LACTATE

Diagnostic reagent for determination of Lactate concentration.
Liquid. Monoreagent. Store at +2/+8°C. For in Vitro Diagnostic Use (IVD). **Do not freeze.**

Ref No	Package	Ref No	Package	Ref No	Package	Ref No	Package
TB2440 TB2441	200 mL 120 mL	T2440 T2441	315 mL 203 mL	ZA30	125 mL	ZA30-1	75 mL

Changes made in the instructions for use are marked as grey.

INTENDED USE

The test is applied for the quantitative determination of lactate in plasma and cerebrospinal fluid (CSF).

GENERAL INFORMATION

Lactic acid, an intermediate in carbohydrate metabolism, is predominantly derived from white skeletal muscle, brain, skin, renal medulla and erythrocytes. The blood lactate concentration depends on the rate of production in these tissues and the rate of metabolism in the liver and kidneys. Approximately 65% to 70% of total basal lactate production is utilized by the liver, mainly in gluconeogenesis. Hepatic lactate clearance increases with a moderate increase in lactate production, but uptake by the liver becomes saturable when plasma concentrations exceed 2 mmol/L. For example, during intense exercise, lactate concentrations can increase significantly from an average concentration of about 0.9 mmol/L to over 20 mmol/L within 10 seconds. There is no uniformly accepted concentration for the diagnosis of lactic acidosis; however, lactate concentrations exceeding 5 mmol/L and pH less than 7.25 indicate a significant degree of lactic acidosis.[1]

Measurement of lactic acid or lactate in blood is important as the concentration of this analyte is related to tissue oxygenation.[2] Lactic acid is considered to be a reliable indicator of severity, prognosis and efficacy of treatment in patients with sepsis and multiple organ failure.[3,4]

In general, lactic acidosis, caused by the accumulation of lactate and protons (H^+) in the body, occurs in two clinical settings. These are: (1) type A (hypoxic) associated with decreased tissue oxygenation such as shock, hypovolemia and left ventricular failure; (2) type B (metabolic), which occurs as a result of disease (e.g. diabetes, neoplasia, liver disease), drugs and/or toxins (e.g. ethanol, methanol, salicylates, anti-retroviral agents, propofol) or inborn errors of metabolism (e.g. methylmalonic aciduria, propionic acidemia, fatty acid oxidation defects).[5] Type A is much more common and the majority of cases involve shock, severe heart failure, severe trauma and sepsis. The mechanism of type B lactic acidosis is unknown; however, it is speculated that there is a primary defect in mitochondrial function with impaired oxygen utilization. This leads to reduced ATP and NAD^+ stores with accumulation of NADH and H^+. In the case of reduced liver perfusion or liver disease, lactate removal from the blood is reduced, thus intensifying lactic acidosis.

In many patients with lactic acidosis, the anion gap may not increase and it is important to recognize that a normal anion gap does not exclude lactic acidosis.[1]

Evidence from a multicenter study in 2008 supports the measurement of lactate from fetal scalp blood during labor for the treatment of intrapartum fetal stress to prevent severe ascemia at birth.[7]

Except in children, lactate levels in cerebrospinal fluid (CSF) are normally parallel to blood concentrations.[8] However, with biochemical changes in the central nervous system (CNS), CSF lactate values change independently of blood values. CSF lactate concentrations may increase in cerebrovascular accidents, intracranial hemorrhage, bacterial meningitis, epilepsy, congenital defects in electron transport chain and other CNS disorders.[1] There are various studies on the relationship between CSF lactic acid levels and CNS infections.[9,10] Increased CSF lactate is usually associated with decreased CSF glucose concentrations due to increased anaerobic glucose utilization. Elevated lactate levels (>4.0 mmol/L) are associated with bacterial, fungal and mycobacterial CNS infections; however, they are not viral based.[9,10] In aseptic (viral) meningitis, CSF lactate concentrations are usually not increased; CSF lactate may help in the differential diagnosis between viral and bacterial meningitis, although its clinical utility is questioned.[11] In known bacterial meningitis, decreased CSF lactate levels reflect successful treatment of the disease. Increased CSF lactic acid may also result from impaired CNS blood flow due to space-occupying tumors.[2] In very few children with inherited metabolic disease, CSF lactate concentrations may be increased despite plasma lactate in the reference range.[8]

Unless otherwise indicated, lactic acidosis refers to acidosis caused by L-lactic acid. This is the normal isomer produced in humans. Production of D-lactic acid by bacteria in the colon can cause D-lactic acidosis. In patients undergoing jejunoileal bypass surgery, plasma concentrations of this metabolite can be extremely high during acidotic episodes as a result of increased production by intestinal bacteria due to malabsorption of carbohydrates.[12]

TEST PRINCIPLE

Enzymatic photometric method

A measurement method is used in which lactic acid is oxidized to pyruvate by lactate oxidase. The produced H2O2 oxidizes a chromogen system and the absorbance of the resulting dye complex, measured with a spectrophotometer at 546 nm, is directly proportional to the concentration of lactate in the sample.

