05403383001V6.0

LACT2

cobas®

Lactate Gen.2

Order information

REF	CONTENT		Analyzer(s) on which kit(s) can be used
05401666 190	Lactate Gen.2 (2 × 50 tests)		**cobas c** 111
Materials required (but not provided):			
10759350 190	Calibrator f.a.s. (12 × 3 mL)	Code 401	
10759350 360	Calibrator f.a.s. (12 × 3 mL, for USA)	Code 401	
12149435 122	Precinorm U plus (10 × 3 mL)	Code 300	
12149435 160	Precinorm U plus (10 × 3 mL, for USA)	Code 300	
12149443 122	Precipath U plus (10 × 3 mL)	Code 301	
12149443 160	Precipath U plus (10 × 3 mL, for USA)	Code 301	
05117003 190	PreciControl ClinChem Multi 1 (20 × 5 mL)	Code 391	
05947626 190	PreciControl ClinChem Multi 1 (4 × 5 mL)	Code 391	
05947626 160	PreciControl ClinChem Multi 1 (4 × 5 mL, for USA)	Code 391	
05117216 190	PreciControl ClinChem Multi 2 (20 × 5 mL)	Code 392	
05947774 190	PreciControl ClinChem Multi 2 (4 × 5 mL)	Code 392	
05947774 160	PreciControl ClinChem Multi 2 (4 × 5 mL, for USA)	Code 392	

English

System information

LACT2: ACN 040

Intended use

In vitro test for the quantitative determination of lactate in human plasma on the **cobas c** 111 system.

Summary

Anaerobic glycolysis markedly increases blood lactate and causes some increase in pyruvate levels, especially with prolonged exercise. The common cause for increased blood lactate and pyruvate is anoxia resulting from such conditions as shock, pneumonia and congestive heart failure. Lactic acidosis may also occur in renal failure and leukemia. Thiamine deficiency and diabetic ketoacidosis are associated with increased levels of lactate and pyruvate.

In recent years, enzymatic methods for the determination of lactate have gained favor over colorimetric and titrimetric methods. Enzymatic methods are generally simple and provide greater specificity, accuracy, and reproducibility.

The first enzymatic method described for the determination of lactate was based on the transfer of hydrogen from lactate to potassium ferricyanide by lactate dehydrogenase. However, the procedure was cumbersome and did not receive wide acceptance.

Subsequent methods involved the UV measurement of the formation of NADH. In 1974, Gutmann and Wahlefeld[1] described a lactate procedure that measures the NADH formed by the oxidation of lactate catalyzed by LD, using hydrazine as a trapping agent for pyruvate. A method described by Noll[2] is also based on the catalytic action of LD but includes ALT in the reaction mixture to more rapidly remove the pyruvate formed from the conversion of lactate.

The method presented here uses an enzymatic reaction to convert lactate to pyruvate. The hydrogen peroxide produced by this reaction is then used in an enzymatic reaction to generate a colored dye.[3,4] This method offers longer reagent stability than the previous UV enzymatic methods.

Test principle

Colorimetric assay

L-lactate is oxidized to pyruvate by the specific enzyme lactate oxidase (LOD). Peroxidase (POD) is used to generate a colored dye using the hydrogen peroxide generated in the first reaction.[3,4]

$$\text{L-lactate} + O_2 \xrightarrow{\text{LOD}} \text{pyruvate} + H_2O_2$$

$$2\,H_2O_2 + \text{H donor} + \text{4-AAP} \xrightarrow{\text{POD}} \text{chromogen} + 2\,H_2O$$

The intensity of the color formed is directly proportional to the L-lactate concentration. It is determined by measuring the increase in absorbance.

Reagents - working solutions

R1 Hydrogen donor: 1.75 mmol/L; ascorbate oxidase (cucumber): 501 µkat/L; buffers; preservatives

SR 4-Aminoantipyrine: 5 mmol/L; lactate oxidase (microbial): 251 µkat/L; peroxidase (horseradish): 401 µkat/L; buffers; preservatives

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

Reagent handling

Ready for use

Storage and stability

Shelf life at 2-8 °C:	See expiration date on reagent
On-board in use and refrigerated on the analyzer:	4 weeks

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Serum: **Do not use serum specimens.**

Plasma: Na-fluoride/K-oxalate and Na-fluoride/Na-heparin plasma

Centrifuge within 15 minutes of collecting the specimen.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

See the limitations and interferences section for details about possible sample interferences.

Note

1. The lactate level increases rapidly with physical exercise. The time required for return to normal lactate values depends on the physical fitness of the subject. 30 minutes at rest is usually sufficient for this purpose.
2. Blood samples should be drawn from a stasis-free vein. However, minimal hemostasis (less than 30 seconds) will not affect lactate levels. Avoid the use of a tourniquet, if possible.[5]
3. Glycolysis in blood samples can rapidly increase lactate levels. Cells contribute to the glycolysis and their quick removal is essential for accurate lactate analysis.[6] Heparinized plasma is acceptable, but precautions must be taken to retard glycolysis by keeping the whole blood on ice and then separating the plasma from the cells within 15 minutes of collection.

