No. 23-2001

Hyperbilirubinemia and Requirements to the Determination of the Concentration of Bilirubin

By Anne Skurup, MSc

1 Introduction

60 % of all neonates develop jaundice during their first week of life and thereby run a risk of getting hyperbilirubinemia. 5-10 % require therapy. Although several different approaches for establishing treatment criteria have been suggested, treatment of neonatal hyperbilirubinemia is usually still based on the measurement of the concentration of total bilirubin [1].

This Bulletin covers the following topics: bilirubin metabolism, jaundice in the neonate, therapy, action limits for therapy, use of action limits, analytical requirements to the measurement of bilirubin, bilirubin measuring systems in general and a discussion.

2 Bilirubin metabolism

The major part (80 %) of bilirubin is produced by the degradation of the heme group of hemoglobin. The rest is derived from erythrocyte precursors or from the degradation of other heme-containing proteins. Hemoglobin is released from the erythrocytes and metabolized into bilirubin. Bilirubin is then transported to the liver by albumin. In the liver it is conjugated with glucoronic acid to form conjugated bilirubin which is then excreted via the bile ducts into the intestines and finally excreted with the feces. Small amounts are reabsorbed into the blood stream.

The following types of bilirubin are found in plasma:
- *Unconjugated bilirubin* – also called *indirect bilirubin*. It is poorly soluble in water and a high concentration of this is toxic due to its solubility in fatty tissue.
- *Unconjugated bilirubin bound to albumin*. It is water-soluble and non-toxic.
- *Conjugated bilirubin* – also called *direct bilirubin*. It is water-soluble and non-toxic.
- *Delta (δ) bilirubin*. It is covalently bound to plasma proteins, is water-soluble and non-toxic.

The ratio between conjugated and unconjugated bilirubin is usually constant and delta bilirubin is present in 0-2 %. It usually applies in neonates that total bilirubin = unconjugated bilirubin + conjugated bilirubin.

3 Hyperbilirubinemia/jaundice in the neonate

60 % of all neonates develop jaundice during their first week of life due to factors such as:

- Natural rate of bilirubin production being greater per body mass compared with adults
- Shorter lifetime of the red blood cells
- Immature conjugating system in the liver
- Reduced bilirubin transport by albumin

Hyperbilirubinemia may in severe cases lead to kernicterus. Kernicterus is characterized by a deep yellow staining of various tissues in the brain, causing severe neural symptoms and abnormal developmental outcome. This should of course be avoided by all means by initiating correct therapy.

The introduction of phototherapy in the 1960s [1] virtually eliminated kernicterus for decades. Unfortunately it has reoccurred in many western countries in the last 5 year and is probably caused by a "why worry?" attitude amongst some neonatologists [2,3] in combination with an early discharge of mother and child after birth (see also Discussion).

4 Therapy

In mild cases of jaundice, therapy may not be necessary. Since the early 1960s more severe jaundice has been treated with phototherapy, and in critical situations exchange transfusion is initiated.

The optimal *phototherapy* is obtained by exposing the neonate to blue light with a wavelength of 450 nm in a specially designed bed or blanket. When bilirubin absorbs the blue light, a photochemical reaction occurs which converts bilirubin to a water-soluble isomer which can then be excreted [4]. This type of therapy is relatively harmless. The major disadvantage is the disruption of the contact between the mother and the child and a mild dehydration.

Exchange transfusion is a very critical step to take and a decision of this has to be considered extremely carefully. The actual exchange transfusion is performed by taking out a small volume of the neonate's blood in exchange of donor blood several times until the neonatal blood is close to being completely replaced. There is a relatively high risk of unwanted effects like fever, hemolysis, blood incompatibility, etc. Exchange transfusion is only performed in very critical situations where phototherapy has either been initiated too late or is insufficient.

5 Action limits for therapy

The severity of hyperbilirubinemia is not only affected by the concentration of bilirubin, but also by other conditions such as

- Birth weight
- Gestational age
- Respiratory condition
- Acid-base status
- Immunization

However, decision on therapy is usually based on the measurement of total bilirubin together with a visual inspection of the color of the neonate.

