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A Survey Report on the Determination of Total Bilirubin in Neonatal Samples

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Summary: Three surveys were organized in the Rotterdam area with respect to the determination of total bilirubin in neonatal sera. Fifteen hospital laboratories participated. The coefficients of variation dropped from about 15% (survey 1) to about 8% (survey 3). This improvement was reached by discussing the techniques used, and in some cases by changing them.

Bericht über Ringversuche zur Bestimmung des Gesamt-Bilirubins im Serum Neugeborener

Zusammenfassung: Drei Ringversuche, an denen 15 Krankenhaus-Laboratorien im Gebiet von Rotterdam teilnahmen, wurden zur Bestimmung des Gesamt-Bilirubins im Serum Neugeborener organisiert. Die Variationskoeffizienten nahmen von Ringversuch 1 von 15% auf etwa 8% im Ringversuch 3 ab. Diese Verbesserung wurde durch Diskussion der angewandten Technik als auch teilweise deren Änderung erreicht.

Introduction

Surveys are nowadays accepted in clinical chemistry as useful tools in improving laboratory performance. Sometimes those tools are the only means available. We met this situation when we wished to know the state of the art in the Rotterdam area with respect to the determination of total bilirubin in neonatal sera. For several reasons we were interested in the correlation between the methods used in the various laboratories:

1. From the development of our ACA-method for neonatal bilirubin the well known "clinical chemical rule" seemed to be fulfilled i. e. one method – one result (1). Fortunately the situation in our discipline is not always that negative, and our present study seems to bear this out. Nevertheless not all correlation studies gave identical results.

2. The assumed widespread popularity of direct reading methods or instruments nowadays, in contrast to older surveys where, in most cases, some modification of the *Jendrassik-Grof* technique was applied (2, 3, 4). As a direct reading method or direct spectrometric method we define a method where the spectrophotometric measurement is based on the bilirubin colour itself. The sample to be analyzed is generally diluted with a buffer. A special part of the direct reading methods is formed by the so called direct reading instruments. These instruments are designed for use with undiluted serum or plasma.

3. Sometimes a situation can occur in which more advanced medical care is needed. This can mean that a neonate has to be transferred from one hospital to another. Ideally, this transfer means that the results of both hospital laboratories must be considered as comparable. It was interesting to know how far we were from such a situation in the Rotterdam area.

4. The application of phototherapy or exchange transfusion is at least partly based on the laboratory result of the determination of total bilirubin. Both points 3 and 4 refer to the medical side of the bilirubin determination. To a certain extent, the clinical chemist has to deal with a physician who does not demand strict precision and accuracy with respect to the determination of bilirubin in neonatal blood. That same physician, however, handles decision schemes on phototherapy and exchange transfusion (5, 6) and reads literature in which it is said that:

. . . "The indications for starting phototherapy and the length of exposure are not universally agreed . . ." (7) and

. . . "The indications for exchange transfusion should be individualized to some extent . . ." (8).

Therefore there is a need to translate those laboratory requests in to terms of accuracy and precision, as in all other aspects of clinical chemistry.

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Materials and Methods

Bilirubin

Standard bilirubin preparations in human serum, and human albumin were prepared according to the Recommandations of the well known Joint Committee (10). Bilirubin was purchased from E. Merck Co, catalogue number 24519.

Albumin

Human albumin solutions (50 g/l) were used for the preparation of some bilirubin standard samples. The stock albumin solution (200 g/l, salt-poor) was obtained from Institut Mérieux S. A. (France).

Specimens

Sera from newborn babies (not older than 4 days) were pooled and refrigerated at – 20 °C. The serum pools were 1–4 weeks old.

Commercial bilirubin preparation

The following commercial samples were used:

1. Versatol Pediatric (General Diagnostics), based on human serum
2. Precibil (Boehringer), based on human serum
3. Bilirubin Control (Dade), based on human albumin.

Cobalt sulphate

A $Co(II)SO_4$ solution (1.39 mol/l) in 0.09 mol/l H_2SO_4 (H_2SO_4) was used for wavelength and absorbance check (12).

Results

During a period of five months three surveys were organized in which fifteen hospital laboratories participated. The longest distance between the organizing laboratory and a participating laboratory was 20 km.

It was agreed, because of this small distance, that the samples should be prepared early in the morning of the "survey day", transported afterwards in well closed containers and analyzed in the afternoon of the same day in all laboratories.

