Useful tips to avoid preanalytical errors in blood gas testing: metabolites

January 2004

Gitte Wennecke
Radiometer Medical ApS
Åkandevej 21
DK-2700 Brønshøj
Denmark

Measurement of glucose and lactate after 30 minutes of storage at room temperature can give deviating results of up to 5 % [1,2] and 29 % of the reference value [3] respectively [1,4], due to in vitro glycolysis

Glucose and lactate are both important parameters in the surveillance of patients in critical care [5,6,7]. Glucose has always been one of the most frequently measured parameters in laboratory medicine, used for the diagnosis and monitoring of diabetes mellitus and other metabolic disorders. Diabetes mellitus directly and indirectly cost USD 132 billion in the US in 2002 [8]. Preanalytical errors when measuring glucose, demanding repeated measurements, also contributed to increasing the costs.

Since lactate became available as a STAT parameter on e.g. blood gas analyzers, it has become an indicator of circulatory impairment and the overall state of oxygenation of patients in critical care [6]. A preanalytical error of 29 % has serious consequences in the interpretation of the critical condition of a patient due to the small span from the reference value to the value where immediate action is needed.

This article provides useful tips for avoiding preanalytical errors on glucose and lactate measurements; tips, which you can incorporate in the standardized procedures and training of your staff. The article is a part of a series of articles dealing with preanalytical errors in blood gas and related parameters.

Introduction

For the purpose of this article, a preanalytical error is defined as a deviating result caused by one of the following steps in the preanalytical phase:

- Patient preparation
- Blood sampling
- Sample handling
- Sample transport and storage

This article focuses on preanalytical issues concerning the metabolite parameters available on a modern blood gas analyzer (cGlu and cLac) when measured on an arterial whole-blood sample together with blood gas parameters. However, the focus of the article is not restricted to this application only, as metabolites are also measured on

venous blood, capillary blood, plasma and serum.

Analytical factors causing a deviation in results and biological variations are not dealt with in this article.

The article starts by giving a general description of the main reasons behind preanalytical errors when measuring metabolites and the biochemical background of their influence on measurements.

Next, it provides lists with useful tips on how to avoid preanalytical errors when measuring metabolite parameters on blood gas analyzers. The lists can be used as checklists when a specific problem is encountered, or as a tool to supplement or expand the knowledge of the staff involved in laboratory medicine, e.g. when updating procedures or when conducting refresher training.

Preanalytical issues – main reasons of preanalytical errors
The three main reasons of preanalytical errors when measuring metabolites on a blood gas analyzer are:

1. Storage time
2. Storage temperature
3. Abnormal cell count

1. Storage time

The glycolysis continues in whole blood after sampling. This means that the cells continue to metabolize glucose with the purpose of producing energy for the cellular functions. The glycolysis is a metabolic pathway that converts one glucose molecule into two molecules of pyruvate. Depending of the type of cell and the availability of oxygen, pyruvate will be further metabolized by one of two pathways.

If oxygen is present (aerobic metabolism), pyruvate will be oxidized to CO_2 and H_2O in a regulated process that creates energy (see Fig. 1). Under anaerobic conditions and in the erythrocytes, pyruvate will be reduced to lactate; a process that creates much less energy (see Fig. 2). This means that the glycolysis will consume glucose and produce lactate in a whole-blood sample stored for a longer period of time.

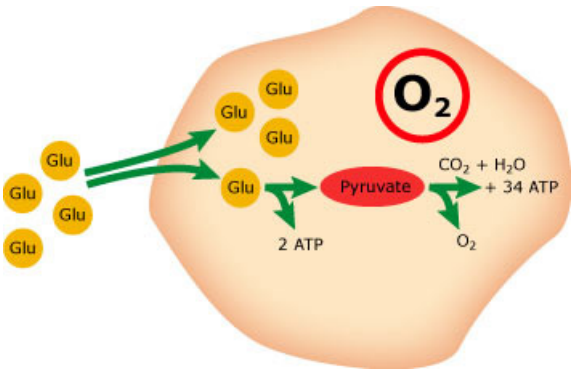

FIG. 1: Aerobic metabolism

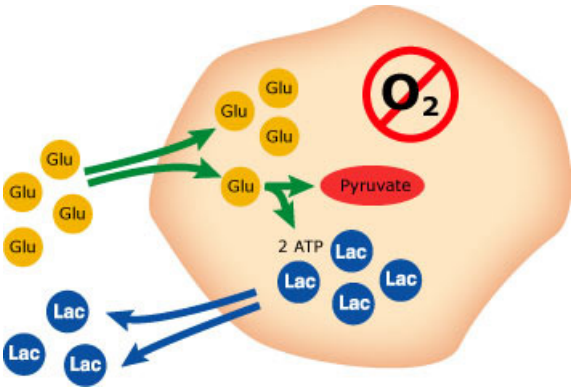

FIG. 2: Anaerobic metabolism

Example:

