrate should not be used.
2. Protect the reagents from direct sunlight.
3. Samples with values greater than 500 mg/dl must be diluted 1:1 with saline and re-assayed. Multiply the result by two.

INTERFERENCE
1. All interference studies were conducted according to the procedures recommended in NCCLS guideline No. EP7-P for interference testing in clinical chemistry. Hemoglobin at levels up to 400 mg/dl, Bilirubin at levels up to 20 mg/dl and Triglycerides to 1500 mg/dl were found to exhibit negligible interference (<5%) on this method. Samples with levels of interfering substances higher than the upper limits should be diluted with physiological saline before assaying. Multiply the result obtained from the manual dilution by the appropriate dilution factor.

2. Refer to the work of Young for a review of drug and comprehensive list of substances which have an effect on serum LDL cholesterol levels.

EXPECTED VALUE

The following NCEP recommendations for patient classifications are suggested for the prevention and management of coronary heart disease:

LDL Cholesterol	Classifications
<100mg/dl (2.586mmol/L)	Optimal
100-129mg/dl (2.586-3.34mmol/L)	Near Optimal/Above Optimal
130-159 mg/dl (3.36-4.11mmol/L)	Borderline High Risk
160-189mg/dl (4.14-4.89mmol/L)	High Risk
≥190 mg/dl (4.91mmol/L)	Very High Risk

It is highly recommended that each laboratory establishes its own range of expected values.

PRECAUTIONS:

1. For in vitro diagnostic use only.
2. Since all specimens are potentially infectious, they should be handled with appropriate precautions and practices in accordance with Biosafety level 2 as recommended by USA NIH Biosafety in Microbiological and Biomedical Laboratories manual, and in accordance with national or local regulations related to the safety precautions of such materials.
3. Each laboratory has to perform the quality control tests to ensure the results are reliable before testing the specimens.

PROCEDURES

Test Name:	LDL	R1:	190
Full Name:	Direct LDLCholesterol	R2:	57
Pri. Wave:	578 nm	Sample volume:	2.0
Sec. Wave:	700 nm	Calibration Type:	2 point linear
Assay/ Point:	2 point end	K Value:	/
Start - End:	10 - 24	Point:	2
Decimal place:	1	Blank Type:	Water
Unit:	mg/dL	Point 0 (Blank) Con.:	0.0
Linearity Range:	2.00 - 500.00	Point 1 (STD) Con.:	Standard/
Correlation Factor:	1.0000 - 0.0000		Calibrator

PERFORMANCE CHARACTERISTICS

Analytical Range: 2-500 mg/dl

For Direct LDL Cholesterol analysis by Direct LDL Cholesterol Reagent on TC Matrix System, this method has been demonstrated to be linear from 2- 500 mg/dl.

Accuracy: Comparison study was performed on TC Matrix System for 40 samples. Beckman Coulter Direct LDL Cholesterol Reagent was used to compare with Direct LDL Cholesterol Reagent. The results of this study yielded a correlation coefficient of 0.98 with a regression equation of y = 0.98X + 3.7

Precision: Within Run precision for Direct LDL Cholesterol Reagent Set was determined following a modification of NCCLS EP5-A. Two commercial human serum samples with Direct LDL Cholesterol Calibrator were assayed on TC Matrix System for 25 times.

Sample	Sample 1	Sample 2
N	25	25
Mean (mg/dl)	73	170
Standard Deviation (mg/dl)	3.3	0.7
Coefficient of Variation (%)	1.8	1.9

Run-Day precision for Direct LDL Cholesterol Reagent was determined following a modification of NCCLS EP5-A. Two commercial human serum samples with Direct LDL Cholesterol Calibrator were assayed on TC Matrix Systems five times per day for five days for the total of 25 values.

Sample	Sample 1	Sample 2
N	25	25
Mean (mg/dl)	74	171
Standard Deviation (mg/dl)	4.1	0.9
Coefficient of Variation (%)	2.5	2.7

REFERENCES

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