Lab.med. 15: 10–15 (1991)

Improvement of the Accuracy of Bilirubinometer Results

Verbesserung der Richtigkeit von Bilirubinometer-Ergebnissen

H. Schlebusch[1], G. Röhle[3], Ch. Schneider[1], N. Liappis[2], W.-J. Geilenkeuser[3], R. Genz[1]

Frauenklinik[1], Kinderklinik[2] und Institut[3] für Klinische Biochemie der Universität Bonn

Summary:

Using two methods, i. e. the reference method of Doumas and measurement with a bilirubinometer, bilirubin was measured in 80 neonatal sera, and in 30 sera from healthy adults containing added, unconjugated bilirubin.

In the range 150–350 µmol/l, the regressions of the analytical values were identical. Thus, correct values for neonatal sera can be obtained by the bilirubinometer after calibration with a pool of adult sera (containing added bilirubin and standardized with the reference method). However, all bilirubinometers show a more or less curved calibration line, so that deviations from accuracy are to be expected at certain analytytical concentrations, depending on the bilirubin content of the calibrator. A calibrator concentration of 250 µmol/l is suggested as optimal.

Using the procedure described here, it is possible to test the linear range of individual bilirubinometers in the routine laboratory.

Keywords:

bilirubin – neonates – bilirubinometer – reference method – accuracy

Zusammenfassung:

Die Bilirubinkonzentration in 80 Neugeborenenseren und 30 Seren von gesunden Erwachsenen, die mit unkonjugiertem Bilirubin aufgestockt waren, wurde mit der Referenzmethode nach Doumas und einem Bilirubinometer gemessen.

Die Regressionsfunktionen durch die Meßpunkte sind für beide Gruppen im Bereich 150–350 µmol/l identisch. Daraus folgt, daß richtige Ergebnisse in Neugeborenenseren mit Bilirubinometern erzielt werden können, die mit einem Pool von aufgestockten Erwachsenenseren unter Verwendung des Referenzmethodenwertes kalibriert sind.

Da alle Bilirubinometer eine mehr oder weniger stark gebogene Kalibrationskurve haben, sind jedoch, abhängig von der Kalibratorkonzentration, Abweichungen von der Richtigkeit in bestimmten Meßbereichen zu erwarten. Als optimale Kalibratorkonzentration wird 250 µmol/l vorgeschlagen.

Mit dem hier beschriebenen Verfahren ist es auch für Routinelaboratorien möglich, den Linearitätsbereich des eigenen Gerätes zu überprüfen.

Schlüsselwörter:

Bilirubin – Neugeborene – Bilirubinometer – Referenzmethode – Richtigkeit

Introduction

Bilirubinometers or „bilimeters" are widely used for the determination of bilirubin in neonatal sera.

These are simple filter photometers, which measure the absorbance of undiluted serum or plasma at two wavelengths, and directly compute the concentration of bilirubin. A glass capillary serves as the cuvette. Since no pipetting is involved, and the procedure is not prolonged by chemical reactions, the bilirubinometer gives rapid results, even in the hands of less experienced personnel.

The calibration of bilirubinometers, however, represents a significant problem. Generally „control sera" are used for this purpose, with assigned values stated by the supplier. How these assigned values have been determined is often unclear. Moreover, it has recently become apparent that analytical results may vary, depending on the standard used, and differences have even been observed between batches of the same standard (1, 2).

In addition, interlaboratory collaborative studies showed a wide scatter of results and considerable deviations of accuracy. These interlaboratory studies involved the analysis of control samples, whose bilirubin content had been determined with the reference method (3).

