LACTATE ASSAY - COMPARISON AMONG PHOTOPETTE®, SPECTROPHOTOMETER AND MICROPLATE READER

Pei She Loh and Volker Patzel, Department of Microbiology & Immunology, *National University of Singapore, Singapore.*

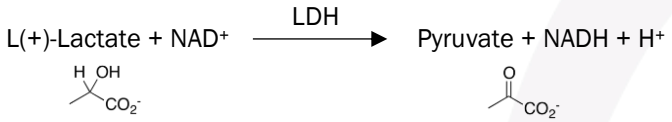
- *The Photopette® enables efficient determination of lactate concentration with an accuracy that is comparable with that of benchtop instruments.*
- *The enzymatic assay can be used for serum, plasma, urine, cell culture/fermentation media or in food & beverages.*

OBJECTIVE

In this application note we compare different readout instruments for the lactate assay – The Photopette® device, a Shimadzu spectrophotometer and a BioTek microplate reader.

INTRODUCTION

During the lactic acid or Cori cycle, L(+)-lactate produced by anaerobic glycolysis in muscles is transported to the liver and converted to D(+)-glucose via pyruvate. The glucose then returns to the muscles and is cyclically metabolized back to lactate. In this process, lactate dehydrogenase (LDH) catalyses the oxidation of lactate to pyruvate and an equimolar amount of β-nicotinamide adenine dinucleotide (NAD^+) is reduced to its reduced form NADH. The amount of NADH produced is directly proportional to the lactate concentration in the sample [1]. The produced NADH can be measured by its absorbance at 340 nm.



This enzymatic assay is applicable to quantify lactate in beverages, food and various biological samples including serum. Here, Photopette® Cell or Bio devices were used to quantify lactate concentrations in fermentation media at 340 nm.

MATERIALS AND APPARATUS

Instruments:

- Photopette® Cell or Bio with 340 nm wavelength.
- Spectrophotometer UV-1800 (Shimadzu).
- Microplate reader Synergy H1 (BioTek).
- Incubator (37°C).

Reagents:

- L-lactic dehydrogenase (Sigma Aldrich #L3916)
- Glycine buffer (Sigma Aldrich #G5418)
- 10 mg β-nicotinamide adenine dinucleotide (NAD^+) in pre-weighed vials (Sigma Aldrich, #N8285)
- Sodium L(+)-lactate (Sigma Aldrich, #L7022)

METHOD

EXPERIMENTAL PROCEDURE

Stock solution: To prepare a 20 mM lactate stock solution, 224 mg sodium lactate powder was dissolved in 100 mL deionized water. Thereof, standard lactate solutions with concentrations between 0.5 mM and 12 mM were prepared by serial dilution.

Reaction mixture: 2 mL of glycine buffer, 4 mL of water and 0.1 mL of L-lactate dehydrogenase were pipetted into an NAD vial and mixed thoroughly by inverting the vial several times (do not shake).

Protocol: Each 1.45 mL of the reaction mixture were pipetted into 2 mL reaction tubes labelled BLANK, CALIBRATION and SAMPLE. Subsequently, 50 µL of deionized water, standard lactate solution or sample were pipetted into the 'BLANK', 'CALIBRATION' or 'SAMPLE' reaction tubes. and incubated for 15 minutes at 37 °C.

Spectrophotometer: The double beam Shimadzu benchtop spectrophotometer was connected to the PC based software. Measurements were started 10 min after the device was switched on to allow the UV lamp to warm up and reach constant light output. The sample volume of 1.5 ml was placed in a plastic cuvette (10 mm pathlength) and A340 was measured. The blank sample was placed in the reference beam as negative control. The results of the measurements are displayed in Table 1.

Photopette: The Photopette® device was connect to the Photopette® iOS/Android app and '*Lactate Assay*' was selected as the measurement type. '*Dataset*' was selected (additional settings might apply) and the measurement was started (*'Start Measurement'*). A pre-warming phase is not required for this LED-based device. A user manual for operating and safety precautions referring to the Photopette® is available online in video-tutorials at **www.tipbiosystems.com** [2]. A CuveTip™ was placed firmly on the device probe and was dipped into the blank sample to perform an auto-zero measurement. Subsequently the samples with different lactate concentrations were measured using the same CuveTip. Between measurements, the CuveTip was washed by dipping into deionized water and dried by gentle contact with a wipe. All measurements were performed directly in the 2 mL cuvettes. The results of the measurements are displayed in Table 1.

