

## SOP: Lactate Determination Assay Using Spectrophotometry

### Approvals:

Preparer: Jason McMillan

Date: 26JUN14

Reviewer: Dr. Maggie Bryans

Date: 27JUN14

### 1. Purpose:

1.1. Use of the Lactate Determination Assay.

### 2. Scope:

2.1. Applies to the quantitative determination of Lactate in Conditioned Media.

### 3. Responsibilities:

3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.

3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.

### 4. References:

4.1. <http://www.pointscientific.medtest.com/uploads/inserts/L7596-01-984.pdf>

### 5. Precautions:

5.1. Reagents contain sodium azide as preservative. Upon disposal flush with large volumes of water.

5.2. Do not use the reagents beyond the expiration date printed on the label.

### 6. Materials:

6.1. P-20 and P-1000 micropipette and tips

6.2. Micro centrifuge tubes

6.3. Timer

6.4. Spectrophotometer able to read at 550nm

6.5. Cuvettes

6.6. Heating block (37°)

6.7. Lactate reagents R1 and R2

6.8. Lactate standard

6.9. Control with known normal range

### 7. Procedure:

#### 7.1. Running Assay

7.1.1. Turn on heating block and set to 37°C.

7.1.2. Label micro centrifuge tubes "Blank," "Control," "Standard," "Sample Name #'s."

7.1.3. Prepare Lactate working reagent by combining R1 and R2 using a 3 to 2 ratio. Ex: Mix 3ml of R1 reagent with 2ml of R2 reagent.

7.1.4. Pipette 1.0ml of working reagent to all of the tubes and place in the 37°C heating block for 5 minutes.

7.1.5. Remove micro centrifuge tubes from the heating block.

7.1.6. Add 10µl of control solution to the "Control" tube, 10µl of Lactate standard to the "Standard" tube, and 10µl of sample to each of their respective "Sample" micro centrifuge tubes and mix by gently aspirating and dispensing the solution with the micropipette.

7.1.7. Place all of the micro centrifuge tubes except for the "Blank" micro centrifuge tube back into the 37°C heating block for 5 minutes.

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- 7.1.8. Remove the micro centrifuge tubes from the 37°C heating block and immediately remove 1ml from each microfuge tube and place it in a corresponding labeled cuvette.
- 7.1.9. Read and record the absorbance of the tubes at 550nm using the “Blank” tube to zero the spectrophotometer.
- 7.1.10. Record absorbance values for each of the tubes and calculate the concentration of Lactate.
- 7.2. **Calculate Lactate Concentration.**
  - 7.2.1. Formula to determine lactate concentration:  
$$\text{Lactate (mmol/L)} = \frac{\text{Abs of sample}}{\text{Abs of standard}} \times \text{Concentration of standard (mmol/L)}$$
  - 7.2.2. If the result exceeds 20 mmol/L, the sample should be diluted 1:1 with normal saline, ran again, and the result multiplied by 2.

#### **8. History:**

Name	Date	Amendment
Jason McMillan	26JUN14	Initial release