The rate of change of cGlu and cLac at room temperature (22-25 °C (72-77 °F)) found in various studies are presented in the table below. All studies are based upon samples with values within the reference range:

	Rate of change mmol/L/h	Rate of change mg/dL/h	Rate of change in percentage of reference value*	Reference
cGlu	-0.5	-9	10 %	[1]
	-0.5	-9	10 %	[2]
	-0.3	-5	6 %	[4]
	-0.4	-6	8 %	[9]

TABLE I

	Rate of change mmol/L/h	Rate of change mg/dL/h	Rate of change in percentage of reference value*	Reference
cLac	+0.6	+5	57 %	[1]
	+0.6	+5	57 %	[4]
	+0.4	+4	38 %	[10]
	+0.4	+4	38 %	[11]

TABLE II

*Normal value is calculated as the mean reference value [3].

Reference values

*c*Glu(fPt)(aP):

- 3.9-5.8 mmol/L
- 70-105 mg/dL

cLac(aP):

- 0.5-1.6 mmol/L
- 4.5-14.4 mg/dL

fPt = fasting patient
aP = arterial plasma

Based on Tables I and II, it can be concluded that the average rate of change per hour for *c*Glu and cLac at room temperature is –0.40 mmol/L/h and 0.50 mmol/L/h, respectively.

2. Storage temperature

The on-going in vitro glycolysis in samples that cannot be measured immediately can be avoided or reduced to a minimum by different procedures. Two common methods are to add an antiglycolytic agent, such as a fluoride salt, to the sample, or simply to store the sample at or below 4 °C (34 °F).

However, fluoride salts only have a significant effect after a minimum of one hour [12,13,14,15] of storage and may even have a hemolytic effect on the erythrocytes [1,16]. Besides, fluoride is not compatible with the biosensors used for glucose and lactate determinations in blood gas analyzers [17].

Immediate analysis (defined by Kost *et al* [1] to be within 15 minutes after the sample has been taken) or cool storage are the preferred and easiest options for avoiding glycolysis. Storage at cool temperatures slows down the glycolytic processes, having a significant effect on especially lactate.

Example:

The influence of storage temperature on lactate (Table III) and glucose (Table IV) found in various studies are presented in the table below. All studies are based upon samples with values within the normal range:

cLac (mmol/L) changes after one hour of storage		Reference
At 4°C (34 °F)	At room temperature	
+0.06	+0.63	[4]
+0.4	+0.4	[10]
+0.09	+0.42	[11]
+0.21	+0.72	[18]
+0.12	+0.45	[19]
Mean: 0.18 mmol/L	Mean: 0.52 mmol/L	

TABLE III

*c*Glu (mmol/L) changes after one hour of storage		Reference
At 4°C (34 °F)	At room temperature	
-0.03	-0.32	[4]

TABLE IV

There is a huge discrepancy in the level of changes shown in the various studies concerning lactate. The reason may be that different measuring and preparation methods have been used. However, the effect of storage temperature on cLac and *c*Glu cannot be questioned!

3. Abnormal cell count

The glycolysis continues in vitro, because of the presence of energy-consuming cells in the sample. The cells continue to metabolize glucose to produce energy for various cellular functions, e.g. the Na-K pump [20]. The cells are divided into three main groups and their numbers are determined by a complete blood cell count (CBC):

1. Erythrocytes or red blood cells
2. Thrombocytes or platelets
3. Leukocytes or white blood cells

An abnormally high blood cell count will influence the rate of glycolysis and thereby the stability of cGlu and cLac over time. Especially leukocytosis [18] but also an increase of erythrocytes (high hematocrit) [13,15,21] have been reported to affect the rate of the glycolysis.

Example:

The influence of leukocytosis (Table V) and high hematocrit (Table VI) on cLac and cGlu at room temperature is often mentioned in the literature [1,10,18,22,13,15,21]. However, only few studies present actual data. All studies in Tables V and VI are based upon samples with values within the normal range:

Leukocyte count	Rate of change of cLac mmol/L/h	Reference
23–53 x 109 cells/L	+0.8*	[10]
> 60 x 109 cells/L	+0.8*	[18]

TABLE V

*This value should be compared with the average rate of change for cLac at room temperature with a normal cell count (refer to Tables II and III) at +0.5 mmol/L/h.

