Pointe Lactate Reagent Set

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Intended Use
For the quantitative determination of lactate in human plasma. For *in vitro* diagnostic use only. **Rx Only**

Clinical Significance
Lactate determinations are used in the diagnosis of lactate acidosis. Shock is the most widely recognized cause of lactic acidosis although, it is possible for elevated lactate levels to precede shock. Myocardial infarction, severe congestive heart failure, pulmonary edema and blood loss are the common causes of shock which will produce lactic acidosis. Lactic acidosis may also result from renal failure and leukemia. Thiamine deficiency and diabetic ketoacidosis will usually result in increased levels of lactate.

Method History
Originally lactic acid determinations were performed by either titrametric or colorimetric methods.
The first enzymatic method for lactic acid was based on the transfer of hydrogen from lactate to potassium ferrocyanide by lactate dehydrogenase (LD). This procedure was very cumbersome and did not gain wide acceptance. More current enzymatic methods involved the measurement of NADH formed from the oxidation of lactate by LD.[1,2] This method has become more widely used, but still suffers from instability in many analyzer systems. The current enzymatic method is based on the action of lactate oxidase. This method is fast, accurate and is considerably more stable than previous enzymatic methods.

Principle
Lactate oxidase catalyzes the oxidation of lactic acid to pyruvate and hydrogen peroxide. Peroxidase then catalyzes the reaction of hydrogen peroxide with a hydrogen donor, in the presence of 4-aminoantipyrene, to form a dye. Color intensity, measured at 550nm, is proportional to the lactate concentration in the sample.

$$\text{Lactate} + O_2 \xrightarrow{\text{Lactate Oxidase}} \text{Pyruvate} + \text{Hydrogen Peroxide}$$

$$\text{Hydrogen Peroxide} + \text{TOOS} + \text{4-AAP} \xrightarrow{\text{Peroxidase}} \text{Dye (550 nm)}$$

Reagents
Lactate Reagent (R1): TRIS Buffer 100mM, 4-aminoantipyrene 1.7mM, Peroxidase (Horseradish) > 10,000 U/L, Surfactant, Stabilizer, Sodium Azide (0.09%) as preservative.
Lactate Reagent (R2): TRIS Buffer 100mM, Lactate Oxidase (Microbial) > 1,000 U/L, TOOS 1.5mM, Surfactant, Stabilizer, Sodium Azide (0.09%) as preservative.

Precautions
1. This reagent is for *in vitro* diagnostic use only.
2. Reagents contain sodium azide as preservative. Upon disposal flush with large volumes of water.
3. All specimens used in this test should be considered potentially infectious. Universal precautions as they apply to your facility should be used for handling and disposal of materials during and after testing.
4. Do not use the reagents beyond the expiration date printed on the kit label.

Reagent Preparation
Lactate reagents R1 and R2 are ready to use for instruments suitable for two reagent analysis.

Reagent Storage
All reagents are stable until the expiration date on the label when stored at 2-8°C.

Specimen Collection and Storage
Plasma collected in sodium fluoride/potassium oxalate is the recommended specimen. The specimen should be immediately placed on ice and the cells must be separated within 15 minutes.[3] The sample should be drawn from a stasis-free vein.[4] If not analyzed promptly, specimens may be stored at 2-8ºC for up to 2 days. If specimens need to be stored for more than 2 days, they may be stored for one month frozen at –20°C.[5]

Interferences
All interference studies were conducted based on the procedures recommended in NCCLS guideline No. EP7-P.[6] Hemoglobin at levels up to 500 mg/dl and Bilirubin at levels up to 20 mg/dl were found to exhibit negligible interference (<5%) on this method. Samples with levels of interfering substances higher than the upper limits should be diluted with physiological saline before assaying. Multiply the result obtained from the manual dilution by the appropriate dilution factor. For a comprehensive review of drug interference on lactate levels see Young et al.[7]

Materials Provided
Lactate (Liquid) Reagent Set

Materials Required but not Provided
1. Lactate standard or suitable serum-based calibrator.
2. Controls with normal and elevated levels of lactate.
3. Automated clinical chemistry analyzer capable of accommodating two-reagent assays.

Procedure
Below is a general example of the lactate test procedure for an automated analyzer. For assistance with applications on automated analyzers, please contact the manufacturer's Technical Service Department.