The concentration of L-Lactate in the sample is determined according to the following reaction:

$$\text{L-Lactate} + O_2 \xrightarrow{\text{Lactate oxidase}} \text{Pyruvate} + H_2O_2$$

$$2H_2O_2 + \text{4-aminoantipyrine} + \text{TOOS} \xrightarrow{\text{Peroxidase}} \text{Purple product} + 4H_2O$$

TOOS = N-ethyl-N-(2 hydroxy-3-sulfopropyl)m-toluidine

REAGENT COMPONENTS

Reagent 1:

Pipes buffer	≤80 mmol/L
TBHB	≤15 mmol/L
LOX	≤0.8 KU/L
POD	≤7 KU/L
4-aminoantipyrine	≤0.7 mmol/L

REAGENT PREPARATION

Reagent is ready for use.

REAGENT STABILITY AND STORAGE

Reagent is stable at +2/+8°C till the expiration date stated on the label which is only for closed vials.

Once opened vials are stable for 15 days at +2/+8°C in optimum conditions. On board stability is strongly related to auto analyzers' cooling specification and carry-over values.

Reagent stability and storage data have been verified by using Clinical and Laboratory Standards Institute (CLSI) EP25-A protocol.[13]

SAMPLE REQUIREMENTS

Plasma (Na-fluoride/K-oxalate and Na-fluoride/Na-heparin plasma) and CSF can be used and are collected according to the standard procedures. Multiple sample freezing and thawing should be avoided. The sample should be homogenized before testing.

Stability of lactate in plasma (separated) [27]:
8 hours at +20/+25ºC
14 days at +2/+8ºC

Stability of lactate in plasma (with heparin) [28]:
38 days at -20ºC

Stability of lactate in CSF[29]:
24 hours at +2/+8ºC
30 days at -20ºC

Annotation:

- To obtain accurate lactic acid measurements, samples must be processed meticulously as blood cells metabolize glucose to lactic acid, resulting in a positive bias.[14,15] This metabolism is time and temperature dependent. Increases in lactate of 0.4 mmol/L when placed on ice 1 hour after collection and 0.7 mmol/L at room temperature have been reported.[16]
- Maintenance of lactate concentrations by deproteinization of whole blood or serum is inappropriate and unreliable.[17]
- Studies on the effect of sodium fluoride and sodium iodoacetate revealed that an inhibitory effect on glycolysis has been obtained with both compounds, and complete inhibition has been obtained with a mixture of 1% sodium fluoride and 1% iodoacetate.[15]
- Because sodium fluoride can partially inhibit clotting, samples from sodium fluoride phlebotomy tubes tend to be contaminated with protein clots. Iodoacetate does not affect clotting and therefore a suitable sample can be obtained.[2]
- One study has shown that lactic acid levels (final concentration, 0.5 g/L blood) in samples collected with sodium iodoacetate can be stable for up to 2 hours at room temperature.[18]
- Plasma samples can also be collected in tubes containing fluoride and oxalate (60 and 12 mmol/L, respectively). In tubes collected this way, lactate is stable for up to 8 hours at room temperature.[16]
- Heparinized plasma can be used for lactic acid analysis, but the sample must be placed on ice and the plasma separated within one hour of collection.[2]
- For convenience and accuracy, the recommended sample for lactic acid analysis is a sodium iodoacetate phlebotomy tube. The sample should preferably be collected without a tourniquet. If a tourniquet is required, it should be released after venipuncture and the phlebotomist should allow a few minutes to pass before drawing blood into the blood collection tube. Patients should avoid overuse of their hands or arms before sampling.[2]

CALIBRATION AND QUALITY CONTROL

Calibration: The assay requires the use of an Arcal Auto Calibrator.

Arcal Auto Calibrator-Lyophilized
Ref.No: A39052
Ref.No: A39054
Ref.No: A39055 (For Olympus AU series.)

Calibration stability is 7 days. Calibration stability depends on the application characteristics and cooling capacity of the autoanalyzer used.

Control: Commercially available control material with established values determined by this method can be used. We recommend:

Arcon N Level 1 Control- Lyophilized
Ref.No: A3910
Ref.No: A3912 (For Olympus AU series.)
Ref.No: A3913 (For BS series.)
Ref.No: A3914 (For Erba.)

Arcon P Level 2 Control- Lyophilized
Ref.No: A3920
Ref.No: A3922 (For Olympus AU series.)
Ref.No: A3923 (For BS series.)
Ref.No: A3924 (For Erba.)

At least two level controls must be run once in every 24 hours. Each laboratory should determine its own quality control scheme and procedures. If quality control results are not within acceptable limits, calibration is required.

REFERENCE INTERVALS / MEDICAL DECISION LEVELS

Plasma: :4.5 – 19.8 mg/dL

CSF:

New born	:10 – 60 mg/dL
3-10 days old	:10 – 40 mg/dL
> 10 days old	:10 – 25 mg/dL
Adults	:10 – 22 mg/dL

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

Reference interval has been verified by using CLSI EP28-A3c protocol.[19]

PERFORMANCE CHARACTERISTICS

Measuring Interval

According to CLSI EP34-ED1:2018, "Measuring Interval" refers to the interval where the analyte concentration is measured with intended accuracy in terms of medical and laboratory requirements without dilution, concentrating or any kind of pre-treatment that is between the analyte's lower limit of quantitation (LLoQ) and upper limit of quantitation (ULoQ).[20]

The determined analytic measuring interval for Lactate is 2 – 140 mg/dL.

