UMA CO., LTD.

2-19-6 Yokosuka

Matsudo, Chiba, Japan

MEASURE TC

Reagent for determination of Total Cholesterol

CHOD/POD Method

2 - 8°C	**IVD** *In vitro* Diagnostics	**QUALITY MANAGEMENT SYSTEM (BY TUV)**
DO NOT freeze	18 months/block from light	**ISO 13485:2016**

1. PURPOSE OF USE

Providing a quantitative in vitro assay for the Total Cholesterol (TC) concentration in serum or plasma.

2. GENERAL INSTRUCTION

a. For in vitro diagnostics use only.

b. Diagnosis should be made in a comprehensive manner, in accordance with other related test results and clinical symptoms by the doctor in attendance.

c. For guaranteed results, usage of this product must comply with the instruction in this manual.

d. If you use automatic analyzers, follow their instructions carefully.

SUMMARY

It is well established that high serum total cholesterol (S-TC) is associated with greater all-cause and cardiovascular mortality in middle-aged adults. A recent meta-analysis of 61 prospective observational studies involving almost 12 million person - years at risk at ages between 40 and 89 years confirmed that S-TC is a strong risk factor of mortality from ischaemic heart disease (IHD). Age, however, seemed to attenuate the relative effect of total cholesterol on IHD mortality. Interestingly, the association between total cholesterol and total stroke mortality was inverse in the age group 70 - 89 years and among those with systolic blood pressure above 145 mmHg. In addition, some observational studies of persons aged 65 years or older suggest that cholesterol might be inversely associated with total mortality. In the European guideline, in general, the optimal goal for S-TC is < 5.0 mmol/l, and for patients with clinically established cardiovascular disease and patients with diabetes, the optimal treatment goal is < 4.5 mmol/l.

Cholesterol levels seem to decrease with age Low S-TC in the elderly has often been seen as a marker of frailty. The inverse association between S-TC and mortality has also been interpreted to be due to confounding by chronic diseases such as dementia or malnutrition. However, some epidemiological studies have shown that the inverse association remains obvious among the oldest old, even when adjusted for comorbid diseases.

The aim of this population-based cohort study was to examine the relationship between S-TC and six-year all-cause mortality in home-dwelling elderly aged 75 years or older with concomitant diseases.

3. MATERIALS REQUIRED BUT NOT INCLUDED

- Saline 0.9 % and high grade purified water

- Micropipet and other basic laboratory equipment.

- Lipids Calibrator and Lipids Control / MEASURE Human Lyo L-1 and MEASURE Human Lyo L-2

4. REAGENT COMPOSITION & PREPARATION

- Reagent R-1: Cholesterol esterase (CHER); Peroxidase; N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline sodium salt (HDAOS); Ascorbate Oxidase

Reagent R-1 is ready for use

- Reagent R-2: Cholesterol oxidase (CHOD); Peroxidase; 4-Aminoantipyrine (4-AA)

Reagent R-2 is ready for use

- Once open, Reagent stored on board the instrument is stable for 30 days with Hitachi 7180 Analyzers.

- Applicable to various automated analyzers.

- Calibrator Lipids Calibrator (separately sold): Put 2 mL of purified water to the vials of calibrator (Lipids Calibrator); leave at room temperature for 120 minutes and sometimes gently invert the vial before use. After reconstituting, Calibrator can be used without dilution.

- Control Lipids Control (separately sold): Put 2 mL of purified water to the vials of control (Lipids Control); leave at room temperature for 120 minutes and sometimes gently invert the vial before use. After reconstituting, controls can be used without dilution.

- Controls MEASURE Human Lyo L-1 and MEASURE Human Lyo L-2 (separately sold): Put 5 mL of purified water to the vials of controls (Lyo L-1 and Lyo L-2); leave at room temperature for 45 minutes and sometimes gently invert the vial before use. After reconstituting, controls can be used without dilution.

5. SAMPLE PREPARATION & STORAGE

- Serum: Wait until sample completely coagulated. Take the supernatant to use as specimen. .

- Plasma: Collect blood after 12 - 14 hours fasting. Treat sample by anticoagulant (Li-heparin, K2-EDTA); leave sample to stand for 3 hours or centrifuge at 2000 rpm for 2 minutes; take the plasma layer (supernatant) and use as specimen.

- Analyze sample soon after collection.

- Stability

  - 8 hours at 15 - 25°C
  - 3 days at 2 - 8°C
  - 30 days at < -20°C

- See interferences section for details about possible sample interferences.

