SOP: Measurement of Glucose and Lactate Concentrations in Cell Culture Media using the YSI 2500 Biochemistry Analyzer.

Approvals:

Preparer: Isaiah Dennis Date: 10DEC21
Reviewer: Robin Zuck Date: 20DEC21
Reviewer: Dr. Maggie Bryans Date: 20JAN22

1. Purpose:

1.1. Measurement of Glucose and Lactate Concentrations in Cell Culture Media using the YSI 2500 Biochemistry Analyzer

2. Scope: Applies to determining the Glucose and Lactate levels in conditioned cell culture media using manual sampling.

3. Summary of Methods:

3.1. Preparation of standards for analysis.
3.2. Preparation of samples for analysis.
3.3. Analysis of glucose and lactate data using Microsoft excel.

4. References:

4.1. YSI 2500 Biochemistry Analyzer manual.

5. Definitions:

5.1. Station 2- the location of the test tube holder on the front of the machine. (See Figure 1 for location of station 2)
5.2. Sample Module- The resting position of the sipper where samples are measured.

6. Precautions:

6.1. Routine care should be exercised in the handling of buffers and samples of biological materials, which may have harmful biological activity in the case of accidental ingestion, needle stick, etc.
6.2. Gloves, a lab coat, and protective eyewear should be worn when handling buffers and samples.
6.3. Do not use reagents beyond their expiration date, this may cause probes A and B to become fouled.
6.4. Samples with large particles should be avoided, this can cause the sipper to become clogged.

7. Responsibilities:

7.1. 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.
7.2. It is the responsibility of the students/technician to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.

8. Materials:

8.1. YSI 2363 Potassium Ferrocyanide (FCN)
8.2. YSI 1531 D-Glucose standard 9.00 g/L
8.3. YSI 1530 L-Lactate standard 30.0 mmol/L
8.4. 1.5 mL microfuge tubes and tube rack
8.5. Cell culture media samples.
8.6. YSI 2500 Biochemistry Analyzer
8.7. P-1000 micropipette and tips

9. Procedure:

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9.1. **Priming the fluid System**

Note: The priming of the calibrator bottle is performed daily before calibrating or sampling to remove air bubbles from the tubing

9.1.1. Confirm that the buffer solution and calibrator solution are present in the bottle rack on the right of the instrument and are not expired.

9.1.2. Tap the middle of the Biochemistry Analyzer display to turn it on. The display should show a blue home screen with six different icons. If it does not tap the red 'X' on the top left to get to the home screen.

9.1.3. Touch the service icon on the home screen to go to Service Screen.

9.1.4. From the Service screen, touch the "Module" tab.

9.1.5. Touch the button under B1 Pump to turn the buffer pump on.

9.1.6. Allow the pump to run for 2 minutes

9.1.7. Touch the button under B1 pump to stop the pump.

9.1.8. Touch the button under Cal 1A pump to turn it on.

9.1.9. Allow the pump to run for 3 minutes.

9.1.10. Touch the button under Cal 1A pump to stop the pump.

9.2. **Analysis of YSI standards.**

To ensure proper operation of YSI 2500 Bioanalyzer, the membrane integrity and linearity checks are performed daily before running the samples

Note: When a sample is being run, the YSI may attempt a scheduled calibration. Allow the system to calibrate, if the sample is not taken contact an instructor (the YSI may have failed a calibration and needs proper maintenance) (Refer to section 9.1 of SOP: Operation of YSI Biochemistry Analyzer for calibration solution replacement)

9.2.1. Confirm that the buffer solution and calibrator solution are present in the bottle rack on the right of the instrument and are not expired.

9.2.2. Label a 1.5 mL microfuge tube FCN. Transfer 1,000 μL of YSI 2363 Potassium Ferrocyanide into the labeled tube. Close the cap of the tube and place it in a tube rack.

9.2.3. Label a 1.5 mL microfuge tube glucose standard. Transfer 1,000 μL of YSI 1531 D-glucose standard 9.00 g/L into the labeled tube. Close the cap of the tube and place it in a tube rack.

9.2.4. Label a 1.5 mL microfuge tube lactate standard. Transfer 1,000 μL of YSI 1530 L-Lactate standard 30.0 mmol/L into the labeled tube. Close the cap of the tube and place it in a tube rack.

9.2.5. Tap the middle of the Biochemistry Analyzer display to turn it on. The display should show a blue home screen with six different buttons. If it does not tap the red 'X' on the top left to get to the home screen.

9.2.6. Tap the Run button in the middle of the top row. A screen with four different tabs will appear: Run Batch, Status, Results, and Run Stat. Tap the Run Batch tab.

9.2.7. Us the green arrows below the four tabs to navigate to the tube labeled FCN. Uncap microfuge tube labelled FCN, then place and hold the tube under the top lip of Station 2.

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(See Figure 1 for location of station 2.) Press the green start button on the bottom right corner of the display.

9.2.8. The display will automatically switch to the Results tab and the instrument will begin to run. The sipper will move towards station two. A sound will be heard when the sipper is just above the tube, this is the sipper dispensing air so that it can take a sample. It will then dip into the liquid. After the sipper has aspirated a small amount of FCN, it will analyze the FNC. Close tube and return to rack.

