Trinity Biotech

LACTATE

REF 735-10 10 x 10 ml

Pour d'autres langues	Para outras línguas
Für andere Sprachen	Για τις άλλες λώσσες
Para otras lenguas	För andra språk
Per le altre lingue	For andre språk
Dla innych języków	For andre sprog

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INTENDED USE

Lactate Reagent is for the quantitative, enzymatic determination of lactate in plasma at 540 nm.

SUMMARY

Lactic acid is a by-product of carbohydrate metabolism. Blood lactate arises primarily from muscle cells and erythrocytes and is metabolized by the liver. Therefore, blood lactate levels reflect both production and metabolism.

Severe tissue oxygen deprivation leads to "lactic acidosis" characterized by weakness, stupor, fatigue and coma. The liver can normally remove more lactate than the body can produce, hence treatment for hypoxia and removal of the initiating condition usually results in lowering of the blood lactate. Lactate measurement also can be useful clinically in the diagnosis of angina pectoris or in liver function testing where reduced liver function is suspected.

Both lactate and pyruvate levels provide an index of severity of circulatory failure.[1] Several types of lactic acidosis based on association with tissue hypoxia, systemic disorders, drug and toxin ingestion, neoplastic proliferative disorders and inborn errors of metabolism have been described.[2,3]

Lactate metabolism and post-exercise oxygen consumption have been extensively studied from as early as 1900.[1,4,5] The contemporary view holds that "Lactate metabolism regardless of pathways is occurring at all times after exercise and thus cannot be associated with a particular phase of the post-exercise VO_2".[6]

Early analytical methods for lactate lacked both specificity and sensitivity. The availability of highly purified nicotinamide adenine dinucleotide (NAD) and its reduced form (NADH), and lactate dehydrogenase (LD) led to the development of highly specific assays for both lactate and pyruvate.[7] An enzymatic procedure for lactate based on the reduction of NAD to NADH by lactate dehydrogenase is detailed in Trinity Biotech Procedure No. 826-UV. That procedure is carried out at 340 nm.

PRINCIPLE

In another enzymatic approach described herein, lactic acid is converted to pyruvate and hydrogen peroxide (H_2O_2) by lactate oxidase. In the presence of the H_2O_2 formed, peroxidase catalyzes the oxidative condensation of chromogen precursors to produce a colored dye with an absorption maximum at 540 nm.[8,9] The increase in absorbance at 540 nm is directly proportional to lactate concentration in the sample.

REAGENT

LACTATE REAGENT, 10 x 10 ml, Catalogue No. 735-10

When reconstituted according to directions, reagent contains the following concentrations of active ingredients:

Lactate oxidase (microbial)	400 µ/l
Peroxidase (horseradish)	2400 µ/l
Chromogen precursors	As required
Buffer	pH 7.2

Also contains stabilizers and fillers.

PRECAUTIONS

The Lactate Reagent is for "*in vitro* diagnostic use." Normal precautions exercised in handling laboratory reagents should be followed. Dispose of waste observing all local, state and federal laws.

CAUTION: Avoid contact and inhalation of Lactate Reagent.
Refer to Material Safety Data Sheets for any updated risk, hazard or safety information.

The following instruction should be adhered to when opening the red flip-seal cap as it has a sharp edge after opening:

- A tweezers, needle-nose pliers, forceps, de-cappers, spatula or similar type of object should be used to open and peel off the flip-seal from the vial. When doing this action, ensure it is done outwards, away from the body.
- Latex gloves should also be worn to provide further protection to the user.

PREPARATION

Reconstitute Lactate Reagent with volume of deionized water indicated on vial label. If the reagent is to be used in a discrete analyzer, please refer to the respective application procedure. After addition of water, stopper vial and immediately mix several times by gentle inversion. DO NOT SHAKE.

STORAGE AND STABILITY

Store the dry unopened Lactate Reagent refrigerated (2–8°C). Reagent is stable until expiration date shown on the label.

Reconstituted reagent is stable for 8 hours at room temperature (18–26°C) and for 7 days in the refrigerator (2–8°C). Freezing will extend stability for at least one month. Store reagent in an amber bottle.

DETERIORATION

The Lactate Reagent is suitable for use if the absorbance of reconstituted solution measured in a 1-cm lightpath at 540 nm vs water as reference does not exceed 0.08. Discard vial if the dry reagent exhibits caking due to possible moisture penetration, does not dissolve completely upon reconstitution or if the solution appears turbid.

