LACTATE

OSR6193	**4 x 10 mL**	**R1 Buffer**
	4 x	**R1 Lyo**

Intended Use
System reagent for the quantitative determination of L-Lactate in human plasma and cerebrospinal fluid (CSF) on Beckman Coulter AU analyzers.

Summary[1,2,3]
L-lactate is the end product of anaerobic glycolysis. It is derived predominantly from white skeletal muscle, brain, skin, renal medulla and erythrocytes. Lactate dehydrogenase catalyses the reduction of pyruvate to lactate.

There are two major clinical settings in which lactic acidosis occur:
(1) Conditions associated with hypoxia e.g. shock, congestive heart failure, myocardial infarction, blood loss and pulmonary edema.
(2) Metabolic or drug/toxin related disorders. Examples of metabolic disorders include diabetes mellitus, hepatic disease and neoplasia.

Congenital metabolic disorders include type I glycogen storage disease. Examples of drugs/toxins which give rise to elevated lactate are methanol, ethanol, epinephrine and acetaminophen.
L-lactate levels in CSF will generally mirror those in blood/plasma. However, increased lactate levels in CSF in the absence of increased blood/plasma lactate concentration have been reported in cases of bacterial meningitis, cerebral hypoxia, ischemia and in certain inborn errors of metabolism e.g. pyruvate dehydrogenase deficiency, mitochondrial myopathies and biotinidase deficiency.

Methodology[4,5]
L-lactate is oxidized to pyruvate and hydrogen peroxide by lactate oxidase (LOD). A colored product is produced by the reaction of peroxidase (POD), hydrogen peroxide, 4 -aminoantipyrine and a hydrogen donor (TOOS). The colored product is measured photometrically. The color intensity is proportional to the concentration of lactate in the sample under examination.

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

$$H_2O_2 + \text{4–AA} + \text{H donor} \xrightarrow{\text{POD}} \text{Chromogen} + 2\,H_2O$$

System Information
For AU400/400^e/480, AU600/640/640^e/680 and AU2700/5400 Beckman Coulter Analyzers.

Reagents
Final Concentration of Reactive Ingredients

R1		
Lactate oxidase	≥ 0.2	kU/L
Peroxidase	≥ 1	kU/L
Good's Buffer (pH 7.0)	50	mmol/L
4-aminoantipyrine	0.1	mmol/L
TOOS*	≥ 0.3	mmol/L

Also contains preservatives
*N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline

Precautions
1. For *in vitro* diagnostic use.
2. Do not ingest. Harmful if swallowed.
3. To avoid the possible build-up of azide compounds, flush waste-pipes with water after the disposal of undiluted reagent and calibrator.
4. Dispose of all waste material in accordance with local guidelines.

Preparation of Reagents
R1: Dissolve the contents of one vial of R1 Lyo completely with the contents of one vial of R1 Buffer. Mix by gentle inversion and place on board the instrument.

Storage and stability
1. The unopened reagent is stable until the expiration date printed on the label when stored at 2 – 8°C.
2. Working reagent is stable for 30 days when stored in the refrigerated compartment of the Beckman Coulter AU analyzers.

Indications of Deterioration
Visible signs of microbial growth, gross turbidity, precipitate in the reagent, may indicate degradation and warrant discontinuation of use.
A slight pink coloration of the working reagent will not influence performance. The reagent can still be used providing the reagent blank is within specification.

Specimen Collection and Preparation[1,6,7,8]
Plasma or cerebrospinal fluid.
Do not use serum.
Plasma: Use plasma from blood collected into sodium fluoride-potassium oxalate tubes.
Glycolysis resulting from physical exercise gives rise to an increased lactate concentration in the bloodstream. Therefore, the patient should be at rest before taking the sample. In particular, movement of the hand or arm should be avoided. Keep the sample on ice and separate plasma from cells within 15 minutes of collection. Analyze the sample immediately. Avoid hemolysis. Note whether the sample is venous or arterial.

Lactate

CSF: Beckman Coulter recommends that CSF samples be collected in plain collection devices or sodium fluoride-potassium oxalate tubes. Care should be taken to avoid blood contamination during collection.

Sample Storage and stability[7,8]
Plasma: Analyze fresh. Samples are stable stored at 15 - 25°C for up to 8 hours or at 2 - 8°C for up to 14 days.
CSF: Analyze fresh. Samples are stable stored at 2 - 8°C for up to 24 hours.

Interfering Substances
Results of studies show that the following substances interfere with this Lactate procedure:
The criteria for no significant interference is recovery within 10% of the initial value.

