CATACHEM

LACTATE (LACTIC ACID)
MANUAL OR AUTOMATED ASSAY
Cat.No. C454-01

Intended Use
For **In Vitro Diagnostic** use in the quantitative determination of Lactate in plasma and spinal fluid.

Clinical Significance (2-5)
Lactate as an intermediary product of carbohydrate metabolism is present in the blood entirely as lactate ion. The Lactate blood concentration is dependent on the rate of metabolism by the liver and its production by the erythrocytes and muscle cells. During exercise Lactate may increase significantly up to ten times higher than the normal level. Pyruvate levels increase under these conditions as well and the normal ratio of Pyruvate to Lactate is approximately 10:1.
Lactate in spinal fluid normally parallels blood levels. In case of biochemical alteration in the central nervous system, Lactate changes independently of blood values. Lactate levels in spinal fluid are increased in bacterial meningitis. Also, increased values are observed in trauma, seizure, brain abscess, and multiple sclerosis.

Method Principle (1)
Lactate, by the action of Lactate Oxidase is oxidized to pyruvate and hydrogen peroxide. The hydrogen peroxide thus produced is quantitatively determined by coupling 4-aminoantipyrine with N-ethyl-N-(2-hydroxy-3-sulphopropyl)-m-toluidine (TOOS) (3-4), where a quinonemine dye with maximum absorption at 550 nm is produced. The following reaction scheme illustrates the reactions that occur in this method:

$$\text{l - Lactate} + O_2 \xrightarrow{\text{L. OXIDASE}} \text{Pyruvate} + H_2O_2$$

$$H_2O_2 + 4AA + TOOS \xrightarrow{\text{PEROXIDASE}} \text{Quinoneimine Dye} + 4H_20$$

LACTATE REAGENT
Each liter contains:

Buffer	
4-Aminoantipyrine (4AA)	0.15 mol
TOOS	1.2 mmol
Lactate Oxidase	≥1000 Units
Peroxidase	≥10000 Units
Stabilizer and nonreactive ingredients	

Precautions
Handle this reagent using good laboratory practice. **DO NOT PIPETTE REAGENT BY MOUTH**. Avoid contact with skin and eyes. If contact occurs, wash affected area with plenty of cold water. Clean spills immediately.

Reagent Storage and Stability
Store the Catachem Lactate reagent at 2-8°C. When stored as directed the reagent is stable until expiration date stated on the label.

Working Reagent Preparation
The Catachem Lactate reagent is packaged in ready-to-use form. No preparation is required. Once opened the Catachem Lactate Working Reagent is stable for 60 days at 2-8°C.

Reagent Indications of Deterioration
- Turbidity
- Absorbance > 0.5 OD, 1 cm light path, 550nm
- Quality control values out of assigned ranges

If these reagent characteristics are observed contact Catachem technical service.

Specimen Collection and Stability (2, 8)
To maintain sample integrity and avoid changes in Lactate concentrations, care should be taken to collect the sample specimens. Fasting patients should be completely at rest for two hours prior to sampling to allow the Lactate concentrations to reach steady state.
Venous specimens should be collected without the use of a tourniquet or immediately after a tourniquet has been applied.
Specimens should be collected in tubes with heparin/fluoride or heparin/iodoacetate as anticoagulants. Separate immediately from the cells and analyze promptly or store at 2-8°C.
CSF samples should be collected with addition of glycolysis inhibitor, e.g. sodium fluoride. Lactate in CSF is stable for 3 hours at room temperature, for 24 hours at 2-8°C and 2 months at -20°C.

Procedure
These instructions are outlined for performing the Lactate assay by manual or automated procedures. Read the original instrument manufacturer's instructions and procedures before performing this as an automated lactate procedure.

Materials Provided
Catachem Lactate Reagent

Materials required but not Provided
- Analyzer (spectrophotometer) with 550 - 600nm wavelength
- Calibrator material with assigned lactate value
- Quality control material with assigned lactate values

Calibration
Catachem Lactate Calibrator C454-10 or Catacal C1200-10 with assigned lactate value is recommended.

Calibration Schedule
Calibration should be performed when this method is run manually or implemented on an automated analyzer for the first time. Recalibration is required after changes of reagent lot number, major instrument service, and when quality control values are out of the indicated range.

Calibration Procedure
Instructions for calibrating any automated analyzer are provided by the specific instrument manufacturer. Read the entire recommended calibration procedure before proceeding with the instrument calibration.

Quality Control
To monitor the quality performance of the procedure, Catachem Catatrol Level I and Catatrol Level II with assigned lactate control ranges should be included in the assay procedure each time the assay is performed.

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Directions for Use
The Catachem Lactate method requires one reagent.

Procedure Limitations
Samples with Lactate values greater than 25 mmol/L should be diluted 1:2 with physiological saline and reassayed. Multiply results obtained by 2 to adjust for the sample dilution.

Interfering Substances
The following substances have no significant effect on the accuracy of this Lactate procedure at the concentrations stated.

- Hemoglobin ≤ 200 mg/dl
- Triglycerides ≤ 1000 mg/dl
- Bilirubin ≤ 2.2 mg/dl

Other substances and certain drugs are also known to influence the lactate values. (7)

Expected Values - Human (5) 0.5-2.2 mmol/L
The values given here are only to be used as a guideline. It is recommended that each laboratory establish the normal range for the geographical area in which it is located and for its patient population and species.

Analytical Parameters

Wavelength	600nm
Temperature	37˚ C
Path length	1 cm
Reaction Mode	End Point
Reaction Time	5 minutes
Reaction Volume	0.6 ml
Sample Volume	0.005 ml
Total Volume	0.605 ml
Sample-to-reagent ratio	1:121

Assay Procedure
1. Pour the required volume of Lactate Working Reagent needed dependent on the number of samples to be assayed.
2. Set spectrophotometer temperature at 37ºC, wavelength at 600nm.
3. Pipette 0.6 ml of Lactate reagent into each cuvette marked “Blank”, “Test”, “Calibrator” and “Control”.
4. Pipette 0.005 ml of Blank (water), Control or Test sample into their respective cuvettes.
5. Mix all cuvettes and incubate all cuvettes for 5 minutes.
6. Read absorbance (A) of each cuvette marked “Blank”, “Test”, “Calibrator” and “Control” at the set wavelength of 600nm.

Lactate Procedure Scheme				
	RGT BLANK	**CAL**	**CONTROL**	**TEST**
Lactate Rgt.	**0.6 ml**	**0.6 ml**	**0.6 ml**	**0.6 ml**
Water (Blank)	**0.005 ml**	**-**	**-**	**-**
Sample	**-**	**0.005**	**0.005**	**0.005**
Mix	**All cuvettes**			
Incubate	**All cuvettes at 37ºC for 5 minutes**			
Calculate	**Lactate mmol/L**			

Calculations and Results:

$$\text{Lactate (mmol/L)} = \frac{\text{(A) Sample - (A) Blank}}{\text{(A) Calibrator - (A) Blank}} \times \text{Calibrator (mmol/L)}$$

Example:

(A) Blank	(A) Test	(A) Calibrator
0.05	0.25	0.50

Calibrator = 0.5 mmol/L

$$\text{Lactate} = \frac{0.25-0.05}{0.50-0.05} \times 5 = 2.2 \text{ mmol/l}$$

Method Performance Characteristics
Sensitivity: Using a pathlength of 1 cm, a Δ-absorbance of 0.05-0.08 per mmol/L should be obtained.
Linearity: This procedure is linear over the range of 0-25 mmol/L.

Bibliography

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