5.1 Examples of action limits

Action limits are often established on an empirical basis. Below is an example of action limits based on age and another based on birth weight and condition.

From the American Academy of Pediatrics [5] in μmol/L (mg/dL) based on *age*:

Age, hours	**Consider phototherapy**	**Phototherapy**	**Exchange transfusion, if phototherapy fails**	**Exchange transfusion and phototherapy**
< 24	...	...	...	...
25-48	170 (10)	260 (15)	340 (20)	430 (25)
49-72	260 (15)	310 (18)	430 (25)	510 (30)
>72	290 (17)	340 (20)	430 (25)	510 (30)

Table 1: units: μmol/L (mg/dL)

From the University Hospital in Copenhagen based on *weight* and *condition:*

Condition of newborn	Phototherapy	Exchange transfusion
Healthy	10 % of birth weight (g) + 50 μmol/L (2.9 mg/dL) Limit 350 μmol/L (20.5 mg/dL)	10 % of birth weight (g) + 150 μmol/L (8.8 mg/dL) Limit 450 μmol/L (26.4 mg/dL)
Mildly ill	10 % of birth weight (g) Limit 300 μmol/L (17.5 mg/dL)	10 % of birth weight (g) + 100 μmol/L (5.8 mg/dL) Limit 400 μmol/L (23.5 mg/dL)
Critically ill	10 % of birth weight (g) – 50 μmol/L (3 mg/dL) Limit 250 μmol/L (14.6 mg/dL)	10 % of birth weight (g) + 50 μmol/L (3 mg/dL) Limit 350 μmol/L (20.6 mg/dL)

Table 2: 10 % of birth weight for bilirubin in μmol/L.

5.2 Use of bilirubin action limits

Due to the extensive use of action limits, accuracy and precision of the measurement of bilirubin are important.

The overestimation of bilirubin could potentially lead to unnecessary therapy. The side effects from phototherapy are not detrimental, but phototherapy may cause mild dehydration, rehospitalization if the mother and baby have been discharged and disruption of the contact between the mother and the baby.

In worst-case scenarios unnecessary exchange transfusion, which is a very aggressive treatment, is performed. As mentioned previously this entails the risk of fever, hemolysis, blood incompatibility, etc.

The underestimation of bilirubin could potentially lead to the withholding of necessary therapy and in a worst-case situation lead to kernicterus.

Both scenarios should by all means be avoided and one way to assure this is to have a reliable measurement of bilirubin so that it can justify the use of specific action limits.

6 Analytical requirements to the measurement of bilirubin

The requirements to the measurement vary slightly depending on its use, e.g. whether it is for screening or for decisions on initiating, continuing or stopping therapy.

According to many clinicians a total error of ±10 % is acceptable with less strict requirements, when bilirubin is below 100 μmol/L (5.8 mg/dL)[1].

Below are examples of analytical requirements for total bilirubin from the literature:

- Analytical goal as % CV derived from all known data on biological variation in healthy individual: 11.3 % [6].

- HCFA proficiency testing grading criteria for acceptable performance: Target value ±3.0 mg/L or 20 %, whichever is greater [7].

1 Source: Author's interviews with various neonatologists.

- Proposed quality specifications for total analytical imprecision as % CV: 11.3 %, and inaccuracy as % maximal allowable deviation: 9.8 %. These should be achieved in at least 95 % of experiments performed [8].

- Estimates of allowable imprecision from clinical and analytical outcome criteria with a decision level of 137 μmol/L (8 mg/dL):
  - Maximal allowable clinical imprecision: 11.2-15.7 %
  - Maximal allowable analytical imprecision: 9.7-13.0 % [9]

- Guidelines for providing quality STAT Laboratory Services Analytical Goals: Bilirubin at a level of 342 μmol/L (20 mg/dL): SD 9 μmol/L (0.5 mg/dL), max. bias: 17 μmol/L (1 mg/dL) [10].

- National Academy of Clinical Biochemistry, Standards of Laboratory Practice: Some clinicians feel that a total analytical error of ±10 % (approximately ±2 SD) is necessary for clinical needs [11].

Please note that in some of these references it is not stated to which age the requirement relates.