9.2.9. The analysis is finished when the tube on the display changes from blue to green. At this time the glucose and lactate concentrations will be displayed in g/L. The FCN limit for glucose concentration should be ≤ 0.05g/L and lactate concentration should be ≤0.03g/L. This confirms the integrity of the enzyme probe membranes. Record the data in the "YSI 2500 Biochemistry Analyzer Standard Reading Log" found in the YSI Equipment Binder.

9.2.10. If the glucose and lactate concentration does not display within the specification mentioned in step 9.2.8. check the FCN solution for cloudiness and/or slight yellow tint. If it is completely clear, the Glucose and lactate membranes need to be replaced. Contact an instructor. (Refer to section 9.3 of SOP: Operation of YSI Biochemistry Analyzer for membrane replacement.)

9.2.11. Tap on the Run Batch tab. Using the green arrows below the four tabs, navigate to the tube labeled Glucose. Uncap your Glucose Standard, then place and hold under the top lip of Station 2. Press the green start button on the bottom right corner.

9.2.12. The display will automatically switch to the Results tab and the instrument will begin to run. The sipper will move towards station two. A sound will be heard when the sipper is just above the tube, this is the sipper dispensing air so that it can take a sample. It will then dip into the liquid. After the sipper has aspirated a small amount of glucose standard, it will analyze the glucose standard. Close tube and return to rack

9.2.13. The analysis is finished when the tube on the display changes from blue to green. On the right of the display, Lactate standard concentration will be displayed as 0.00 g/L and Glucose standard will have a concentration of 9.00 g/L ±0.45 g/L (Range 8.55 to 9.45 g/L). Record the data in the "YSI 2500 Biochemistry Analyzer Standard Reading Log" found in the YSI Equipment Binder

9.2.14. Tap on the Run Batch tab. Using the green arrows below the four tabs, navigate to the tube labeled Lactate. Uncap your Lactate Standard, then place and hold under the top lip of Station 2. Press the green start button on the bottom right corner.

9.2.15. The display should automatically switch to the Results tab and the instrument should begin to run. The sipper will first go to the sample module, then move towards station two. A sound will be heard when the sipper is just above the tube, this is the sipper dispensing air so that it can take a sample. It will then dip into the lactate standard. After the sipper has aspirated lactate standard, it will move back to the sample module and analyze. Close tube and return to rack

9.2.16. The analysis is finished when the tube on the display changes from blue to green. On the right of the display, Glucose will be displayed as 0.00 g/L. Lactate will have a concentration of 2.67 g/L ±0.13 g/L (Range 2.54 to 2.80 g/L). Record the data in the

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"YSI 2500 Biochemistry Analyzer Standard Reading Log" found in the YSI Equipment Binder

9.3. **Analysis of Conditioned Media Sample**

9.3.1. Obtain a sample of conditioned cell media.

9.3.2. Tap on the "Run Batch" tab.

9.3.3. Tap on the edit button on the left side of the screen. A new window will open.

9.3.4. On the top right of the window next to "Batch Name" tap "Manual". A keyboard window should pop up, rename the batch name to the appropriate vessel and day of your sample (e.g."Spinner 1" for day 1 sample from spinner flask), and press the enter key.

9.3.5. On the right side of the window under chemistries, tap both glucose and lactate. A blue line under both will appear, Press the save button below chemistries to exit the window. Uncap your sample tube, then place and hold under the top lip of Station 2 Tap the start button on the bottom right corner.

9.3.6. The display should automatically switch to the Results tab and the instrument will begin to run. The sipper will first go to sample module, then move towards station two. A sound will be heard when the sipper is just above the tube, this is the sipper dispensing air so that it can take a sample. It will then dip into the sample. After the sipper has aspirated a sample, it will move back to the sample module and analyze the sample. Close tube and return to rack

9.3.7. The sample reading is finished when the tube on the display Changes from blue to green. On the right of the display, glucose and lactate concentration is displayed. Record the data in your lab notebook and/or batch record.

9.3.8. Once all the sample are analyzed, tap the red "X" on the top left corner.

9.4. **YSI data collection**

9.4.1. The display will show the Home Screen with six different buttons. If it does not tap the red 'X' on the top left to get to the home screen. Tap on the right button on the top row labeled "Data".

9.4.2. On the top of the display there should be two tabs labeled "Plate" and "Calibration". Tap the plate tab.

9.4.3. Directly below the plate tab is date range. Tap on one gray box and enter today's date then do the same for the other box. On the bottom half of the screen there will be a list of the different plates ran. Find the row named FCN, on the right side of the row, tap the black box. A green "X" will appear, Repeat for the Glucose standard, the Lactate standard, and your sample.

9.4.4. Tap on export on the top right to export it to the connected flash drive. A window will appear once export is complete, tap okay to close the window.

9.4.5. On the computer, locate the file on the flash drive. The name of it should be something like "BioAnalysis_21F102133_26-10-2021_13-49-30", giving you a time and date stamp. Rename your file to your team initials, day of sample and appropriate vessel name. Return the flash drive back to the YSI Biochemistry analyzer.