The first survey took place in mid November 1978.

Because of the reasons mentioned in the Introduction, a questionnaire was enclosed. The most relevant information regarding this article is collected in table 1.

As can be seen from table 1 the situation in the Rotterdam area is rather diverse. Part of it was known from discussion beforehand with several colleagues. Therefore it was decided to include in the first survey a bilirubin determination which was easy to perform for every participant i. e. the direct reading method of *Hertz* (serum dilution with borate buffer). A further advantage of this method is that it is thorougly studied (9). In every laboratory the *Hertz*' method had to be checked with respect to its practical pitfalls i. e. good spectrophotometer setting and a good sample dilution.

This was done with cobalt sulphate solution (see Materials and Methods).

The first survey included five pooled baby sera, two commercial samples (human protein base) and two bilirubin standards in human albumin. In table 2 three data sets are given: one concerning the routine method in the participating laboratories, one concerning the *Hertz*' technique and one concerning the direct reading instruments.

Tab. 1. Information on participants.
n = number of bilirubin determinations per month.

Lab. No.	Method	Photo-therapy Exchange transfu-sion	n	Calibration
1	Direct reading; dilution with buffer	+	250	Own standard (human albumin)
2	Direct reading; serum undiluted	+	300	Commercial standard
3	Direct reading; serum undiluted	+	50	
4	Diazo type technique	+	200	Commercial standard
5	Direct reading; serum undiluted	+	120	Commercial standard
6	Direct reading; serum undiluted	+	100	Commercial standard
7	Direct reading; dilution with buffer	+	100	Calculated
8	Diazo type technique	+	5	Commercial standard
9	Direct reading; serum undiluted	–	65	Commercial standard
10	Direct reading; serum undiluted	+	65	Serum sample elevated bilirubin
11	Direct reading; serum undiluted	+	400	Commercial standard
12	Direct reading; serum undiluted	+	120	Commercial standard
13	Diazo type technique	+	50	Calculated
14	Direct reading; dilution with buffer	+	50	Commercial standard
15	Direct reading; serum undiluted	+	65	Own standard (bovine albumin)

A number of interesting (though sometimes disappointing) remarks can be made when looking at table 2:

1. The coefficients of variation with respect to the routine methods must be considered in our opinion as too high.

2. An important part of the large variation may be caused by the participating direct reading instruments.

3. The *Hertz*' technique seems to be more reliable.

Regarding this "reference method" however, we got the impression, based on the results of the measurements with the cobalt sulphate solution (the data are not given), that improvements could be made, even in its present application.

Tab. 2. Results of survey 1.

Sample	Routine method of the laboratory		*Hertz*' technique		Direct reading; serum undiluted	
	$\bar{x}$ (μmol/l)	CV (%)	$\bar{x}$ (μmol/l)	CV (%)	$\bar{x}$ (μmol/l)	CV (%)
Pooled baby sera	100	15.0	101	4.0	105	10.9
Pooled baby sera	186	15.6	174	5.8	198	11.2
Pooled baby sera	187	13.4	188	4.8	191	10.4
Pooled baby sera	183	16.4	174	5.2	195	11.6
Pooled baby sera	116	13.8	117	5.1	120	11.4
Dade Bilicontrol*	348	14.7	329	5.5	357	14.8
Versatol Pediatric**	320	17.2	294	3.7	325	15.3
Own standard***	98	20.4	108	11.1	105	12.9
Own standard***	135	11.9	141	12.1	134	8.9

* Bilirubin in human albumin stated value 357 μmol/l
** Bilirubin in human serum stated value 296 μmol/l
*** Bilirubin in human albumin

Therefore we found it useful to repeat the survey with the emphasis on three points:

1. The use of the same routine methods in the same set up as in survey 1.

2. Calculation of the results on the basis of standards in human serum (see Materials and Methods) which were prepared by the organizing laboratory and included in the sample container.

3. Improvement of the application of the *Hertz*' technique by each participant (wavelength check, sample dilution).

In table 3 the same data sets of survey 2, which was organized in mid December 1978, are given as in table 2.

Comparing table 2 and table 3 it is clear that we made considerable progress by using the same standards. Therefore it seemed useful that every participant should study his results and his technique. As a consequence, a third survey had to be organized. This survey was held in April 1979.

It included a number of items:

1. A precision study between the participating laboratories with four pooled baby sera (comparable to the results mentioned in tab. 2 and tab. 3).