7 Bilirubin measuring system in general

Do the bilirubin measuring systems available fulfill the above listed criteria? The answer is unfortunately not always yes.

The different bilirubin measuring systems available on the market can be divided into:
- Transcutaneous devices
- Bilirubinometers
- Chemistry analyzers
- Whole-blood bilirubin analyzers

These may report a qualitative result, a semi-quantitative or a quantitative result, and this should of course be taken into consideration.

7.1 Bilirubin performance studies in the literature

It is well-known within the clinical chemistry community that large variations exist in the measurement of bilirubin from one system to another. Quote: "In 1960, Mather stated that the bilirubin determinations are perhaps the most notoriously unreliable of any in clinical chemistry. Twenty-two years later, Watkinson *et al* came to the same conclusion" [12] and this is also the conclusion of Newman *et al* [3] in 1992. A study conducted by Vreman *et al* [13] in 1995-96 reveals that only minor improvements have been achieved since 1960.

The study by Vreman *et al* was a very extensive study over 8 months with 14 laboratories participating and representing 3 different measuring systems.

A lyophilized bovine bilirubin specimen of 3 different levels was measured. Table 3 shows mean lowest and highest results reported by the 14 clinical laboratories over 8 months.

"True value"	Results from 9 different Kodak Ektachem analyzers		Results from 3 different Hitachi analyzers		Results from 3 different Paramax analyzers	
	Min.	Max.	Min.	Max.	Min.	Max.
38 (2.2)	31 (1.8)	53 (3.1)	39 (2.3)	46 (2.7)	31 (1.8)	32 (1.9)
169 (9.9)	146 (8.5)	222 (13.0)	166 (9.7)	211 (12.3)	146 (8.5)	149 (8.7)
253 (14.8)	208 (12.2)	316 (18.5)	248 (14.5)	302 (17.7)	215 (12.6)	216 (12.6)

Table 3: units: μmol/L (mg/dL)

The within-laboratory variability across time, expressed as CV in % was:

- 1.3 % to 15.8 % for the 38-μmol/L (2.2 mg/dL) level standard
- 1.4 % to 15.4 % for the 69-μmol/L (9.9 mg/dL) level standard
- 2.0 % to 17.2 % for the 253-μmol/L (14.8 mg/dL) level standard

The authors conclude:

- The measured values remained fairly constant during the study at each laboratory.

- There was a very large variation from one analyzer to the other, even on the same type of analyzer.

- Because the within-laboratory variability is relatively low, the real problem exists between laboratories, even between those using the same brand of instrument, perhaps because of inconsistent calibrations locally.

- Matrix effect may explain some of the method-specific biases; however, on the same types of instrument this should be expected to be more or less constant. This suggests more or less serious calibration problems in the field.

- Until widely available standardization processes free of matrix effects are introduced, accurate and precise measurements in the clinical laboratories cannot be assured and this fact must be taken into consideration, especially when pediatricians are mandated to respond to proposed bilirubin action guidelines.

- The intralaboratory variability may have consequences for clinical practice on a case-by-case basis.

Other publications support this conclusion, e.g. Röhle *et al* [14]:
"Considering the special importance of bilirubin determination for neonatology, the state of analysis today is unsatisfactory. Substantial improvement could probably be brought about by means that are already available or could, in all probability, shortly become available."

7.2 Limitations of use and interference studies

The problems with poor performance of bilirubin measuring systems can probably to some degree be explained by either limitations of use or interfering substances.

Examples of limitations of use:
The Kodak Ektachem manual recommends that specimens from neonates <14 days old should not be analyzed for TBil because differences of ±10 % will be observed across the concentration range; however, a study by Langbaum does not support this conclusion [15].

Other limitations of use are, e.g. race, birth weight, etc. [16].

Examples of interference:
Various studies have been conducted on the interference from the presence of hemoglobin on the measurement of bilirubin on plasma. A major study was conducted by Grafmeyer *et al* [17] where 15 different analyzers measuring bilirubin were tested. Only three of these did not suffer from interference; the rest showed an interference in the range of –49 % to +73 % (–43 %, +55 %, –60 %, +47 %, +33 %, +36 %, etc). One type of analyzer showed interference from –43 % to +55 %. The hemoglobin concentration tested was 0.24 mmol/L (0.39 g/dL), which for a neonate corresponds to a hemolysis of 2 %. However, it should be noted that the range of bilirubin concentration tested was only 40-50 μmol/L.