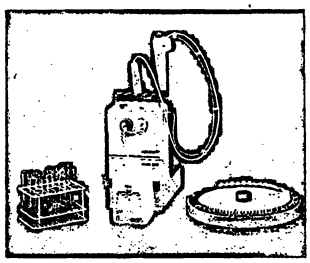
Closer investigation of the control samples or standards showed that the matrix of these samples differed more or less from that of neonatal samples. Bilirubinometer results are, however, strongly dependent on the sample matrix, especially on the protein composition. It is therefore not surprising that standards with human or bovine serum albumin as the protein base should prove unsuitable as calibrators, when their reference method value is used as the assigned calibrator value. It was therefore suggested (4) that bilirubinometers should be calibrated with a pool of neonatal sera, and that the assigned value of this pool should be determined with the reference method. This procedure leads to a marked improvement in accuracy (1, 2), but it is impracticable for most laboratories.

ESAT® 6660

Sekundenschneller Glucoseanalyzer

Gehören Glucose-bestimmungen für Sie zum Routinebetrieb?

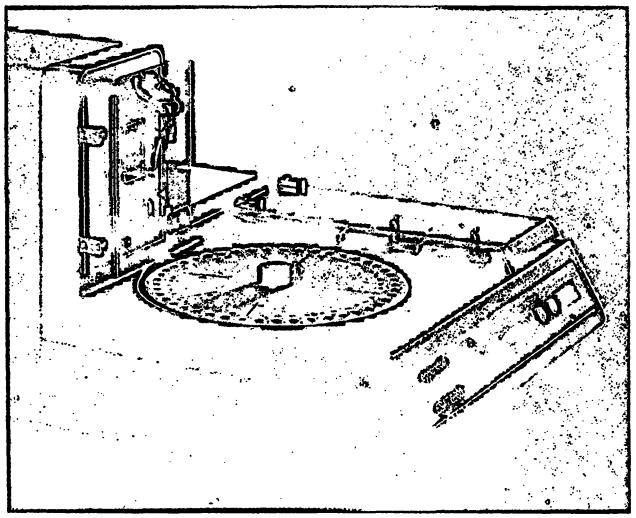
Dann ist ESAT genau das richtige für Sie. Mit ESAT können Sie Glucosebestim-



mungen schnell, präzise und kostengünstig durchführen, und zwar bis zu 120 mal in der Stunde.

Setzen Sie unterschiedliches Probenmaterial ein?

Kein Problem, denn ESAT analysiert die Glucosekonzentration aus Vollblut, Plasma und Serum. Eppendorf liefert außerdem spezielle Gefäße für die Kapillarblutentnahme.



Erwarten Sie ein schnelles Ergebnis für den Notfall?

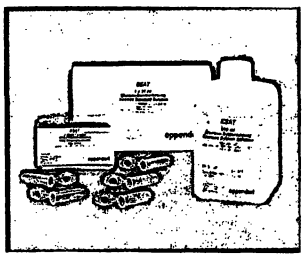
ESAT liefert es für eine Notfallprobe innerhalb von einer Minute.

Gehen zu jeder Zeit Proben bei Ihnen ein?

Der ESAT-Analyzer ist darauf vorbereitet, denn er ist rund um die Uhr einsatzbereit.

Haben Sie nur wenig Zeit für ESAT übrig?

Damit Ihre Zeit trotzdem reicht, ist der Bedienungsaufwand für ESAT minimal. Die Analyse der Glucosekonzentration erfolgt vollautomatisch, und sie wird durch Kontrollfunktionen abgesichert.



Suchen Sie eine Komplettlösung?

Dann ist Eppendorf der richtige Partner für Sie, denn für die rationelle Glucoseanalyse mit dem ESAT-Analyzer erhalten Sie alles aus einer Hand.