6. MEASUREMENT PRINCIPLE

When the sample is reacted with the buffer solution (Reagent R-1), cholesterol esterase (CHER) causes hydrolysis of esterified cholesterol, which generates free cholesterol. Further, through Ascorbic acid oxidase (AOD), Ascorbic acid in the reagent is eliminated. In the second reaction, both the generated free cholesterol and the existing free cholesterol are oxidized and generate hydrogen peroxide. The generated hydrogen peroxide through Peroxidase (POD) causes HDAOS and 4-aminoantipyrine (4-AA) quantitative oxidative condensation and generates blue-violet pigment. Concentration of total cholesterol in the sample is determined by measuring this absorbance.

1st reaction

$$\text{Esterified cholesterol} + H_2O \xrightarrow{CHER} \text{free cholesterol} + \text{fatty acid}$$

$$2\ \text{Ascorbic acid} + O_2 \xrightarrow{AOD} \text{2-dehydroascorbic acid} + 2H_2O$$

2nd reaction

$$\text{Free cholesterol} + O_2 \xrightarrow{CHOD} \text{cholestenon} + H_2O_2$$

$$2\ H_2O_2 + \text{4-AA} + \text{HDAOS} + H_3O^+ \xrightarrow{POD} \text{blue-violet quinine pigment} + 5H_2O$$

7. ASSAY PROCEDURE

This product is compatible with various types of clinical analyzer. An example of the assay procedure is indicated below.

Sample	+	Reagent 1	$\xrightarrow[280s]{37^\circ C}$	Meas. Abs I
2.0µL		150µL		(600/800nm)
$\xrightarrow[18s]{37^\circ C}$		Reagent 2	$\xrightarrow[302s]{37^\circ C}$	Meas. Abs II
		50µL		(600/800nm)

$\longrightarrow$ ΔAbs at 600/800nm $\longrightarrow$ Concentration TC

Perform the assay according to the instructions for operating the automated analyzer Hitachi models. Refer to the **13. INFORMATION FOR AUTOANALYZERS** for the details of the assay method. Contact **HUMA MEDICAL CO., LTD.** for information about the parameters for other automated analyzers.

8. CALCULATION & UNIT CONVERSION

Calculation

- Calculate ΔAbs of specimen & standards vs blank

- Plot a calibration curve TC = f(ΔAbs)

- Calculate TC in specimen using the curve

(doing same procedure for Controls)

Unit conversion

mg/dL x 0.0259 = mmol/L

9. PERFORMANCE & CORRELATION TEST

a. Measuring range

- The assay is linear within an TC concentration range in serum/plasma of 0.08 - 20.72 mmol/L.

- If the concentration of sample exceeds assay range, dilute the sample with saline and repeat the measurement.

b. Detection Limit

Limit of Blank (LoB)	=	0.01 mmol/L
Limit of Detection (LoD)	=	0.03 mmol/L
Limit of Quantitation (LoQ)	=	0.08 mmol/L

The LoB, LoD and LoQ were determined in accordance with CLSI EP17-A2 requirements.

The LoB is the highest apparent analyte concentration expected to be found when replicates of a blank sample containing no analyte are tested. The LoB corresponds to the concentration below which analyte-free samples are found with a probability of 95%.

The LoD is determined based on the LoB and standard deviation of low concentration samples. The LoD corresponds to the lowest analyte concentration which can be detected (value above the LoB with a probability of 95%).

The LoQ is the lowest analyte concentration that can be reproducibly measured with a total error of 20%. It has been determined using low concentration samples.

c. Performance

- Sensitivity: Using purified water, absorbance change is 0.01 - 0.05; using cholesterol 5.18 mmol/L solution, absorbance change is 0.25 - 0.85.

- Accuracy: When measuring a control sample, the result is within ±10% of assigned value.

d. Precision (on Biolis 30i / SK300)

Representative performance data on the analyzers are given below.

Results obtained in individual laboratories may differ.

Precision was determined using controls followed the CLSI Approved Guidline EP5-A2 with repeatability, reproducibility and total precison (1 aliquot per run, 2 run per day, 20 days).The following results were obtained.

Criterion: CV of Repeatability (aka. Within-run precision) is less than 3% and Total Precision is less than 5%.

Repeatability	*Mean mmol/L*	*SD mmol/L*	*CV %*
Control Lyo L-1	4.2	0.05	1.25
Control Lyo L-2	6.1	0.08	1.27

Reproducibility	*Mean mmol/L*	*SD mmol/L*	*CV %*
Control Lyo L-1	4.2	0.10	2.37
Control Lyo L-2	6.1	0.12	1.97

Total precision	*Mean mmol/L*	*SD mmol/L*	*CV %*
Control Lyo L-1	4.2	0.11	2.53
Control Lyo L-2	6.1	0.13	2.16

e. Correlation Test

Same measurement principle

Regression equation: y = 1.0064x - 0.9013 (n = 62)

Correlation coefficient: r = 0.9992

(y: value obtained from this method)

Reference Material for Calibration

ReCCS JCCRM 224

10. EXPECTED VALUES

3.68 - 6.42 mmol/L

Reference range should be established at each facility and judgement should be based on measurement results in a comprehensive manner together with clinical symptoms and other measurement results.