REAGENT REQUIRED BUT NOT PROVIDED

LACTATE STANDARD SOLUTION, Catalogue No. 826-10

L(+)lactic acid, 40 mg/dl (4.44 mmol/l). Sodium azide, 0.1% added as preservative. Store in refrigerator (2–8°C). Reagent label bears expiration date.

OPTIONAL REAGENTS

LACTATE STANDARDS SET, Catalogue No. 735-11

Set contains aqueous standards with lactate concentrations of 20, 80 and 120 mg/dl.

DISCRETE ANALYZER APPLICATIONS

Application procedures using Lactate Reagent are available for various automated instruments. Please contact Trinity Biotech Technical Services Department for more information.

SPECIMEN COLLECTION

It is recommended that specimen collection be carried out in accordance with NCCLS document M29-T2. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.

Large and variable changes in lactate concentration may occur after specimen collection.[10,11] These changes may be minimized by using fluoride-oxalate as anticoagulant, keeping samples on ice and separating the plasma from blood without delay.[10,11] While lactate levels are not stable in whole blood, the separated plasma may be stored at 2–8°C for 48 hours prior to use. Plasma lactate is stable up to at least 1 month stored frozen at –20°C.

SAMPLE PREPARATION:

1. Draw blood with a minimum of stasis from fasting, resting patients into tubes containing fluoride/oxalate as anticoagulant. Mix well by gentle inversion at least 6 times. Cool blood tube in an ice bath.
2. Within 30 minutes separate the plasma from blood by centrifugation at 400 x g for 10 minutes. Avoid excessive forces which contribute to hemolysis.

NOTE: If separation of plasma from blood (kept on ice) is delayed, lactate values may variably increase by about 10% after 1 hour, and up to 15% after 2 hours.

INTERFERING SUBSTANCES

The lactate oxidase used in this procedure is specific for L(+)lactic acid. D(–)lactic acid is nonreactive. Among structurally related compounds tested, ß-hydroxybutyrate, malate, acetoacetate and α -ketobutyrate did not interfere.

Expected therapeutic levels of aspirin (up to 30 mg/dl plasma)[12] and acetaminophen (up to 2 mg/dl plasma)[13] do not interfere in this procedure.

While ascorbate may interfere, it is mostly cleared through the urine within 4 hours of ingestion. At tissue saturation levels, ascorbate is reported to have a plasma concentration of 1–1.5 mg/dl.[14] Our findings indicate that at this level ascorbate does not interfere.

Slight or moderate hemolysis (approximately 200 mg/dl hemoglobin) does not interfere. However, markedly hemolyzed samples may contribute to spuriously higher values and should be avoided.

Slight or moderately turbid samples cause insignificant interference. The effect of highly turbid samples may be corrected by use of a saline sample blank.

MANUAL PROCEDURE

MATERIALS PROVIDED

Lactate Reagent

MATERIALS REQUIRED BUT NOT PROVIDED

Lactate Standard Solution, Catalogue No. 826-10
Spectrophotometer capable of accurately measuring absorbance at 540 nm.
Test tubes or cuvettes.
Pipetting devices for the accurate delivery of volumes required for the assay
Centrifuge
Timer

PROCEDURE

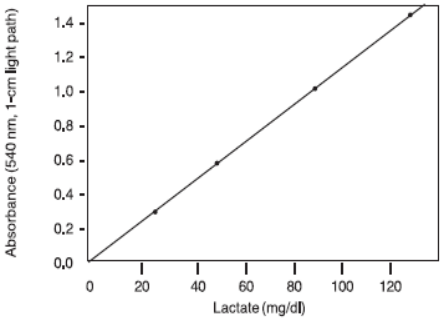
NOTE: Reaction may be carried out at any temperature between 25–37°C. Constant temperature control is not required. Protect from strong light.

1. Prepare Lactate Reagent Solution according to instructions under "Reagent" section.
2. Pipette 1.0 ml Lactate Reagent Solution into test tubes or cuvettes labeled BLANK, STANDARD, TEST 1, TEST 2, etc.
3. To STANDARD add: 10µl Lactate Standard Solution, Catalogue No.826-10. To TEST add: 10 µl plasma.
4. Incubate tubes for 5–10 minutes.
5. Read and record absorbance (A) of STANDARD and TEST vs BLANK as reference at 540 nm. Complete readings within 10 minutes following incubation.
6. To determine lactate concentration, refer to "Calculations" section.

