Lactate Dry-Fast

CE

Determination of lactate with an enzymatic-colorimetric method in serum, plasma and cerebrospinal fluid (CSF)

REAGENT 1a: 1 x 50 mL - REAGENT 1b: (lyophilized) 5 x 10 mL - STANDARD: 1 x 5 mL	**REF** 17285

IVD : in vitro diagnostic medical device
REAGENT : the term refers to the single reagent
STANDARD / CALIBRATOR : the term refers to the standard / the calibrator
CONTROL : the term refers to the control

PRECAUTIONS IN USE

- In addition to the possible risk indications regarding the reactive components, reagents may contain non-reactive components such as preservatives (i.e. sodium azide or other) and detergents. The total concentration of these components is lower than the limits reported by the 67/548/EEC and 88/379/EEC directives and following modifications and amendments about classification, labelling and packaging of dangerous preparations (reagents). However, it is recommended to handle reagents carefully, to avoid ingestion and contact with eyes, skin and mucous membranes and to use laboratory reagents according to good laboratory practice.

SUMMARY

Lactate is produced in Cori cycle, by anaerobic conversion of glucose, mainly in skeletal muscle. Its determination, frequently done together with pyruvate, is useful in discovering lactic acidosis due to reduced tissue oxygenation or enzymatic deficiencies.

PRINCIPLE

Lactate is oxidized by lactate oxidase to pyruvate and hydrogen peroxide, which, in presence of peroxidase (POD), reacts with TOOS* forming a compound, which colour intensity is proportional to the concentration of lactate in the examined sample.

** = N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline*

REAGENTS

Reagents, stored at 2-8 °C in unopened vials, are stable up to expiry date indicated on the package.
Reagents must be limpid; do not use if turbid.
Components of the kit and initial concentration of reactive components:

- **REAGENT 1a**
- **REAGENT 1b** lyophilized
  Good's buffer 50 mmol/L pH 7.0, TOOS ≥ 0.3 mmol/L, lactate oxidase ≥ 0.2 kU/L, peroxidase ≥ 1 kU/L, 4-aminoantipyrine 0.1 mmol/L, sodium azide < 0.1%
  NOTE: the indicated concentrations are referred to the Solution R1 obtained by reconstituting the contents of one vial of REAGENT 1b with 10 mL of REAGENT 1a.
- **STANDARD**
  lactate standard, sodium azide < 0.1%: The lactate concentration (8-10 mg/dL) is reported on the label of the vial

Barcode and bottle code number, if printed on reagent labels, are referred to the use of the product on Hitachi 911/912 analyzers. Please refer to the application and detailed information available upon request.

NOTES AND LIMITATIONS

1) REAGENTS PECULIAR INFORMATION:
   - the STANDARD value is verified using a primary standard (weighed in purified material).
   - A slight pink coloration of the Solution R1 will not influence the reagent performance.

Preparation of reagent solutions

Solution R1: add 10 mL of REAGENT 1a to each vial of REAGENT 1b; let stand for about 10 minutes and gently mix. Stability: 30 days at 2-8 °C after reconstitution, if contamination avoided and vial recapped immediately after use.
STANDARD: ready to use. Reagent in unopened vial is stable up to expiry date indicated on the package.
Stability: 120 days at 2-8 °C after opening, if contamination avoided and vial recapped immediately after use.

QUALITY CONTROL

The use of following control materials at different levels of analyte is recommended to verify test accuracy:

Clin Chem Control 1 — **REF 16150** — **6x5mL**
Lyophilized control serum. For use, follow the instructions contained in the kit.

Clin Chem Control 2 — **REF 16250** — **6x5mL**
Lyophilized control serum. For use, follow the instructions contained in the kit.

SAMPLE[1]**:** serum, plasma or CSF.

SERUM Sample[2]

- Collect samples in tubes containing the sodium-iodoacetate glycostatic agent (final concentration 5mg/10mL of blood). Stability: 2 days at 2-8 °C.

PLASMA Sample[5]

- Collect samples in tubes containing 2.5 mg of sodium fluoride and 2.0 mg of potassium oxalate for each mL of blood. Centrifuge within 15 minutes of collection and separate. Stability: 2 days at 2-8 °C.

Important Notes:

1) Lactate concentration rapidly increases during physical activities. Normal levels are reached again after usually 30 minutes but it may vary according to individuals.
2) Draw blood with lowest venous stasis as possible (max. 30 seconds) from fasting and resting patient and avoid using a tourniquet.
3) Sample glycolysis may rapidly increase lactate concentration and the cells play an important part in glycolysis. For an accurate analysis, it is therefore necessary to promptly proceed with the separation: keep blood on ice and separate plasma from the cells within 15 minutes of collection.