10. Attachments:

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Figure 1:

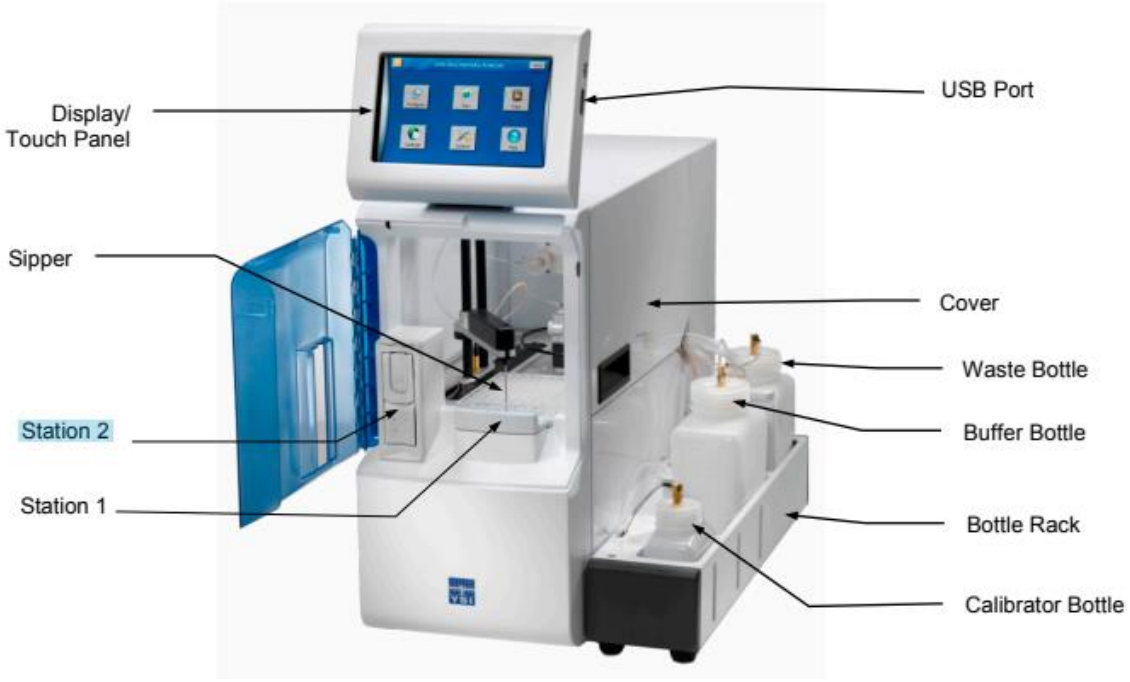
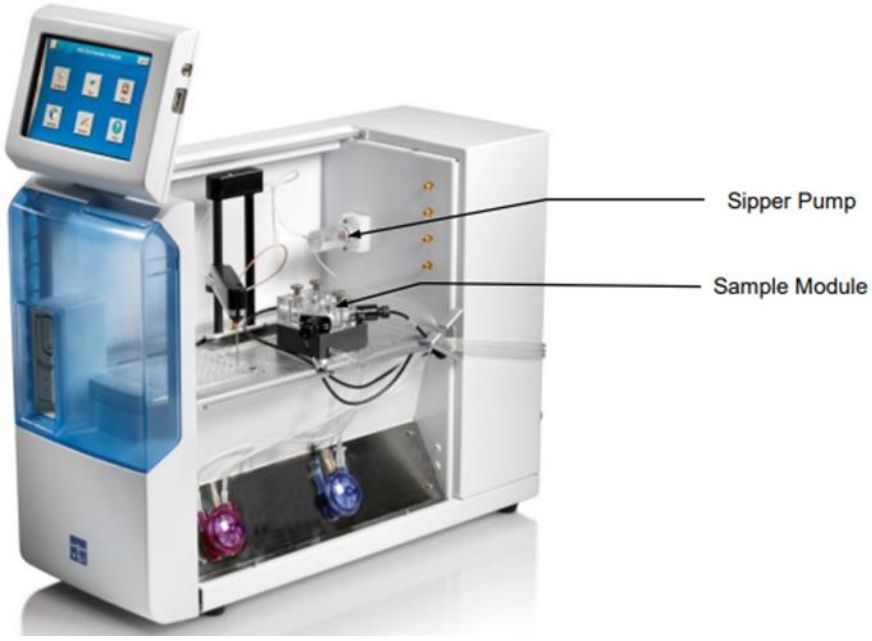


Figure 2:



11. History:

SOP: Measurement of Glucose and Lactate Concentrations in Cell Culture Media using the YSI 2500 Biochemistry Analyzer.

Revision Number	Effective Date	Preparer	Description of Change
0	23JAN22	Isaiah Dennis	Initial release
1	9MAY22	Hetal Doshi	Added Priming of the fluid system and details to the specification for glucose and lactate standard readings
2	11JAN24	Hetal Doshi	Made few changes in the steps for Analysis of Conditioned Media Sample