CALIBRATION

The procedure is linear to lactate concentration of 120 mg/dl. A calibration curve is not required since a standard is included with each series of assays. However, to confirm the linearity of the procedure on your spectrophotometer, a calibration curve may be prepared using Lactate Standards Set, Catalogue No. 735-11, which contains Lactate Standards at concentrations of 20, 80 and 120 mg/dl. A typical calibration curve is depicted in Figure 1.

Figure 1. Typical Lactate Calibration Curve



NOTE: The typical calibration curve depicted cannot be used to derive laboratory results. Each laboratory must prepare a calibration curve.

QUALITY CONTROL

The reliability of test results should be monitored by routine use of a control with known lactate concentration. Lactate concentration should fall within the stated ranges of the controls.

CALCULATIONS

$$\text{Lactate (mg/dl)} = \frac{\text{Absorbance Test}}{\text{Absorbance Standard}} \times 40^*$$

*Concentration of lactate in standard.

EXAMPLE

Absorbance Test = 0.153
Absorbance Standard = 0.408

$$\text{Lactate(mg/dl)} = \frac{0.153}{0.408} \times 40 = 15$$

To convert the results to mmol/l, multiply mg/dl by 0.111. In example above, 15 mg/dl x 0.111 = 1.665 mmol/l.

LIMITATIONS

Trinity Biotech Lactate Reagent is linear to a lactate level of 120 mg/dl. If lactate level exceeds 120 mg/dl, dilute 1 part sample with 1 part water and reassay. Multiply result by 2 to compensate for dilution.

EXPECTED VALUES

Plasma (From Fasting Venous Blood)

3–12 mg/dl (0.33–1.33 mmol/l)

The expected values stated were taken from the literature. It is strongly recommended that each laboratory establish its own expected range, characteristics for the local population.

Increased lactate values in blood (plasma) have been reported to occur in the following conditions:

Tissue Hypoxia [3]
Severe muscular work [17,18]
Neuromuscular disorders [15]
Toxins and drug overdosage by ingestion or infusion (i.e., sorbitol, fructose, methanol, ethanol, salicylates and phenformin)[3,18]
Neoplastic disorders [2,3]
Congenital enzymatic defects that affect pyruvate and lactate metabolism [3,18]

PERFORMANCE CHARACTERISTICS

REPRODUCIBILITY

Between-run replicate assays of 3 samples with mean lactate concentrations of 8.2, 37.8 and 115.3 mg/dl yielded standard deviations of 0.15, 0.76 and 0.96 mg/dl with coefficients of variation of 1.8, 2.0 and 0.8%, respectively.

Within-run replicate assays of samples with mean lactate concentrations of 8.1, 37.3 and 115.3 mg/dl yielded standard deviations of 0.2, 0.5 and 1.3 mg/dl with coefficient of variation of 2.5, 1.3 and 1.1%, respectively.

CORRELATION

A series of 75 samples with lactate concentrations ranging from 4 to 120mg/dl was assayed using Trinity Biotech lactate oxidase system(x) and Trinity Biotech lactic dehydrogenase procedure, No. 826-UV (y). Respective means of 40.31 and 41.97 mg/dl were obtained with a correlation coefficient of 0.999. The equation for the regression line was y = 1.050x – 0.1578.

RECOVERY

Aliquots of plasma sample (7 mg/dl) were augmented with lactate to increase levels to 40, 80 and 120 mg/dl. Recoveries of 97.5, 100 and 97%, respectively, were obtained.

SENSITIVITY

The lowest level of lactate that can be measured by this method is considered to be 2 mg/dl, corresponding to an absorbance change of 0.02.

Trinity Biotech warrants that its products conform to the information contained in this and other Trinity Biotech publications. Purchaser must determine the suitability of the product for its particular use.

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ORDERING INFORMATION

Catalogue No.	Item	Quantity
735-10	LACTATE REAGENT	10x10 ml
REAGENT REQUIRED BUT NOT PROVIDED		
Catalogue No.	**Item**	**Quantity**
826-10	LACTATE STANDARD SOLUTION*	10 ml
OPTIONAL REAGENTS		
Catalogue No.	**Item**	**Quantity**
735-11	LACTATE STANDARDS SET Contains 2x5 ml each of aqueous standards with lactate concentrations of 20, 80 and 120 mg/dl	1 set

* Reagent also required for procedure

GUIDE TO SYMBOLS

Consult Instructions for Use

Store at 2-8°C

REF
Catalogue number

IVD
For *in vitro* Diagnostic Use

Use by

LOT
Batch code

REAG LACTATE
Lactate Reagent

Manufacturer

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