Similar results are observed in a study conducted by Brady *et al* [18] and Scott *et al* [19].

7.3 Correlation studies and reference method

When correlating bilirubin measuring systems to each other the reference method that each of the systems is designed against must be taken into consideration.

Various methods have been suggested as reference method for bilirubin. Often the Jendrassik-Grof method is used or a modification of this referred to as the Doumas method [20]. Others advocate that an HPLC measurement of bilirubin is the "golden standard" [21]. In a major study conducted by Gourly *et al* [22] four different methods were compared with HPLC.

Examples of correlation to HPLC from this study:

Method	**n**	**RMSE**	**r**	**Syx**	**Syx**	**Equation y =**
				μmol/L	mg/dL	
Hitachi	944	0.615	0.988	8.8	0.514	$0.998 \times X + 0.356$
Amer Optical	944	0.574	0.988	9.2	0.538	$1.043 \times X - 0.181$
Ektachem	906	1.267	0.982	10.4	0.609	$0.983 \times X + 1.242$

Table 4: RMSE = root mean square error, and Syx = standard error of the estimate. Where no units given: mg/dL

Correlation results like these can always be evaluated in several ways, e.g. what coefficient of correlation is acceptable. According to Francoual *et al* [23], an r > 0.96 is considered to be a "good correlation" for a bilirubin comparison.

8 Discussion

It does not seem as if there will be any new therapy for neonatal jaundice in the near future. It is, however, clear that the current practice has to be reevaluated. There has been a reoccurrence of kernicterus in the last decade in the USA and other western countries [24]. In Denmark there have been 6 cases of diagnosed kernicterus from 1994-1998 after 20 years with none [25]. In the USA there have been 41 known cases of kernicterus from 1980-2000. 31 of these have been after 1990, representing a dramatic increase [26].

There are various reasons for this reoccurrence and some of the suggested actions to overcome this are:

- Changing the "why worry?" attitude that has been growing
- Introducing screening of all newborn
- Lowering the action limits for therapy
- Etc. [24,25]

With the information given above about the reliability of bilirubin measuring systems and the reoccurrence of kernicterus it could be worthwhile

(re)evaluating whether the bilirubin measuring system in use at each institution provides the requested information and whether it supports the actual action limits used.

For healthy term newborns it may be sufficient with an indication of the actual bilirubin concentration, e.g. by a transcutaneous method, whereas preterm neonates, term neonates with respiratory problems, etc. require a more accurate information on the bilirubin concentration, as minor differences may be important. There is also some evidence that acid-base status [1,27,28] and hypoxia [29] affect the toxicity of bilirubin.

It is not enough to strive for getting an accurate and precise bilirubin determination that can justify the use of specific action limits together with an evaluation of the general status. The systems chosen for bilirubin measurement may have a direct (negative) impact on the patient care. The sample volume is probably the most critical factor for bilirubin measuring systems. Besides that, the results from a bilirubin measurement should be back in due time to take appropriate action. There should be readily access to a measurement both from a time and from a logistical point of view. It should also be possible to measure bilirubin together with other parameters, e.g. the blood gases that can influence the decision on therapy [1,27,28].

9 Conclusion

Experience shows that Mather's statement from 1960 about determinations perhaps being the most notoriously unreliable of any in clinical chemistry may to some degree still be the case.

With this in mind and along the fact about reemerging kernicterus it may be worthwhile that each institution carefully (re)evaluate the performance of the actual method of measurement of bilirubin against current procedures, action limits, etc.

When new methods are taken into use they should be evaluated carefully along with the clinical requirements, action limits, sample volume, limitation of use, turnaround time and other parameters that the result can be reported together with.

References

1 Bratlid D. Criteria for treatment of neonatal jaundice. Journal of Perinatology 1996; 16, 3: S83-S88.

2 Anand RK, Mehta KP, Amdekar YK, Khubchandani RP. Safe bilirubin level for term babies with non-hemolytic jaundice. Indian Pediatrics 1997; 34: 653.

