

DIAGNOSTIC KIT FOR DETERMINATION OF CHOLINESTERASE ACTIVITY



HC – CHOLINESTERASE

INTRODUCTION

There are two cholinesterases (CHE and ACHE) differing in substrate specificity, tissue of origin and biological role. The term cholinesterase (ACHE), also known as acetylcholine acetylhydrolase, is found in erythrocytes, in the lungs, spleen and in grey matter of the brain. The pseudocholinesterase (CHE), also referred to as acylcholine acylhydrolase, is found in serum, the liver, pancreas, heart and in the white matter of brain. The assay of serum cholinesterase (CHE) is useful to diagnose: liver disorders, hepatitis, cirrhosis, carcinoma with metastasis, sensitivity to succinylcholine administration and pesticide poisoning. Levels decrease in all of the diseases above.

METHOD PRINCIPLE

Optimized kinetic method according to Deutsche Gesellschaft für Klinische Chemie (DGKC).

The method uses butyrylthiocholine as the specific substrate for cholinesterase (CHE). Cholinesterase catalyses the hydrolysis of butyrylthiocholine substrate forming butyrate and thiocholine, in presence of potassium hexacyanoferrate (III). Thiocholine reduces potassium hexacyanoferrate (III) (yellow colour) to potassium hexacyanoferrate (II) (colourless). The decrease in absorbance is directly proportional to the CHE activity in the sample.

REAGENTS

Package

1-Reagent	1 x 46.5 ml
2-Reagent	1 x 12 ml

The reagents when stored at 2-8°C are stable up to expiry date printed on the package. The reagents are stable for 8 weeks on board the analyser at 2-10°C. Do not freeze reagents. Protect from direct light, evaporation and avoid contamination!

Concentrations in the test

1-Reagent

pyrophosphate buffer, pH 7.7	65 mmol/l
hexacyanoferrate (III)	2 mmol/l

2-Reagent

Good's Buffer pH 4.0	20 mmol/l
butyrylthiocholine iodide	65 mmol/l

Warnings and notes

- Products for in vitro diagnostic use only.
- The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.
- Immediately after use, recap the bottles and store at 2-8°C.
- Do not use after expiry date.
- Do not interchange caps.
- Reagents should be mixed before use by gentle inverting the bottle several times.
- The appearance of turbidity or control sera values outside the manufacturer's acceptable range may indicate of reagent instability.

SPECIMEN

Fresh serum free from haemolysis, plasma (EDTA, heparin) not hemolyzed.

Serum / plasma should be separated from red blood cells as soon as possible after blood collection. Do not use citrate, borate, oxalate and fluoride as an anticoagulant because it inhibits cholinesterase activity.

It is recommended to follow CLSI procedures regarding specimen collecting and handling.

Sample may be stored for up to 15 days at 2-8°C or 12 months at -20°C.

Nevertheless it is recommended to perform the assay with freshly collected samples!

PROCEDURE

The reagents are ready to use.

These reagents may be used in automatic analyser Hitachi 911/912.

Application should be entered using handheld barcode scanner and attached barcodes sheet, according to procedure described below:

- Delete previous version of application and calibrators assigned to it and restart the analyser.
- Enter codes of calibrators according to the attached list.
- Enter barcoded application and assign proper values to calibrators.
- To activate entered application go to the tab UTILITY | APPLICATION | RANGE and change value of field DATA MODE from INACTIVE to ON BOARD. Confirm the change using UPDATE button.
- Put reagents on board the analyser – they will be assigned to relevant tests automatically. Perform also measurement of level of reagents inside the bottles.
- After calibration analyser is ready to use.

REFERENCE VALUES ^{4,5}

serum / plasma	37°C	
female	4000 – 12600 U/l	67 – 210 µkat/l
male	5100 – 11700 U/l	85 – 195 µkat/l

In infants up to 6 months of age, cholinesterase activity is 40% to 50% higher than in adults. In young adult (< 35 years) women, the enzyme activity is approximately 64% to 74% of that in adult males. The activity decreases during pregnancy.

It is recommended for each laboratory to establish its own reference ranges for local population.

QUALITY CONTROL

For internal quality control it is recommended to use the CORMAY SERUM HN (Cat. No 5-172) and CORMAY SERUM HP (Cat. No 5-173) with each batch of samples.

For the calibration of automatic analysers systems the CORMAY MULTICALIBRATOR LEVEL 1 (Cat. No 5-174; 5-176) is recommended. Calibrator and 0.9% NaCl should be used for calibration.

The calibration curve should be prepared every 8 weeks, with change of reagent lot number or as required e.g. quality control findings outside the specified range.

PERFORMANCE CHARACTERISTICS

These metrological characteristics have been obtained using the automatic analyser Hitachi 912. Results may vary if a different instrument or a manual procedure is used.

- Sensitivity:** 156 U/l (2.60 µkat/l).
- Linearity:** up to 18750 U/l (313 µkat/l).
For higher activity dilute the sample with 0.9% NaCl and repeat the assay. Multiply the result by dilution factor.
- Specificity / Interferences**
Haemoglobin up to 5 g/dl, bilirubin up to 20 mg/dl and triglycerides up to 1000 mg/dl do not interfere with the test.

Precision

Repeatability (run to run) n = 10	Mean [U/l]	SD [U/l]	CV [%]
level 1	5426	25.58	0.47
level 2	7470	21.50	0.29

Reproducibility (day to day) n = 10	Mean [U/l]	SD [U/l]	CV [%]
level 1	2223	55.26	2.49
level 2	7263	139.61	1.92

▪ **Method comparison**

A comparison between CORMAY reagent (y) and another commercially available assay (x) using 46 samples gave following results:

$$y = 0.9461 x + 286.04 \text{ U/l};$$

$$R = 0.998 \quad (R - \text{correlation coefficient})$$

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

1. Burtis C.A., Ashwood E.R., ed. Tietz Textbook of Clinical Chemistry, 3rd ed. Philadelphia, PA: WB Saunders, 708-11, (1999).
2. Alan H.B. Wu: Tietz Clinical Guide to Laboratory Tests, 4th ed. WB Saunders., 250-251, (2006).
3. Use of Anticoagulants in Diagnostic Laboratory Investigations. WHO. Publication WHO/DIL/LAB/99.1 (Rev.2. Jan. 2002).
4. Kaplan LA. Pesce AJ: "Clinical Chemistry", Mosby Ed. 967, (1996).
5. Internal reference range studies.

Date of issue: 12. 2012.

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