

Useful tips to avoid preanalytical errors in blood gas testing: neonatal total bilirubin

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50-75 % of all newborns develop jaundice during their first week of life [1,2,3]. The decision behind which treatment is necessary and when it should be initiated is based upon a measurement of the concentration of total bilirubin (ctBil) in serum, plasma or whole blood.

It is therefore obvious that it is important to consider the requirements to the neonatal total bilirubin analysis, and it is well known that a large contribution to the total uncertainty generally comes from the preanalytical phase [4].

This article provides useful tips for avoiding preanalytical errors on total bilirubin measurements; tips, which you can incorporate in the standardized procedures, training of your staff or whenever method comparisons of ctBil are conducted. The article is a part of a series of articles dealing with preanalytical errors in blood gas testing 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 neonatal total bilirubin measurement available on a modern blood gas analyzer (ctBil) when measured on a capillary whole-blood sample together with blood gas parameters. However, the focus of the article is not restricted to this application only, as total bilirubin is also measured on arterial blood, venous blood, plasma, and serum.

The article starts with a short summary of the bilirubin metabolism and some analytical issues, followed by a description of the main preanalytical considerations when measuring total bilirubin. Next, it provides a list of useful tips on how to avoid preanalytical errors when measuring total bilirubin on blood gas analyzers.

The list can be used as checklist when a specific problem is encountered, a method comparison is conducted 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.

Bilirubin metabolism

Bilirubin is a product of the decomposition of heme groups from e.g. hemoglobin from aged erythrocytes. The bilirubin molecule is insoluble in water and is therefore transported in blood by albumin to the liver. Albumin delivers bilirubin to the liver where it is solubilized by conjugation with glucuronic acid. The conjugated bilirubin is secreted into the bile and ultimately excreted via the intestine. Defects in metabolizing bilirubin give rise to jaundice.

The following types of bilirubin are found in plasma:

- Unconjugated bilirubin – also called indirect bilirubin. It is poorly soluble in water and is toxic in high concentrations due to its solubility in fatty tissue.
- Unconjugated bilirubin transported by albumin. It is water-soluble and non-toxic.
- Mono- or diconjugated bilirubin – also called direct bilirubin. It is water-soluble and non-toxic.
- Delta bilirubin. It is covalently bound to plasma proteins, is water-soluble and non-toxic.
- 50-75 % of all newborns develop jaundice during their first week of life [1,2,3] due to either an increased hemoglobin catabolism or an immature conjugation system in the liver – also called physiological jaundice – or due to pathological conditions such as e.g. hemolytic diseases.

Jaundice is a condition that causes a pronounced yellow tint to the skin and the white part of the eyes because of accumulation of unconjugated bilirubin pigments in the tissue. Infants with high concentrations of unconjugated bilirubin can be treated with phototherapy, which decomposes the accumulated bilirubin in the skin into stereoisomers of bilirubin that can be excreted in the bile without conjugation.

If this treatment is unsuccessful, i.e. if the concentration of bilirubin continues to increase, the infant can be given exchange transfusions.

The decision behind which treatment is necessary and when it should be initiated is based upon a measurement of ctBil in serum, plasma or whole blood. The action limits can be based on a combination of age, birth weight and the clinical condition of the infant, and are often very narrow.

It is therefore obvious that it is important to consider the requirements to the neonatal total bilirubin analysis, often focused on important issues such as analytical performance, necessary blood volume, turnaround time, etc., but it is also well known that a large contribution to the total uncertainty generally comes from the preanalytical phase [4].

Analytical issues

The measurement of bilirubin has always had a rather bad reputation, since Mather in 1960 stated that “bilirubin determinations are perhaps the most notoriously unreliable of any in clinical chemistry” [5], and several studies since then has supported this conclusion, e.g. Watkinson in 1980 [6] and Vreman in 1996 [7].

The latest findings by Lo/Doumas in 2004 [8] are that the analytical precision has improved but that “[t]he evaluation of accuracy is impossible with specimens consisting of bovine serum containing a mixture of unconjugated bilirubin and ditaurobilirubin” since some methods are affected by matrix effects. However, it is not the purpose of this article to deal with analytical factors or biological variations causing a deviation in results.

Preanalytical issues

The three main preanalytical considerations when measuring total bilirubin on a blood gas analyzer are:

1. Bilirubin stability
2. Sampling site
3. Neonatal blood collection

1. Bilirubin stability

When a sample is drawn there is no continued metabolism that will cause a change in the bilirubin concentration. However, it is well known that bilirubin in a sample can be degraded by exposure to light, which is also used as treatment of hyperbilirubinemia. Irradiation of a serum sample changes the structure of the bilirubin molecule into a number of different polar (water-soluble) photoproducts.