Microplate Rader: Sample volumes of 250 µL were loaded into the wells of a 96 well microplate for the A340 reading with the microplate reader. '*Relative Absorbance*' was calculated by deducting the baseline absorbance (blank) from the sample readings. The results of the measurements are displayed in Table 1.

RESULTS

The results obtained from spectrophotometer, Photopette® and microplate reader are summarized in Table 1.

Table 1: Summery of average readings (3 repeats) measured with the spectrophotometer, five Photopette® devices (manufacturing no. 0057, 0058, 0059, Bio A, Bio B and Bio C), and the microplate reader.

Lactate concentration (mM)		0.5	2.0	4.0	8.0	12.0
Spectrophotometer		-0.001	0.040	0.056	0.093	0.137
Photopette®	0057	0.006	0.062	0.100	0.210	0.254
	0058	-0.02	0.046	0.106	0.152	0.254
	Bio A	0.033	0.060	0.108	0.180	0.250
	Bio B	0.009	0.063	0.099	0.181	0.263
	Bio C	0.004	0.019	0.101	0.168	0.261
Microplate reader (after subtraction of blank)		0.006	0.018	0.048	0.083	0.125

The data was used for the generation of a standard curve, and to determine experimental parameters such as limit of detection and linear range.

STANDARD CURVE

Standard curves were measured with all devices using lactate concentrations from 0.5 mM to 12.0 mM (Figures 1 to 3). A linear regression was performed on the data using Microsoft's Excel® software, and the equations of the standard curves along with the R-squared values were obtained. The slopes of the standard curves obtained were 0.0118 (spectrophotometer), 0.0204 (Photopette) and 0.0105 mM (microplate reader) respectively with high coefficients of determination (R^2>0.95) for all instruments.

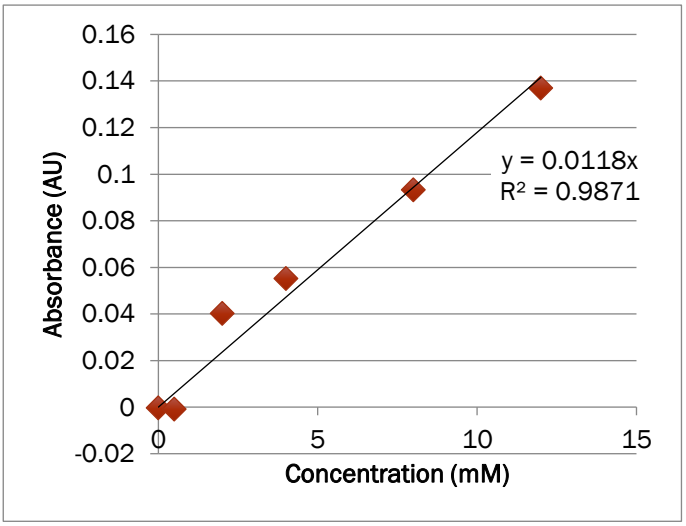


Fig 1: Absorbance (A340) and standard curve for lactate assay using the spectrophotometer (average of 3 repeats).

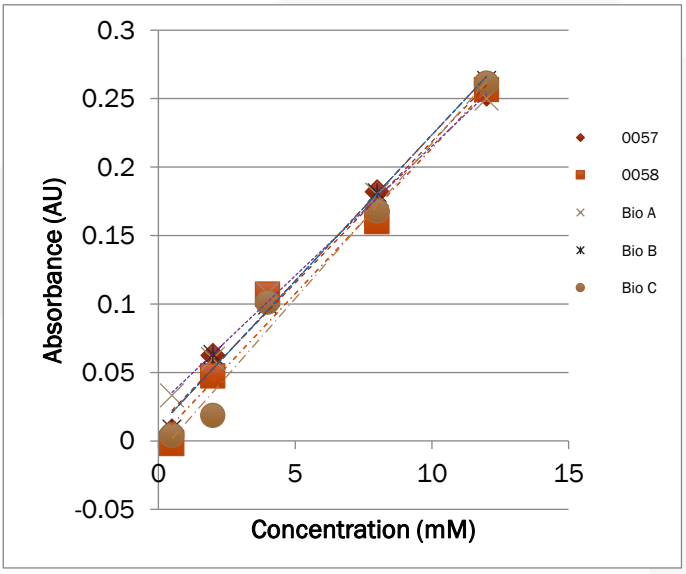


Fig 2: Absorbance (A340) and standard curve for lactate assay using the Photopette® devices.