Detection Capability

Limit of Detection (LoD): 1 mg/dL

Limit of Quantitation (LoQ): 2 mg/dL

Note: LoQ values are based on Coefficient of Variation Percentage (CV) ≤ 20%.

LoD and LoQ values have been verified by using CLSI EP17-A2:2012 protocol.[21]

Linearity

This method shows measurement linearity in the activities up to 140 mg/dL. Autoanaylzer's auto-dilution system can be used if the concentrations have higher values. See device manual for further information.

For the manual dilution procedure, dilute the sample 1:5 using 0.90% isotonic. After this process, multiply the result of the reworked sample by the dilution factor. Do not report the sample result after dilution if it is marked as lower than the linear lower limit. Rerun with a suitable dilution.

Linearity Studies data have been verified by using CLSI EP06-A:2003 protocol.[22]

Precision

Running system has been developed according to 20x2x2 "The Single Site" protocol. Repeatability and Within-Laboratory Precision/Within-Device values have been obtained according to the running results.

According to the protocol in use, 2 separate runs per day have been made for 20 days (no obligation for being consecutive days). This protocol has been applied to each low and high samples separately and 80 results have been obtained for each one. Statistically, the results have been obtained using 2-factor Nested-ANOVA model.[23]

Repeatability (Within Run) and Repeatability (Day to Day) CV% values of Lactate have been given in the table 1 and 2 respectively.

Table 1. Lactate Repeatability (Within Run) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
8.83	0.16	1.76	80
25.4	0.54	2.14	80

Note: This working system has been named "Within-Run Precision" in the previous CLSI - EP05-A2 manual.[24]

Table 2. Lactate Repeatability (Day to Day) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
8.83	0.32	3.62	80
25.4	0.61	2.40	80

Note: This working system has been named "Total Precision" in the previous CLSI - EP05-A2 manual.[24]

Interference

Endogenous interferant and analyte concentrations that have been used in the Lactate scanning tests has been determined according to "CLSI EP37-ED1:2018" and "CLSI EP07-ED3:2018" manuals.[25,26]

The total acceptable error rate, which is going to be used to detect whether the observed differential value obtained from Lactate interference scanning test is appropriate, is determined as ±10%.[27]

In Lactate test results, no significant interaction has been observed in the determined endogenous interferant and analyte concentrations or between interferants and analyte.

Hemoglobin	: ≤ 220 mg/dL
Lipemia	: ≤ 1000mg/dL
Bilirubin	: ≤ 60 mg/dL

It should be noted that endogenous interferants, as well as various medicines and metabolites, anticoagulants (e.g. Heparin, EDTA, citrate, oxalate) and preservatives (e.g. sodium floride, iodoacetate, hydrochloride acide) such as additives, materials that may contact with samples during collection and processing (serum separator devices, sample collection containers and contents, catheters, catheter wash solutions, skin disinfectants, hand cleaners and lotions, glass washing detergents, powder gloves), dietary substances known to affect some specific tests (caffeine, beta-carotene, poppy seeds, etc.), or some substances present in a sample that cause foreign proteins (heterophilic antibodies, etc.), autoimmune response (autoantibodies, etc.), or due to malignancy (for example, interference by paraproteins with phosphate testing and indirect ion selective electrode methods) may show some negative effects that will cause various attempts and some misjudgements.[26]

These performance characteristics have been obtained using an autoanalyzer. Results may vary slightly when using different equipment or manual procedures.

WARNINGS AND PRECAUTIONS

IVD: For in Vitro Diagnostic use only.
Do not use expired reagents.
Reagents with two different lot numbers should not be interchanged.
For professional use.
Follow Good Laboratory Practice (GLP) guidelines.

CAUTION: Human source samples are processed with this product. All human source samples must be treated as potentially infectious materials and must be handled in accordance with OSHA (Occupational Safety and Health Administration) standards.

Danger

EUH032	:Releases a very toxic gas if contacts with acid.
H317	:May cause allergic skin reaction.

Precaution

P280	:Use protective gloves / clothes / glasses / mask.
P264	:Wash your hands properly after using.
P272	:Contaminated work clothes should not be allowed to be used outside of the workplace.

Intervention

P302+P352	:Wash with plenty of water and soap if it contacts with skin.
P333+P313	:Seek medical help if it irritates your skin or develops rash.
P362+P364	:Remove contaminated clothes and wash properly before using.

Disposal

P501	:Dispose the vials and contents according to the local regulations.

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SYMBOLS

IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
R1	Reagent 1
GTIN	Global Trade Item Number
REF	Reference Number
GLP	Good Laboratory Practices
FOR USE WITH	Identifies Products to Be Used Together
PRODUCT OF TURKEY	Product of Turkey
	Manufacturer
	Expiration Date
	Temperature Limits
	Consult Instructions for Use
	Caution
	Number of Tests

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