2. A precision study per laboratory with a standard serum sample (bilirubin content in human serum 210 μmol/l).

3. A calibration check with three standard samples (109, 210 and 311 μmol/l in human serum)

4. A check on the influence of hemoglobin per method (hemoglobin content 0, 50 and 100 μmol/l).

5. A questionnaire with questions concerning the attention being paid to the surveys 1 and 2.

Tab. 3. Results of survey 2.

Sample	Routine method of the laboratory		*Hertz*' technique		Direct reading; serum undiluted	
	$\bar{x}$ (μmol/l)	CV (%)	$\bar{x}$ (μmol/l)	CV (%)	$\bar{x}$ (μmol/l)	CV (%)
Pooled baby sera	291	5.0	278	4.2	290	4.6
Pooled baby sera	122	11.8	114	3.4	129	8.0
Pooled baby sera	242	9.6	226	3.7	251	6.9
Dade Bilicontrol*	349	7.5	335	4.0	356	7.6
Boehringer Precibil**	372	5.3	345	3.2	378	4.4
Versatol Pediatric***	317	8.1	294	2.4	319	5.5
Own standard****	291	5.5	318	6.3	291	3.7
Own standard****	155	7.9	158	2.7	155	7.0

* Bilirubin in human albumin, stated value 357 μmol/l
** Bilirubin in human serum, stated value 333 μmol/l
*** Bilirubin in human serum, stated value 296 μmol/l
**** Bilirubin in human albumin

Tab. 4. Results of survey 3.

Sample	Without correction $\bar{x}$ (μmol/l)	CV (%)	With correction $\bar{x}$ (μmol/l)	CV (%)
Pooled baby sera	294	8.7	291	6.4
Pooled baby sera	268	8.3	263	6.2
Pooled baby sera	205	9.6	197	5.8
Pooled baby sera	259	9.5	255	7.1
Standard*	118	8.9		
Standard**	313	5.4		

* Bilirubin standard in human serum (109 μmol/l)
** Bilirubin standard in human serum (311 μmol/l)

In table 4 a summary is given of the precision between the laboratories without and with corrections on basis of the calibration samples.

Discussion

If we focus our attention first on the methods used in the Rotterdam area (tab. 1), then we see that, roughly speaking, three different techniques were in use at the time of the first survey:

a) direct reading methods with buffer dilution (n = 3)

b) direct reading methods without dilution (n = 9)

c) some modification of the *Jendrassik-Grof* determination (n = 3).

As a result of the surveys 1 and 2 some changes took place. If we take into account the changes that will take place in the near future, then nine colleagues changed their techniques and one modified his standardization procedure. This leads to the following division:

a) direct reading methods with buffer dilution (n = 9)

b) direct reading without dilution (n = 5)

c) the *Jendrassik-Grof* method (n = 1)

The second point we want to discuss regards the progress we achieved and the question of whether this progress is sufficient. As we wrote in the Introduction the physician does not ask for precision and accuracy. To our knowledge only *Barnett* in his article on the medical signifance of laboratory results mentioned a figure of 7.5 as coefficient of variation at decision levels (340 μmol/l) (11). These data refer to full term babies embarking on exchange transfusion. What is the situation with premature infants (lower decision levels) and phototherapy (lower precision)?

We cannot give an answer to those questions. However, we considered the coefficients of variation for the routine methods (table 2) to be too high.

Table 3 shows a welcome improvement even in the *Hertz'* technique. The question remained as to whether this improvement would continue without guidance by the organizing laboratory. From table 4 it is clear that after a reflection period, about the same coefficients of variation were scored (column "without correction").

We still feel that more improvements can be made. If we look at the column "with correction" in table 4 and at the column "*Hertz'* " in table 3 then we see better coefficients of variation. Another point concerns the within-run precision and the influence of hemoglobin. Though the data are not given here it became clear from survey 3 that some collegues needed to pay more attention to the problem of imprecision and inaccuracy then they probably did. This leaves one last item, namely the direct reading instruments. An important part of the imprecision must be attributed to these instruments. This conclusion can be drawn from table 2 and 3, as well as from the accuracy data (hemoglobin interference). The instruments used were:

a) OHC Photo Ictometer, model II and

b) the American Optical Company Bilirubinometer.

The questions that arose with these instruments are under study now and we hope to report on this study in a separate article.

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