Table 2: Summary of standard curve equations and R^2 values obtained for Photopette® devices.

	Slope	R^2
0057	0.0207	0.9886
0058	0.0212	0.9808
Bio A	0.0189	0.9976
Bio B	0.0213	0.9930
Bio C	0.0227	0.9843

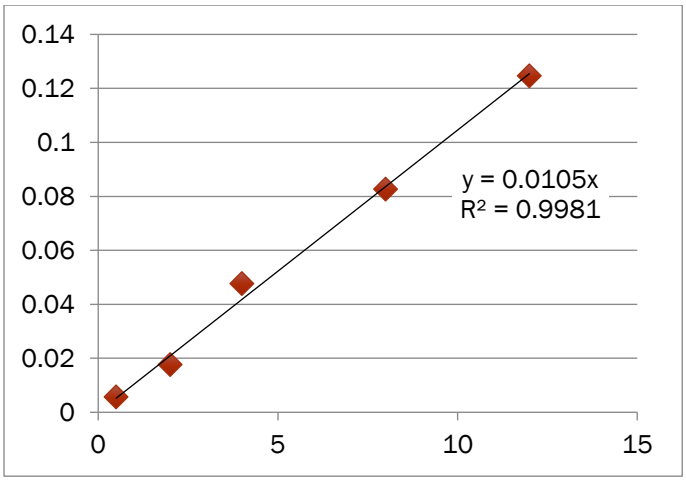


Fig 3: Absorbance (A340) and standard curve for lactate assay using the microplate reader.

DETERMINING THE CONCENTRATION OF SAMPLES USING THE STANDARD CURVES

Using the standard curves, we quantified the concentration of one sample. The concentration of the sample was expected to be 6 mM. For the quantification, the measured absorbance (A340) was entered into the equation of the respective standard curve (y) and the lactate concentration (x) was calculated (Table 3).

	Est. Lactate Conc. (mM)	Error (%)
Spectrophotometer	6.69	11.5
Microplate reader	5.14	-14.3
0057	4.98	-17
0058	5.23	-12.8
Bio A	4.84	-19.3
Bio B	5.63	-6.2
Bio C	6.39	6.5

Table 3: Comparison of Photopette®, spectrophotometer and microplate reader. The Photopette showed an average error of 13.0% which is comparable to the errors of the spectrophotometer of 11.5% and the microplate reader of 14.3%.

SUMMARY & DISCUSSION

Lactate assays were performed using three different devices: a benchtop spectrophotometer, a benchtop microplate reader, and handheld Photopette devices. All instruments performed well but some differences are noted.

Standard curves generated with the Photopette® devices exhibited a higher slope, indicating higher sensitivity of detection compared to the spectrophotometer and the microplate reader.

The five Photopette devices have shown some differences in sloop and in the lactate concentration that was measured. That is acceptable considering the significantly lower price of the Photopette. However, if several spectrophotometer and microplate reader would be used there would be differences as well. From this study we cannot say if the differences would be smaller, similar or higher. Allover, the Photopette exhibited about the same accuracy then the competitor devices. The good quality of Photopette® measurements is attributed to the way how the device works and analyses the data. Each Photopette® measurement is generated from a series of short measurements (LED flashes) and results are calculated by deduction of environmental light. Therefore, a result displayed at the Photopette® app represents an average of several measurements. The LED technique of the Photopette does not call for a warmup period and the device were immediately ready to measure after switching it on.

ADAPTATION FROM MANUFACTURER'S PROTOCOL

Notably, the manufacturer's protocol for the lactate assay is designed for detection using the spectrophotometer or microplate reader. To adapt the protocol for the use of Photopette®, the total volume of each assay can be reduced to 250 µl.

REFERENCES

[1] Sigma Aldrich, "L-Lactic Dehydrogenase from bovine heart #L3916."
[2] Tip Biosystems Pte Ltd, "Photopette® User Manual V1.0.0," *Singapore,* 2017.
[3] Tip Biosystems Pte Ltd, Technical Note "How to use Photopette®'s CuveTip® correctly"

Conflict of Interest Statement

Volker Patzel is shareholder of Tip Biosystems. This shareholding did not affect the results, interpretations and conclusions.

Disclaimer

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