CHOLESTEROL FL

CT F100 CH	2 x 50 ml
CT F400 CH	4 x 100 ml
CT 100F CH	4 x 250 ml

INTENDED USE

Reagent for quantitative in vitro determination of cholesterol in biological fluids.

SUMMARY OF TEST

Although every living organism examined has been found to contain sterols, cholesterol is found almost exclusively in animals and humans, in which it is also the main sterol. Cholesterol is the initial starting point in many metabolic pathways. These include vitamin D synthesis, steroid hormone synthesis, and bile acid metabolism. Cholesterol is presented to the intestinal wall from three sources: the diet, bile and intestinal secretions, and cells.

PRINCIPLE OF THE METHOD

All cholesterol esters present in plasma are hydrolyzed quantitatively into free cholesterol and fatty acids by cholesterol esterase. In the presence of oxygen, free cholesterol is then oxidized by cholesterol oxidase to cholesten-4-ene-3-one and H_2O_2. The H_2O_2 reacts with p-chlorophenol and 4-aminoantipyrine in the presence of peroxidase to form a quinoneimine dye. The intensity of color formed is proportional to the cholesterol concentration and can be measured photometrically between 480 and 520 nm.

KIT COMPONENTS

For in vitro diagnostic use only.
The components of the kit are stable until expiration date on the label.
Keep away from direct light sources.

CHOL R1 **F100: 2 x 50 ml (liquid) blue cap**
F400: 4 x 100 ml (liquid) blue cap
100F: 4 x 250 ml (liquid) blue cap

Composition: Good's buffer pH 7.20, sodium cholate 8 mM, CHE ≥ 400 U/l, CHOD ≥ 200 U/l, POD ≥ 500 U/l, 4-AAP 0.6 mM, 4-chlorophenol 2 mM.

Standard: cholesterol solution 200 mg/dl - 5 ml

Store all components at 2-8°C.

MATERIALS REQUIRED BUT NOT SUPPLIED

Current laboratory instrumentation. Spectrophotometer UV/VIS with thermostatic cuvette holder. Automatic micropipettes. Glass or high quality polystyrene cuvettes. Saline solution.

PRECAUTIONS

Reagent may contain some non-reactive and preservative components. It is suggested to handle carefully it, avoiding contact with skin and swallow.
Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

N-acetylcysteine (NAC), metamizole and acetaminophen may cause interference in the Trinder reaction.[1,2]
To avoid interference, the blood withdrawal should be performed before drug administration.

REAGENT PREPARATION

Use reagent ready to use.
Stability: up to expiration date on labels at 2-8°C.
Stability since first opening of vials: preferably within 60 days at 2-8°C.

SPECIMEN

Serum, plasma EDTA.
Sample is stable 3 days at 2-8°C and 1 month at -20°C.

TEST PROCEDURE

Wavelenght: 510 nm (allowed 480 ÷ 520 nm)
Lightpath: 1 cm
Temperature: 37°C

dispense:	blank	standard	sample
reagent	1 ml	1 ml	1 ml
water	10 µl	-	-
standard	-	10 µl	-
sample	-	-	10 µl

Mix, incubate at 37°C for 5 minutes.
Read absorbances of standard (As) and samples (Ax) against reagent blank.

RESULTS CALCULATION

serum/plasma sample:

cholesterol mg/dl = Ax/As x 200 (standard value)

EXPECTED VALUES

desirable:	140 - 200 mg/dl
borderline/high risk:	200 - 240 mg/dl
high risk:	> 240 mg/dl

Each laboratory should establish appropriate reference intervals related to its population.

QUALITY CONTROL AND CALIBRATION

It is suggested to perform an internal quality control. For this purpose the following human based control sera are available:
QUANTINORM CHEMA
with normal or close to normal control values
QUANTIPATH CHEMA
with pathological control values.
If required, a multiparametric, human based calibrator is available:
AUTOCAL H

Please contact Customer Care for further information.

TEST PERFORMANCE

Linearity
the method is linear up to 700 mg/dl.
If the limit value is exceeded, it is suggested to dilute sample 1+9 with saline and to repeat the test, multiplying the result by 10.

Sensitivity/limit of detection (LOD)
the limit of detection is 1 mg/dl.

Interferences
no interference was observed by the presence of:

hemoglobin	≤ 500 mg/dl
bilirubin	≤ 15 mg/dl
lipids	≤ 850 mg/dl

Precision

intra-assay (n=10)	mean (mg/dl)	SD (mg/dl)	CV%
sample 1	101.50	1.84	1.80
sample 2	176.20	2.74	1.60

inter-assay (n=20)	mean (mg/dl)	SD (mg/dl)	CV%
sample 1	100.99	2.11	2.10
sample 2	176.51	2.23	1.30

Methods comparison
a comparison between Chema and a commercially available product gave the following results:

Cholesterol FL Chema = x
Cholesterol competitor = y
n = 100

y = 0.979x - 1.71 mg/dl $r^2 = 0.995$

WASTE DISPOSAL

This product is made to be used in professional laboratories.
P501: Dispose of contents according to national/international regulations.

REFERENCES

1) N-acetylcysteine interference of Trinder-based assays. Genzen JR, Hunsaker JJ, Nelson LS, Faine BA, Krasowski MD. Clin Biochem. 2016 Jan;49(1-2):100-4
2) Drug interference in Trinder reaction. Wiewiorka O, Čermáková Z, Dastych M. Euromedlab 2017. ISSN 1437-4431
3) Trinder P., - J. Clin. Path. 22, 158 (1969);
4) Allain C.C., Poon L.S., Chan C.S., Richmond W., Fu P.C., - Clin. Chem. 20,470 (1974).
5) National Cholesterol Education Program (NCEP) recommended values for cholesterol. Tietz Textbook of Clinical Chemistry, Second Edition, Burtis-Ashwood (1994).

MANUFACTURER

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SYMBOLS

IVD	*in vitro* diagnostic medical device
LOT	batch code
REF	catalogue number
	temperature limit
	use by date
	caution
	consult instructions for use

CE