Sample	Reagent 1	37°C	Reagent 2	37°C	Measurement of
6ul	300ul	30 sec.	200ul	5 min	absorbance (700/546nm)

↓
Lactate results

Limitations
1. Anticoagulants containing citrate should not be used.
2. Protect the reagents from direct sunlight.
3. Samples with values greater than 15mmol/L must be diluted 1:1 with saline and re-assayed. Multiply the result by two.

Calibration
Use an NIST-traceable lactate standard, or a suitable serum-based lactate standard. The procedure should be calibrated according to the instrument manufacturer's instructions. If control results are found to be out of range, the procedure should be re-calibrated.

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Quality Control
Reliability of test results should be routinely monitored with control materials that reasonably emulate performance of patient specimens. Quality control materials are intended for use only as monitors of accuracy and precision. The recovery of control values within the appropriate range should be the criteria used in evaluation of future assay performance. Controls should be run with every working shift in which lactate assays are performed. It is recommended that each laboratory establish their own frequency of control determination. Quality control requirements should be determined in conformance with local, state, and/or Federal regulations or accreditation requirements.

Results
To convert from S.I. units to conventional units, multiply the S.I. units by 9.01.

Example: mmol/L x 9.01 = mg/dL Lactate

Expected Values
The following reference range is suggested for L-Lactate.[8]

Venous	0.5-2.2 mmol/L
Arterial	0.5-1.6 mmol/L

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

Performance
1. Assay Range: 0-15 mmol/L
2. Comparison: This lactate reagent was compared to the method performed on the Dade Chemistry Analyzer. The study was performed using 57 patient samples ranging from 0.3-10.4 mmol/L. Data was subjected to least-squares linear regression analysis which yielded a correlation coefficient(r) of 0.998 with a regression equation of $y = 0.97 x + 0.1$.
3. Precision: Within-Day precision for the Lactate Reagent was determined following a modification of NCCLS document EP5-T2.[9] Within-Day precision studies produced the following results:

		Within Day		
Sample	**N**	**Mean**	**S.D.**	**C.V.%**
Low	20	1.52	0.04	2.63
Mid	20	3.98	0.07	1.76
High	20	8.89	0.09	1.01

Day To Day precision was also determined following a modification of NCCLS document EP5-T2.[9] Day to Day precision studies produced the following results:

		Day To Day		
Sample	**N**	**Mean**	**S.D.**	**C.V.%**
Low	20	1.51	0.04	2.65
Mid	20	4.12	0.09	2.18
High	20	9.19	0.17	1.85

4. Sensitivity: The analytical sensitivity for lactate was determined to be 0.15 absorbance units per 1 mmol/L of lactate.

References

1. Gutmann, I., Wahlefeld, A., Methods of Enzymatic Analysis. 2nd Ed., Academic Press, New York, 1974, 1464.
2. Noll, F., Methods of Enzymatic Analysis. 2nd Ed., Academic Press, New York, 1974, 1465.
3. Tietz, N.W., Fundamentals of Clinical Chemistry, 4th Ed., W.B. Saunders Company, Philadelphia, 1996, 367.
4. Tietz, N.W., Clinical Guide to Laboratory Tests, 3rd Ed., W.B. Saunders Company, Philadelphia, 1995, 382-383.
5. Westgard, J.O., Lahmeyer, B.L, Birnbaum, M.L, Clin Chem 1972, 18:1334-1338.
6. National Committee for Clinical Laboratory Standards, National Evaluation Protocols for Interference Testing, Evaluation Protocol Number 7, Vol. 4, No. 8, June 1984.
7. Young, D.S., effects of Drugs on Clinical Laboratory Tests, 3rd Ed., AACC Press, Washington D.C., 1990.
8. Tietz, N.W., Fundamentals of Clinical Chemistry, 4th Ed., W.B. Saunders Company, 1996, 801.
9. NCCLS document "Evaluation of Precision Performance of Clinical Chemistry Devices" 2nd Ed., 1992.

Symbol Key
Use by (YYYY-MM-DD)	LOT Lot and batch code
REF Catalog number	Manufacturer
IVD *In vitro* diagnostic medical device	Temperature limitation
Consult instructions for use	**Rx Only:** Prescription Use Only
CE mark	EC REP Authorized representative in the European Community

REF L7596

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2°C 8°C IVD

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Certified to Perform Reagents
The Pointe reagents are certified to be manufactured according to specified parameters. Any Pointe reagent product not meeting specifications through its listed expiration date will be remedied immediately without charge.

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