CSF Sample[5]

- Untreated freshly drawn cerebrospinal fluid. Stability: 1 day at 2-8 °C or 1 month at -20 °C.

Instrumentation and materials required but not provided

- Usual laboratory equipment
- Filters photometer or spectrophotometer

ANALYTICAL PROCEDURE

Wavelength:	550 (540-560) nm
Pathlength:	1 cm
Temperature:	37 °C
Sample/Solution R1:	1/100
Reaction:	end-point (increase)

Allow reagents to reach working temperature before using.
A proportional variation of the reaction volumes indicated in the analytical procedure does not change the result.

Put into cuvette	Reagent Blank	Sample	Standard
Distilled water	0.010 mL	-	-
Sample	-	0.010 mL	-
STANDARD	-	-	0.010 mL
Solution R1	1.0 mL	1.0 mL	1.0 mL

Mix carefully and incubate for 10 minutes at working temperature; read the absorbance of Sample (AS) and Standard (AST) against Reagent Blank. Final colour is stable for at least 1 hour.

CALCULATION

(AS / AST) x [STD]* = mg lactate/dL of sample

[STD]* = concentration of the STANDARD in mg/dL

Conversion Factor:

lactate: [mg/dL] x 0.1110 = lactate [mmol/L]

REFERENCE VALUES[4]

Serum/Plasma (venous):		4.5 - 19.8 mg/dL	(0.5 - 2.2 mmol/L)
CSF:	new-born	10 - 60 mg/dL	(1.1 - 6.7 mmol/L)
	3 to 10 days	10 - 40 mg/dL	(1.1 - 4.4 mmol/L)
	> 10 days	10 - 25 mg/dL	(1.1 - 2.8 mmol/L)
	adult	10 - 22 mg/dL	(1.1 - 2.4 mmol/L)

It is recommended that each laboratory establish its own expected range.

PERFORMANCES (determined on Hitachi automatic analyzer)

Interferences: the test is not affected by the presence of bilirubin up to 30 mg/dL, and triglycerides up to 1000 mg/dL. Ascorbic acid interferes in the test.
Measuring range: 1 - 150 mg/dL. Samples with concentration higher than 150 mg/dL (16.65 mmol/L) must be diluted 1:10 with normal saline and result multiplied by 10.
Intra-Assay Precision: it was determined on 20 replicates of each control (3 levels - L1/L2/L3). Results were as follows:
L1: average 14.6 mg/dL, SD 0.41, CV% 2.83 / L2: average 33.3 mg/dL, SD 0.76, CV% 2.29 / L3: average 55.7 mg/dL, SD 1.25, CV% 2.24.
Inter-Assay Precision: it was determined for 10 days on 2 replicates of each control (3 levels - L1/L2/L3). Results were as follows:

	Mean	Within Run		Run to Run		Total	Total
	mg/dL	SD	CV%	SD	CV%	SD	CV%
L1	14.53	0.33	2.29	0.35	2.44	0.49	3.34
L2	33.29	0.74	2.21	0.78	2.34	1.07	3.22
L3	55.29	1.12	2.03	1.15	2.08	1.61	2.91

Sensitivity: 1 mg/dL. Sensitivity was calculated on 10 replicates of normal saline and reported as the "mean zero value + 3 SD".
Accuracy: this test (y) was compared with a commercially available method (x). Results were as follows:
N = 60, r = 0.99738, y = 1.1152 x - 1.4049

WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

BIBLIOGRAPHY

1) NCCLS Document: “Procedures for the Collection of Arterial Blood Specimens; Approved Standard - Third Edition (1999)”.
2) Kaplan, L.A., Pesce, A.J.: “Clinical Chemistry”, Mosby Ed. (1996).
3) EU-Dir 1999/11 Commission Directive of 8 March 1999 adapting to technical progress the principles of Good Laboratory Practice as specified in Council Directive 87/18/EEC.
4) Jakobs, D.S., Kasten, Jr., B.L., Demmott, W.R., Wolfson, W.L.: “Laboratory Test Handbook”, Lexi-Comp and Williams & Wilkins Ed. (2nd Edition - 1990).
5) Tietz Textbook of Clinical Chemistry (Edited by Burtis CA and Ashwood ER Eds): Third Edition WB Saunders Company (1999).

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Note: significant changes in comparison to the previous version are indicated by a vertical bar in the text margin

SENTINEL DIAGNOSTICS
SENTINEL CH. SpA - Ph. +39 02 3455141
Via Robert Koch, 2 Milano 20152 Italy
www.sentineldiagnostics.com