Stability in plasma:[7]	8 hours at 15-25 °C
	14 days at 2-8 °C
Stability in plasma (heparinized):[8]	38 days at -20 °C

Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

See "Order information" section

General laboratory equipment

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for plasma

cobas c 111 **test definition**

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction direction	Increase
Wavelength A/B	552/659 nm
Calc. first/last	16/23
Units	mmol/L (mg/dL)
Reaction mode	R1-S-SR

Pipetting parameters

		Diluent (H_2O)
R1	125 µL	
Sample	2 µL	20 µL
SR	25 µL	20 µL
Total volume	192 µL	

Calibration

Calibrator	Calibrator f.a.s.
	Deionized water is used automatically by the instrument as the zero calibrator.
Calibration mode	Linear regression
Calibration replicate	Duplicate recommended
Calibration interval	Each lot and as required following quality control procedures

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: This method has been standardized against a primary standard.

Quality control

For quality control, use control materials as listed in the "Order information" section. In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The **cobas c** 111 analyzer automatically calculates the analyte concentration of each sample.

Conversion factors:	mmol/L x 9.009 = mg/dL
	mg/dL x 0.111 = mmol/L

Limitations - interference

Criterion: Recovery within ±10 % of initial value at a lactate concentration of 2.2 mmol/L (19.8 mg/dL).

Icterus:[9] No significant interference up to an I index of 18 for conjugated bilirubin and 50 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 308 µmol/L or 18 mg/dL; approximate unconjugated bilirubin concentration: 855 µmol/L or 50 mg/dL).

Hemolysis:[9] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):[9] No significant interference up to an L index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Highly turbid and grossly lipemic samples may cause Abs. flags.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[10,11] Exception: Calcium dobesilate causes artificially low lactate results at the therapeutic drug level. Glycolate, a metabolite of ethylene glycol, causes a positive interference which is variable from lot to lot of reagent.

Dicynone (Etamsylate) at therapeutic concentrations may lead to false-low results.

Acetaminophen intoxications are frequently treated with N-Acetylcysteine. N-Acetylcysteine at a plasma concentration above 998 mg/L and the Acetaminophen metabolite N-acetyl-p-benzoquinone imine (NAPQI) independently may cause false-low results.

Venipuncture should be performed prior to the administration of Metamizole. Venipuncture immediately after or during the administration of Metamizole may lead to falsely low results. A significant interference may occur at plasma Metamizole concentrations above 0.1 mg/mL.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[12]

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on the **cobas c** 111 analyzer. For information about test combinations requiring special wash steps, please refer to the latest version of the carry-over evasion list found with the CLEAN Method Sheet and the operator's manual for further instructions.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

0.2-15.5 mmol/L (1.8-140 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:10 dilution. Results from

samples diluted using the rerun function are automatically multiplied by a factor of 10.

Lower limits of measurement

Lower detection limit of the test:
0.2 mmol/L (1.8 mg/dL)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying 3 standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

Expected values[5]

Plasma:	0.5-2.2 mmol/L	(4.5-19.8 mg/dL)	venous
	0.5-1.6 mmol/L	(4.5-14.4 mg/dL)	arterial

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in an internal protocol with repeatability (n = 21) and intermediate precision (3 aliquots per run, 1 run per day, 10 days). The following results were obtained:

Repeatability	*Mean mmol/L (mg/dL)*	*SD mmol/L (mg/dL)*	*CV %*
Precinorm U	1.71 (15.4)	0.01 (0.09)	0.85
Precipath U	3.28 (29.6)	0.01 (0.09)	0.24
Plasma 1	1.71 (15.4)	0.01 (0.09)	0.48
Plasma 2	6.74 (60.7)	0.03 (0.27)	0.42

Intermediate precision	*Mean mmol/L (mg/dL)*	*SD mmol/L (mg/dL)*	*CV %*
Precinorm U	1.71 (15.4)	0.02 (0.18)	0.89
Precipath U	3.27 (29.5)	0.01 (0.09)	0.18
Plasma 3	1.70 (15.3)	0.01 (0.09)	0.34
Plasma 4	6.80 (61.3)	0.01 (0.09)	0.15

Method comparison

Lactate values for human plasma samples obtained on the **cobas c** 111 analyzer (y) were compared with those determined on COBAS INTEGRA 400 analyzers (x), using the corresponding reagent.

Sample size (n) = 59

Passing/Bablok[13]	Linear regression
y = 0.994x + 0.025 mmol/L	y = 0.995x + 0.020 mmol/L
τ = 0.990	r = 0.999

The sample concentrations were between 0.94 and 15.0 mmol/L (8.47 and 135 mg/dL).

References

1 Gutmann I, Wahlefeld A. Methods of Enzymatic Analysis. 2nd ed. Bergmeyer HU, ed. New York, NY: Academic Press Inc 1974:1464.

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4 Barhan D, Trinder P. An improved colour reagent for the determination of blood glucose by the oxidase system. Analyst 1972;97:142.

5 Tietz NW, ed. Clinical Guide to Laboratory Tests, 3rd ed. Philadelphia PA: WB Saunders Company 1995;382-383.

6 Burtis CA, Ashwood ER, (eds.). Tietz Textbook of Clinical Chemistry. 2nd ed. Pa: WB Saunders Co 1994;976.

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8 Nelson SR and Kugler KK. Comparison of Lactate Levels in Acid-Treated and Untreated Blood and Spinal Fluid. Biochemical Medicine 1969;2(4):325-332.

9 Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.

10 Breuer J. Report on the Symposium "Drug effects in Clinical Chemistry Methods". Eur J Clin Chem Clin Biochem 1996;34:385-386.

11 Sonntag O, Scholer A. Drug interference in clinical chemistry: recommendation of drugs and their concentrations to be used in drug interference studies. Ann Clin Biochem 2001;38:376-385.

12 Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.

13 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
REAGENT	Reagent
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

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Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

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