Some studies have shown that in vitro bilirubin may be degraded up to as much as 50 % [9] by light degradation.

Only very few spectrophotometric methods measure total bilirubin on whole blood, thus no publications have yet described the light degradation in whole blood. It has been speculated that the erythrocytes protect the sample from irradiation, but this theory still needs confirmation.

Example:

The conversion rate of ctBil at room temperature at different irradiation wavelengths found in various studies are presented in the table below: All results are based upon bilirubin-enriched serum/plasma or whole blood.

tBil – initial conc.	% of initial conc. after one hour	Wavelength of irradiation	Reference
436 µmol/L	95 % tBil 63 % unconj. Bil	340-700 nm	[10]
350 µmol/L	~94 %	>380 nm	[11]
700 µmol/L	92.5 % tBil (plasma) 96.5 % tBil (whole blood)	~375 nm	Unpublished study by Radiometer

TABLE I. Conversion rate of ctBil caused by light exposure.

An analysis of the results in the table above shows that to reproduce the 50 % [9] degradation of the ctBil caused by light it is necessary to be aware of the exact study conditions. The majority of the results indicate only minor changes, which may be due to different study protocols.

Some studies have shown that the conversion rate is larger for some subfractions (e.g. unconjugated bilirubin) than others [10] and that the degradation is dependent on the irradiation intensity and wavelength [12], which often differ from one study to another. Another problem when comparing different studies are that they are based on different methods, and some methods are less specific than others, thus measuring the photoisomers as well [10,11].

The latter is, however, only a problem in in vitro studies where the photoisomers are present in the sample after degradation whereas the photoisomers in neonates

receiving light treatment are excreted very fast and are thus not present in the sample [11].

The general recommendation must therefore be either to measure the sample immediately as a whole-blood sample on a blood gas analyzer in the POC setting or to draw the sample in an amber-colored tube to protect the serum/plasma sample from light. [13]

2. Sampling site

Should the sample be an arterial, venous or capillary sample?

From a theoretical point of view, various factors may cause a difference in the reported bilirubin concentration from an arterial, venous and capillary specimen. This may be a true physiological difference such as:

- Subcutaneous bilirubin may increase the bilirubin

- concentration if picked up during capillary sampling
- Phototherapy; after phototherapy the bilirubin content of the capillary sample might be lower than the 'true' value, as the bilirubin has migrated from the subcutaneous tissue into the circulation
- The different measuring methods may experience interference from substances that are present in varying concentrations at the various sampling sites, e.g. oxyhemoglobin
- or it may be caused by preanalytical and analytical factors.

Example:

Various studies on the difference in bilirubin concentration between the different sampling sites have been published in the literature and some of the results are as follows:

Conclusion	Explanation
Venous = capillary	There is no clinically significant difference whether the sample is venous or a capillary sample from the heel. There is no influence from postnatal age, weight or treatment with phototherapy [14].
Capillary (7 %) > arterial	The bilirubin content of capillary samples tends to be 7 % higher than that of arterial samples, but this does not produce any clinical significant difference, and the results from the two sampling sites can be used interchangeably [15].
Arterial = capillary	There is no clinically significant difference between arterial and capillary samples (heel prick). However, there is a small tendency that the bilirubin content of capillary samples is higher than that of arterial samples [16].
Arterial = capillary	Capillary and arterial results showed a linear correlation ($r = 0.9$) suggesting no influence of environmental light on peripheral bilirubin isomerization [17]
Capillary > venous	The bilirubin content of capillary samples tends to be higher than that of venous samples. It is speculated that this could be caused by a mixture of tissue bilirubin in the capillary samples and a possible higher protein content in capillary blood [18].
Venous > capillary	The bilirubin content of the capillary samples is lower (clinically significant) than that of venous samples for samples with a bilirubin concentration > 170 $\mu\text{mol/L}$. The opposite was expected and the difference may be explained by the influence of ambient light: light may penetrate the infant's skin and subcutaneous tissue and thereby subject the capillary blood to the direct effects of light [19].

TABLE II. Conclusions from studies on the difference in ctBil between different sampling sites.

Opinions differ on the subject and studies show opposite results. There may be multiple explanations of this, such as different patient populations, the state and treatment of the involved infants and different sampling and measuring methodologies [18].