Hematocrit %	Rate of change of cGlu %/h	Reference
35 %	11 %	[2]
55 %	19 %	[2]
43 %	7 %	[15]
75 %	12 %	[15]

TABLE VI

Reference values [23]

Leukocytes:

- 4.5–11 x 109 cells/L

Hematocrit:

- (male) 42-52 %
- (female) 35-47 %

3.1 Neonatal blood

Neonatal blood is known to have a very high hematocrit level and is therefore expected to have a higher rate of glycolysis [14,16,15,24]. There is some discrepancy in the reporting of the rate of glycolysis in neonatal blood. A recent study [24] showed a rate of change for glucose levels in neonate blood of –0.36 mmol/L/h (~ 8 %), which is very similar to the rate of change for adult blood (~-0.40 mmol/L/h).

However, an older study [14] showed a rate of change per hour of 23.7 %. In any case, it must be emphasized that the changes are significant for newborn infants with the risk of hypoglycemia.

Storage conditions – conclusion

The effect of storage time on cGlu and cLac in whole blood depends on time, temperature, blood cell count, pO_2 and tHb [1,10,18]. However, if the general recommendation for storage of samples for blood gas measurement is followed, the influence of these factors will be negligible [18].

Useful tips

Below you will find detailed lists of preanalytical issues affecting cGlu and cLac. The information found in the lists is based on observations reported in various works relating to the preanalytical phase. Some of the observations have a larger impact on the measurement result than others. Nevertheless, no observation should be ignored.

The lists can be used as checklists for training in the different steps of sampling, or whenever a specific problem is encountered during:

- Patient preparation
- Blood sampling
- Sample handling
- Sample transport and storage

Useful tips when measuring glucose (cGlu)

Make sure that:	To avoid:
Patient preparation	
The sample is not drawn from a patient to whom an infusion solution containing glucose has been given within the last hour [16,25]	cGlu↑
Blood sampling	
The sample type is specified when reporting the result, as there is a difference between arterial, venous and capillary blood [26,27,28]	cGlu↑↓
Sample handling	
Samples containing fluoride [11] as a stabilizer are not measured on a blood gas analyzer	cGlu↓
Sample transport and storage	
Samples that are not measured immediately are stored at 0-4 °C (32-39 °F)	cGlu↓

Useful tips when measuring lactate (cLac)

Make sure that:	To avoid:
Patient preparation	
The sample is not drawn from a patient to whom an infusion solution containing lactate has been given within the last hour [16,25] The patient has not exercised prior to the sampling [16,29]	cLac↑
Blood sampling	
The venous stasis is released after max. one minute to avoid anaerobic metabolism [4,16,25] The sample type is specified when reporting the result, as there is a difference between arterial, venous and capillary blood [30,31]	cLac↑ cLac↑↓
Sample handling	
The sample is not hemolyzed, as this will release intracellular lactate dehydrogenase, which promotes the conversion from pyruvate to lactate [2] Samples containing fluoride [11] as a stabilizer are not measured on a blood gas analyzer	cLac↑
Sample transport and storage	
Samples that are not measured immediately are stored at 0-4 °C (32-39 °F)	cLac↑

Discussion

Storage time and temperature are the two most important preanalytical issues to be aware of when measuring cGlu and cLac. If the samples are stored correctly, or as generally recommended for blood gas samples, the rate of glycolysis will not influence the measurement result even if the sample has an abnormal blood cell count. However, if the storage time is prolonged, all of these factors will have a significant influence on the measurement result.

Other issues that are important to mention here, even though they are analytical problems rather than preanalytical, are to know what is measured and what is reported.

There are a number of different measuring methods available for cGlu and cLac, e.g.:

- Biosensors on blood gas analyzers
- POC glucometers
- Chemistry analyzers

The different methods measure on different specimen types, such as the plasma phase of whole blood, whole blood, plasma or hemolysate. It can be difficult to distinguish between preanalytical errors and analytical variations that come from different measuring techniques [32,33] – especially if the different methods are compared with one another. To learn more about different ways of reporting glucose results, read [34].

Conclusion

Measurement of plasma glucose is the single most frequent measured parameter in laboratory medicine today, while lactate is becoming an important prognostic parameter in intensive care.

This article has described the three main causes of preanalytical errors related to measurements on these two parameters and provided tips that will help you prevent errors when measuring these parameters on blood gas analyzers.

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