3 Newman TB, Maisels MJ. Evaluation and treatment of jaundice in the term newborn: A kinder, gentler approach. Pediatrics 1992; 89, 5: 809-818.

4 Torres-Torres M, Tayaba R, Weintraub A, Holzman IR. Perspectives on neonatal hyperbilirubinemia. The Mount Sinai Journal of Medicine 1994; 61, 5: 424-428.

5 American Academy of Pediatrics. Practice parameter: Management of hyperbilirubinemia in the healthy term newborn. Pediatrics 1994; 94, 4: 558-565.

6 Fraser CG. The application of theoretical goals based on biological variation data in proficiency testing. Arch Pathol Lab Med 1988; 112: 402-415.

7 Ehrmeyer SS, Laessig RH, Leinweber JE, Oryall JJ. 1990 Medicare/CLIA Final rules for proficiency testing: Minimum intralaboratory performance characteristics (CV and bias) needed to pass. Clin Chem 1990; 36, 10: 1736-1740.

8 Fraser CG, Petersen PH, Ricos C, Haeckel R. Proposed quality specifications for the imprecision and inaccuracy of analytical systems for clinical chemistry. Eur J CLin Chem Clin Biochem 1992; 30:311-317.

9 Westgard JO, Seehafer JJ, Barry PL. Allowable imprecision for laboratory test based on clinical and analytical test outcome criteria. Clin Chem 1994; 40, 10: 1909-1914.

10 Vanderline RE, Goodwine J, Koch D, Scheer D, Steindel S, Cembrowski G. Guidelines for providing quality stat laboratory services. Laboratory Quality Assurance Commitee 1987.

11 National Academy of Clinical Biochemistry, Standards of Laboratory Practice. Guidelines for the evaluation management of the newborn infant. National Academy of Clinical Biochemistry publication. 1998.

12 Doumas BT, Eckfeldt JH. Errors in the measurement of total bilirubin: A perennial problem. Clin Chem 1996; 42, 6: 845-48.

13 Vreman *et al*. Interlaboratory variability of bilirubin measurements. Clin Chem 1996; 42:6, 869-73.

14 Röhle G, Schlebusch H, Geilenkeuser WJ, Kruse R. External quality control in the determination of neonatal bilirubin. An approach to the improvement of results. Eur J Clin Chem Clin Biochem 1988; 26: 441-446.

15 Langbaum ME, Farber SJ, Rosenthal P. Automated total and neonatal bilirubin values in newborns: Is a distinction clinically relevant? Clin Chem 1992; 38: 1690-1693.

16 Svejgaard A. Guide to Evaluating Bilirubin Measurements – Designing the Optimal Test. RADIOMETER Publication Bulletin No. 24-2001. Radiometer Medical A/S 2001. Code No. 918-628.

17 Grafmeyer D, Bondon M, Manchon M, Levillan P. The influence of bilirubin, haemolysis and turbidity on 20 analytical tests performed on automatic analyser. Eur J Clin Chem Clin Biochem 1995; 33: 31-52.

18 Brady J, O'Leary N. Interference due to hemolysis in routine photometric analysis – a survey. Ann Clin Biochem 1998; 35: 128-134.

19 Scott M, Lau BWC, Koening JW, Walton KG, Hinds BJ, Ladenson H. Improved total bilirubin method for the Olympus AU 5000 that decreases interferences by hemolysis or azotemia. Clin Chem 1988; 34, 9: 1921.

20 Doumas BT, Kwok-Cheung P, Perry BW, Jendrzejczak B, McComb RB, Schaffer R, Hause LL. Candidate Reference method for determination of total bilirubin in serum: Development and validation. Clin Chem 1985; 31, 11: 1779-1789.

21 Bhuthani VK, Gourley GR, Adler S, Kreamer B, Dalin C, Johnson LH. Noninvasive measurement of total serum bilirubin in a multiracial predischarge newborn population to assess the risk of severe hyperbilirubinemia. Pediatrics 2000; 106, 2: e 17.