Bilirubin:	No significant interference up to 16 mg/dL Bilirubin
Hemolysis:	No significant interference up to 500 mg/dL Hemolysate
Lipemia:	No significant interference up to 1000 mg/dL Intralipid*
Ascorbate	No significant interference up to 10 mg/dL Ascorbic Acid

* Intralipid, manufactured by KabiVitrium Inc., is a 20% IV fat emulsion used to emulate extremely turbid samples.

The information presented is based on results from Beckman Coulter studies and is current at the date of publication. Beckman Coulter Inc. makes no representation about the completeness or accuracy of results generated by future studies. For further information on interfering substances**,** refer to Young[9] for a compilation of reported interferences with this test.

Procedure
A complete list of test parameters and operational procedure can be found in the User's Guide appropriate to the analyzer.

Materials Provided
Lactate Reagent

Materials required but not provided
Chemistry Calibrator (Cat. No. DR0070 Level 2)

Stability of Final Reaction Mixture
The Beckman Coulter AU analyzers automatically compute every determination at the same time interval.

Calibration
The frequency of calibration for the Lactate procedure is every 30 days. Calibration of this procedure is accomplished by use of the Chemistry Calibrator (Cat # DR0070 Level 2), which is traceable to a gravimetrically prepared primary standard.

Recalibration of this test is required when any of these conditions exist:
1. A reagent lot number has changed or there is an observed shift in control values.
2. Major preventative maintenance was performed on the analyzer.
3. A critical part was replaced.

Quality Control
During operation of the Beckman Coulter AU analyzer at least two levels of an appropriate quality control material should be tested a minimum of once a day. In addition, these controls should be tested after calibration with each new lot of reagent and after specific maintenance or troubleshooting steps described in the appropriate User's Guide. Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure.

Results
Automatically printed out for each sample in mg/dL at 37°C.

Dynamic Range
The Lactate procedure is linear from 2 to 90 mg/dL. Samples exceeding the upper limit of linearity should be diluted with purified water and repeated. The sample may be diluted, repeated and multiplied by the dilution factor automatically utilizing the AUTO REPEAT RUN.

Expected Values[6]

4.5 – 19.8 mg/dL (0.5 – 2.2 mmol/L)	Plasma
10 - 60 mg/dL (1.1 – 6.7 mmol/L)	CSF, neonate
10 - 40 mg/dL (1.1 – 4.4 mmol/L)	CSF, 3-10 days old
10 - 25 mg/dL (1.1 – 2.8 mmol/L)	CSF, >10 days old
10 – 22 mg/dL (1.1 – 2.4 mmol/L)	CSF, adult

Expected values may vary with age, sex, diet and geographical location. Each laboratory should determine its own expected values as dictated by good laboratory practice.

Specific Performance Characteristics
The following data was obtained using the Lactate reagent on Beckman Coulter AU analyzers according to established procedures. Results obtained in individual laboratories may differ.

Precision[11]
Estimates of precision, based on CLSI recommendations[10], are consistent with typical performance. The within run precision is less than 5% CV and total precision is less than 5% CV. Assays of control material were carried out and data reduced following CLSI guidelines above.

N = 80	Within run		Total	
Mean, mg/dL	SD	CV%	SD	CV%
10.4	0.06	0.6	0.19	1.8
39.2	0.31	0.8	0.81	2.1
121.6	1.02	0.8	2.08	1.7

Method Comparison[11]

Plasma samples were used to compare this Lactate Reagent. The table below demonstrates representative performance on AU analyzers.

Y Method	AU640
X Method	Method 2
Slope	1.05
Intercept (mg/dL)	-1.1
Correlation Coeff. (r)	0.999
No. of Samples (n)	103
Range (mg/dL)	4.2 – 119.2

Patient CSF samples were used to compare this Lactate Reagent on the AU400/400^{e} against another commercially available Lactate Method (Method 2). Results of linear regression analysis were as follows:

Y Method	AU400/400^{e}
X Method	Method 2
Slope	1.06
Intercept (mg/dL)	-1.4
Correlation Coeff. (r)	0.999
No. of Samples (n)	56
Range (mg/dL)	10.9 – 66.0

Sensitivity

The typical change in absorbance for 1 mg/dL of Lactate is 0.01 mA.

References

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9. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.
10. CLSI/NCCLS. Evaluation of precision performance of clinical chemistry devices; approved guideline. CLSI/NCCLS document EP5-A;1999.
11. Data is on file for specific AU analyzers.

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