

# Lactate – Liquizyme

REF: 274 001 (5 x 20 ml) 100 test

# **Intended Use**

Spectrum Diagnostics liquizyme Lactate reagent is intended for the in-vitro quantitative, diagnostic determination of lactate in human Plasma and CSF on both automated and manual systems.

### Background

Lactic acid, present in blood entirely as lactate is an intermediary product of carbohydrate metabolism and is derived mainly from muscle cells and erythrocytes. The blood lactate concentration is affected by its production in muscle cells and erythrocytes and its rate of metabolism in the liver. During exercise, blood lactate can increase up to ten times of normal levels. Under normal conditions, the ratio between lactate and pyruvate is constant(10:1). The liver can normally metabolize more lactate than is produced. In the case of decreased perfusion of the liver , however, removal

In the case of decreased perfusion of the liver, however, removal of lactate by the liver may be significantly reduced. The amount of lactate in cerebrospinal fluid normally parallels blood levels. CSF lactate level is increased in bacterial meningitis, epilepsy, and intracranial hemorrhage. CSF lactate level may be an aid to distinguish between bacterial from viral meningitis.

### Method

Enzymatic colorimetric method (LOX / PAP)with lactate oxidase and 4-aminoantipyrine.

### **Assay Principle**

Lactate is oxidized to pyruvate and hydrogen peroxide  $(H_2O_2)$  by lactate oxidase (LOX). In the presence of peroxidase (POD), hydrogen peroxide reacts with 2,4,6-tribromo-3-hydroxybenzoic acid (THB) and 4-aminoantipyrine (4-AAP) to form a red quinoneimine dye.

Lactate	LOX	Pyruvate
+	>	+
0 <sub>2</sub>		$H_2O_2$
2H <sub>2</sub> O + 4-AAP	POD	quinoneimine dye
THB		+ 4H <sub>2</sub> O

The color intensity of the formed red quinoneimine dye is directly proportional to the lactate concentration. It is determined by measuring the increase in absorbance at 546 nm.

### Reagents

Standard lactate (ST)	10 mg/dL
<b>Reagent 1 (R1 Buffer)</b> Tris buffer 2,4,6-tribromo-3-hydroxybenzoic acid 4-Amino antipyrine	100 mmol/L 2.0 mmol/L 0.8 mmol/L
<b>Reagent 2 (R2 Enzyme)</b> Lactate oxidase Peroxidase Sodium Azide	>20 U/L >15 U/L 0.02 %

For further information, refer to the Lactate reagent material safety data sheet.

# **Precautions and Warnings**

Do not ingest or inhalate. In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.

Reagent (R2) contains sodium azide which may react with copper or lead plumbing.

# SYMBOLS IN PRODUCT LABELLING



### **Reagent Preparation**

### Prepare working solution as following:

REF:274 001:add 2 ml from R2 to one bottle of R1; mix gently.

Or prepare the working solution according to the number of tests required by mixing 9 volumes of reagent 1 (R1) and 1 volume of reagent 2 (R2), e.g. 900  $\mu$ l R1 +100  $\mu$ l R2.

# **Reagent Storage and Stability**

All reagents are stable until expiration date stated on label when stored refrigerated at 2 - 8 °C. Working solution is stable for 3 months at 2 - 8 °C or 1 week at 15 - 25 °C.Once opened, the reagent and the standard vials are stable for 3 months at the specified temperature if contamination is avoided.

# Deterioration

The working reagent is normaly clear or pale pink. Do not use liquizyme lactate reagent if it is turbid or if the absorbance is greater than 0.1 at 546 nm.

# **Specimen Collection and Preservation**

Plasma and CSF. Do not use serum specimens. Avoid icteric and haemolytic specimens. The only acceptable anticoagulants are fluoride/heparin and iodoacetate/heparin. Collection of satisfactory specimen for lactate analysis requires special procedures to prevent changes of lactate both while and after the specimen is drawn. The patient should be fasting and at complete rest and exercise of the arm or hand should be avoided before or during collection of the specimens. The collected blood should be cooled on ice immediately and separated from the cells, lactate values are stable.

is separated from the cells, lactate values are stable. Use the CSF samples with addition of glycolysis inhibitor, e.g.sodium fluoride. Lactate in CSF is stable for 3 hours at  $20 - 25^{\circ}$ C, for 24 hours at  $4 - 8^{\circ}$ C, and for 2 months frozen at -20  $^{\circ}$ C, stable in plasma for 2 hours at  $20 - 25^{\circ}$ C and 2 days at  $4 - 8^{\circ}$ C.