22 Gourley GR, Bhutani V, Johnson B, Kreamer B, Kosorok MR, Dalin C. Measurement of serum bilirubin in newborn infants: Common clinical laboratory methods versus high performance liquid chromatography (HPLC). Pediatric Research 1999; 45: 283A.

23 Francoual J, Myara A, Benattar C, Ravaud A, Paumier AR, Magny JF, Messaoui C, Trivin F. Investigation of total and conjugated bilirubin determination during the neonatal period. Eur J Clin Chem Clin Biochem 1993; 31: 499-502.

24 Bhutani VK. Prediction of risk of significant hyperbilirubinemia in term and near-term newborns. Abstract from REaSoN abstract booklet, Manchester England, 2000.

25 Ebbesen F. Recurrence of kernicterus in term and near-term infants in Denmark. Abstract from REaSoN abstract booklet, Warwick, England, 1999.

26 Newman TB, Maisels JM. Less aggressive treatment of neonatal jaundice and reports of kernicterus: Lessons about practice guidelines. Pediatrics 2000; 105: 242-245.

27 Bowling F. Bilirubin and acid-base/blood gas parameters in the newborn. RADIOMETER Publication AS 130. Radiometer Medical A/S 1999. Code No. 918-595.

28 Ebbesen F, Knudsen A. The risk of bilirubin encephalopathy, as estimated by plasma parameters, in neonates strongly suspected of having sepsis. Acta Pædiatr 1993; 82: 26-29.

29 Johnson LH, Sivieri E, Bhutani V. Apgar scores influences neurological outcome in infants with hyperbilirubinemia. Pediatric Research. 1999; 1189: 203A.

Subject	Title	Code No.
No. 1-1998:	Critical Limits	918-555
No. 2-1998:	The ABL700 Oximeter: A Technical Overview	918-556
No. 3-1998:	The ABL700 Oximeter: New Standards in Interference Detection	918-557
No. 4-1998:	Radiometer's Hemolyzing Cuvette	918-558
No. 5-1998:	The ABL700 Series' Lift'n'Go Inlet	918-559
No. 6-1998:	Guideline to Measurement on the ABL555 Using the User-specified Fluid Mode	918-560
No. 8-1998:	Glucose Measuring System - What Is Measured And What Is Reported?	918-562
No. 9-1998:	Year 2000	918-563
No. 10-1999:	Calibration of pH and pCO_2 Sensors on the ABL70	918-577
No. 11-1999:	Acid-Base Chart - easy interpretation of the acid-base status on the ABL700 Series	918-578
No. 12-2000:	The ABL700 Series' Autocheck Module: An Innovative Piece of QC Technology	918-594
No. 13-2000:	Save Time in STAT Testing	918-592
No. 14-2000:	RADIANCE - Remote Control Functions for Radiometer ABL Analyzers	918-597
No. 15-2000:	Measuring Bilirubin on Whole Blood	918-596
No. 17-2000:	The Radiometer Transcutaneous pO_2 Electrode, E5250	918-616
No. 18-2000:	Discover QualityGuard: NPT7's Internal Quality Control System	918-618
No. 19-2000:	Preparation of Bilirubin Calibration Solutions	918-619
No. 20-2000:	NPT7 Measuring Principles	918-620
No. 21-2001:	NPT7 Clinical Laboratory Improvement Act (CLIA) Compliance	918-625
No. 22-2001:	Frequently Asked Questions Concerning NPT7 Quality Control/Quality Assurance	918-626
No. 23-2001:	Hyperbilirubinemia and Requirements to the Determination of the Concentration of Bilirubin	918-627
No. 24-2001:	Guide to Evaluating Bilirubin Measurements - Designing the Optimal Test	918-628
No. 25-2001:	Comparison of the QC Methods of the ABL700 Series	918-630
No. 27-2001:	Development of Radiometer Quality Control Products	918-640
No. 28-2001:	Production and Metrological Traceability of Quality Control Products	918-641
No. 29-2002:	Establishment of insert control ranges for quality control products	918-665
No. 30-2004:	*Safe*CLINITUBES versus CLINITUBES	918-684
No. 31-2004:	Storage recommendations for blood gas samples	918-686