11. INTERFERENCES

- Icterus: No significant interference of conjugated/free bilirubin concentration up to 20 mg/dL

- Hemolysis: No significant interference of hemoglobin concentration up to 500 mg/dL

- Lipemia (Intralipid): No significant interference of Triglyceride concentration up to 3000 FTU

- Ascorbic Acid: No significant interference of ascorbic acid concentration up to 50 mg/dL

- For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings. Please use another methods if the result is affected by any factors

12. HANDLING, USAGE & DISPOSAL

Handling

1. Specimen can be potentially positive for infectious agents including hepatitis B virus and HIV. Wear glove and goggle when needed.

2. In case reagents got into skin, eye or mouth by mistake, wash it immediately with plenty of water and consult the doctor if needed.

3. If reagents are spilled, dilute with water and wipe it out. If specimen is spilled, spray 80% of alcohol over the specimen and wipe it out.

Usage

1. Store reagents under specified condition. Do not use after expiration date.

2. Do not use the container and auxiliaries included in this kit for other purposes.

3. Do not mix reagents of different lot for use.

4. Do not add to the reagent being used even if it is the same lot number.

Disposal

1. All specimens, as well as all instruments (e.g. test tubes) that come in contact with the specimens, must be treated by the following methods, or they must be treated according to the manual for infectious medical waste provided in each facility.

- Sterilize with an autoclave, subjecting them to high pressure saturated steam at 121 °C for more than 20 minutes. Do not process waste containing sodium hypochlorite solution with an autoclave.

- Immerse at least one hour in sodium hypochlorite solution (active chloride concentration of over 1000 ppm).

2. This reagent contains sodium azide. Sodium azide can react with lead pipe and/or steel pipe and can generate explosive metal azide. Make sure to use plenty of water at disposal. Concentration of sodium azide in R-2 is 0.05%.

13. INFORMATION FOR AUTOANALYZERS

❖ **For Hitachi Model**

Calculation Method		Two point
Temperature		37°C
Volume (µL)	Specimen	2.0
	R1	150
	R2	50
Wavelength (nm)	Main	600
	Sub	800
Measurement (cycle)	Point 1	10
	Point 2	16
	Point 3	34
Calibration type		Linear
Unit		mg/dL

14. OTHER INSTRUCTIONS AND CAUTION

- Results may differ depending on the sample/reagent ratio. Adjust parameters for different analyzer.

- Perform the QC procedure on the day of determination.

15. PACKING AND KIT CONFIGURATION

Code	Package	Test/Kit*	Test/Kit**
11T013A	1x60mL; 1x20mL	310	540
11T013A2	2x60mL; 2x20mL	620	1080
11T013A3	3x60mL; 3x20mL	930	1620
11T013A4	4x60mL; 4x20mL	1240	2160
11T003A	5x60mL; 5x20mL	1550	2700
11T013A6	6x60mL; 6x20mL	1860	3240
11T013	1x90mL; 1x30mL	470	810
11T013-2	2x90mL; 2x30mL	940	1620
11T003	3x90mL; 3x30mL	1410	2430
11T013-4	4x90mL; 4x30mL	1880	3240
11T013-5	5x90mL; 5x30mL	2350	4050

* For middle-scale automatic analyzers such as: SK300; BS series; BA200; BA400. Chemwell Series; Dirui Series; Biolyzer series, HumanStar 300, Erba Series; Bioelab Series, BX 3010; Pictus P500;…

** For large-scale automatic analyzers such as: CA800; CA400; Randox Imola; Randox Modena+; BM 6010; Biolis50i; SK500; AU Series; Pictus P700; C series; Ci series; HumanStar 600; Kenolab series ...

The above-mentioned test's number are calculated base on technical specifications of each analyzer. The real number of test per kit may higher than the calculation's number.

The above-mentioned test's number cover the loss of the dead volume of reagent bottles but not cover the loss of Calibrator and Control.

Please feel free to contact authorized distributor for further confirmation.

16. REFERENCES

1. Päivi Tuikkala,, Serum total cholesterol levels and all-cause mortality in a home-dwelling elderly population: a six-year follow-up, 2010
2. CLSI/NCCLS Evaluation of Precision Performance of Clinical Chemistry Devices, EP05-A2, 2004
3. CLSI EP17 · Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, 2nd Edition, 2017
4. In house data, UMA Diagnostics

17. MANUFACTURER

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