However, the overall conclusion must be that as no agreement can be reached in the literature as to whether there is any difference in relation to the choice of

sampling site or not, each department has to determine which sampling site suits them the best, as it may not matter which sampling site is used.

However, for studies where two methods are compared it is of course vital that the samples for the two different methods are obtained from the same sampling site and at the same time to eliminate any possible sampling-site variations.

3. Neonatal blood collection

Capillary sampling is the preferred procedure to collect blood from neonates, except if repeated tests are necessary and an indwelling catheter is available. The capillary sampling procedure ensures that only small sample volumes are obtained to avoid iatrogenic anemia, but it also introduces additional preanalytical factors [20], such as hemolysis and clot formation in the sample.

Example:

Hemolysis may occur for several reasons; the erythrocytes of neonatal blood are more fragile during the first 13 days of life, the skin puncture sampling technique may lead to both hemolysis and contamination of the sample by tissue fluid, and poor sample handling, e.g. storage directly on ice, may also lead to hemolysis [21].

Hemolysis interferes with diazo measuring methods in different ways, the Jendrassik-Grof procedure underestimates the ctBil whereas other methods overestimate the ctBil [9,20].

Capillary samples frequently contain microclots, most likely because tissue factor which initiates the coagulation pathway is released during skin puncture. Different brands of capillary tubes are coated with heparin in different concentrations, but it may be difficult to mix the sample properly.

In order to protect the sample pathway of the instrument, which is getting smaller to meet the need for smaller sample volumes, preheparinized capillary tubes with an ultrahigh heparin concentration can be considered for neonatal testing.

Useful tips

Below you will find detailed lists of preanalytical issues affecting total bilirubin. 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

Discussion

Bilirubin stability, sampling site and capillary sampling are the main preanalytical issues to be aware of when measuring neonatal total bilirubin. Besides the preanalytical considerations it is also important to be aware of other issues that may affect the interpretation of the result, such as:

- Racial differences in bilirubin levels [9,27]
- Birth weight [3]
- Gestational age [3]
- Gender [3]
- Nutrition [3]
- Altitude [3]
- Geographic location [3]
- Phototherapy [1]
- Exchange transfusion [1]
- Infant's age [1]

These factors have to be considered when it is determined whether the hyperbilirubinemia is caused by physiological or pathological factors.

There are also some preanalytical issues that may affect different analytical methods. These are:

- High concentration of hemoglobin [9]
- Hemolysis [9,20]
- Lipemia [9]
- Photoisomers [10,11]
- pH [26]

Useful tips when measuring total bilirubin:

(ctBil)

Make sure that:	To avoid:	Due to:
Patient preparation		
The light is turned off before sampling from a baby in phototherapy to protect the sample from the blue light	ctBil↓	Bilirubin stability section 1
The same sampling site is used for continuous measurements (trend monitoring) or method comparisons, as there may be a difference between sites (see Table II)	ctBil↑↓	Sampling site section 2
The sample site is arterialized and that the blood is free flowing to avoid tissue fluid in sample	ctB↑↓	Neonatal blood collection section 3
Blood sampling		
The sample type is specified when reporting the result, as there may be a difference between arterial, venous and capillary blood (see Table II)	ctBil↑↓	Sampling site section 2
A sample device is used that minimizes the risk of hemolysis [22], as hemolysis can affect some analytical methods [23,24]	ctBil↑↓	Neonatal blood collection section 3
If the sample is obtained from a peripheral or umbilical arterial line, make sure that it is flushed properly [20,25]	ctBil↓	
Sample handling		
Clots are avoided as this may affect the test result or obstruct the analyzer	ctBil↑↓	Neonatal blood collection section 3
The sample is protected from light exposure [9,10,11]	ctBil↓	Bilirubin stability section 1
Sample transport and storage		
The sample storage does not exceed three days at room temperature [9]. If blood gas and pH are measured as well, the sample should be processed within 30 minutes [26]	ctBil↑↓	Bilirubin stability section 1
The contact with air is reduced as much as possible to avoid evaporation [23]	ctBil↑	Bilirubin stability section 1

Conclusion

Measurement of neonatal total bilirubin on blood gas analyzers is one of the most recent advantages introduced with point-of-care testing, as this not only decreases the turnaround time but also reduces the blood volume needed for neonatal testing.

This article has described the two main preanalytical considerations related to neonatal bilirubin measurements and provided tips that will help you prevent errors when measuring total bilirubin on blood gas analyzers.

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