### System Parameters

Wavelength	546 nm
Optical path	1 cm
Assay type	End-point
Direction	Increase
Sample : Reagent Ratio	1 : 100
e.g.: Reagent volume	1 ml
Sample volume	10 µl
Temperature	37 <sup>o</sup> C or 15 – 25 <sup>o</sup> C
Zero adjustment	Reagent blank
Incubation time	5 minutes at 37 <sup>o</sup> C or
	10 minutes at 15 – 25 <sup>O</sup> C
Reagent Blank Limits	Low 0.00 AU
	High 0.25 AU
Sensitivity	0.3 mg/dL (0.033 mmol/L)
Linearity	90 mg/dL (9.99 mmol/L)
Procedure	

	Blank	Standard	Sample	
Working				
Reagent	1.0 ml	1.0 ml	1.0 ml	
Standard		10 µl		
Sample			10 µl	

Mix and incubate for 5 minutes at 37  $^{\circ}$ C or 10 minutes at 15 – 25  $^{\circ}$ C. Measure absorbance of specimen (Aspecimen) and standard (Astandard) against reagent blank within 30 minutes.

# Calculation

Lactate conc. (mg/dL) =

A<sub>specimen</sub> A<sub>standard</sub>

x 10

# **Quality Control**

Normal and abnormal commercial control serum of known concentrations should be analyzed with each run

# **Methods Comparison**

A comparison between Spectrum Diagnostics Lactate reagent and a commercial reagent of the same methodology was performed on 20 human sera. A correlation of 0.992 was obtained.

# Sensitivity

When run as recommended, the minimum detection limit of the assay is 0.3 mg/dL (0.033 mmol/L).

### Linearity

The reaction is linear up to lactate concentration of 90 mg/dL (9.99 mmol/L), specimens showing higher concentration should be diluted 1+1 using physiological saline, reassayed and the result multiplied by two

# Interfering substance

# Haemolysis

Haemoglobin levels higher than 2.5 g/L (0.16 mmol/L) increase the apparent lactate concentration significantly.

#### Icterus

Bilirubin levels higher than 4.0 mg/dL (68 mmol/L) decrease apparent lactate concentration significantly.

### Lipemia

No significant interference.

### Ascorbic acid

Physiological ascorbic acid concentrations do not interfere with the test. Ascorbic Acid levels higher than 5 mg/dL (284 mmol/l) decrease the apparent lactate concentration significantly.

# **Expected Values**

Plasma	Venous	4.5 – 19.8 mg/dL	0.5–2.2 mmol/L
	Arterial	4.5 – 14.4 mg/dL	0.5–1.6 mmol/L
CSF	Adult	10 – 22 mg/dL	1.1 – 2.4 mmol/L
	Neonates	10 – 60 mg/dL	1.1 – 6.7 mmol/L

Spectrum Diagnostics does not interpret the results of a clinical laboratory procedure; interpretation of the results is considered the responsibility of qualified medical personnel. All indications of clinical significance are supported by literature references.

### **Analytical Range**

0.3 - 90 mg/dL ( 0.033 - 9.99 mmol/L).

### Waste Disposal

This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal. **S56:** dispose of this material and its container at hazardous or special waste collection point.

S57: use appropriate container to avoid environmental contamination. S61: avoid release in environment. refer to special instructions/safety data sheets.

# Reference

1.Bailey EM, Domenico P, Cunha BA. Bacterial or viral meningitis? Measuring lactate in CSF can help you know quickly. Meningitis. 1990;88:217-223.

2.Field M, Block JB, Levin R, Rall DP. Significance of blood lactate elevations amoung patients with acute leukemia and other neoplastic proliferative disorders. Am J Med. 1996;40:528-547.

3.Klein TO. Nervensysteme. In:Greiling H, Gressner AM,eds. Lehrbuch der Klinischen Chemie und Pathobiochemie. Stuttgart:Schattauer; 1987:859-893.

4.Sacks DB. Carbohydrates. In: Burtis CA, Ashwood ER, eds. Tietz Fundamentals of Clinical Chemistry. 4 th ed. Philadelphia:WB Saunders;1996:351-374.

5.Sacks DB. Carbohydrates in: Burtis CA, Ashwood ER, eds. Tietz Textbook of Clinical Chemistry. 2 nd ed. Philadelphia:WB Sander; 1994;928-1001. 6.Tietz NW, ed. Clinical Guide to laboratory tests. 3 rd ed.

Philadelphia: WB Saunders;1995:351-374

ORDERING INFORMATION		
CATALOG NO.	QUANTITY	
274 001	5 x 20 ml	