Eppendorf -
Netheler - Hinz GmbH
Postfach 650670
2000 Hamburg 65
Telefon (040) 53801-0
Teletex-Nr. 403061 = EGHAM
Telefax (040) 53801556

eppendorf

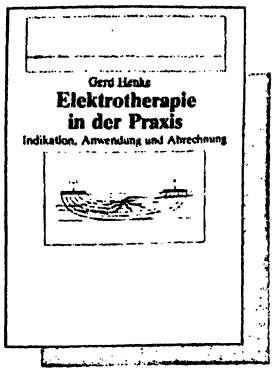
Denn Qualität schafft Sicherheit.

eppendorf® ist ein eingetragenes Warenzeichen

Praxishilfen

Wissen, Tips und Service für den Arzt

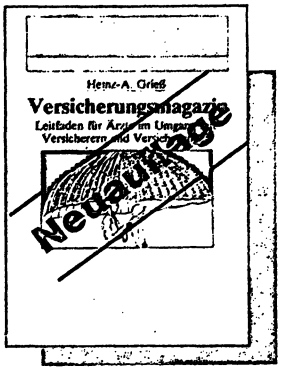
Herausgegeben von Frank H. Mader



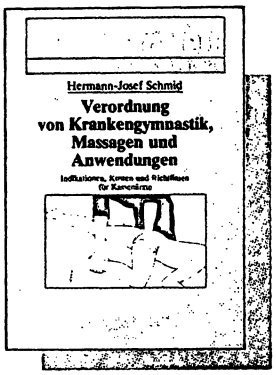
Indikation, Anwendung und Abrechnung, 15 Tabellen und 37 Abbildungen, 80 Seiten, 3. Auflage, 24,80 DM.

Katalog + Kommentar für die Allgemeinpraxis, gültig für BMÄ '87 und E-GO, 7. Auflage, 43,80 DM.

Katalog + Kommentar für die Allgemeinpraxis, GOÄ '88, Stand 1. 10. 88, 6. Auflage, 158 Seiten, 43,80 DM.



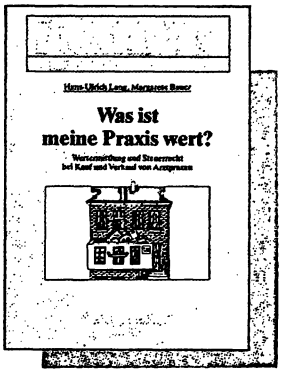
Leitfaden für Ärzte im Umgang mit Versicherern und Versicherungen, 24 Tabellen mit 12 ausklappbaren Seiten, 4 Abb., 52 Seiten, 3. Auflage, 22,80 DM.



Indikationen, Kontraindikationen, Kosten und Richtlinien für Kassenärzte, 64 Seiten, 3. Auflage, 24,80 DM.

Durchführungen – Auswertung – Abrechnung, 17 Abbildungen, 56 Seiten, 3. Auflage, 24,80 DM.

Ein Leitfaden für Verordnung, Materialien und Kosten, 44 Seiten, 2. Auflage, 24,80 DM.

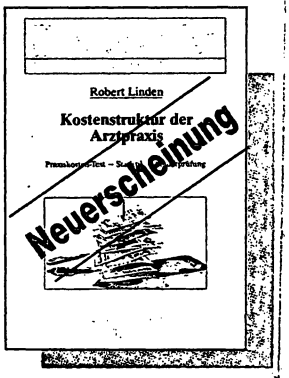


Wertermittlung und Steuerrecht bei Kauf und Verkauf von Arztpraxen, 50 Seiten, 25,80 DM.

Vordruckmuster, Richtlinien und Hilfen, 102 Seiten, 49,80 DM.

Gesetze, Todeszeit, Attest und Abrechnung, Gewebeproben und Abstriche, Entnahme und Versand, 52 Seiten, 25,80 DM.

Geld-Anlage-Tips für Anfänger und Fortgeschrittene. Die Zukunftssicherung des Kassenarztes, 80 Seiten, 36,00 DM.



Praxiskosten-Test, Statistik, Steuerprüfung, 60 Seiten, 24,80 DM.

Bitte senden Sie mir die angekreuzten Praxishilfen

Name: _____

Straße: _____

PLZ/Ort: _____

Datum: _____ Unterschrift: _____

①	100137	Henke, 24,80 DM
②	100172	Weber, 43,80 DM
②a	100158	Weber GOÄ, 43,80 DM
③	100149	Grieß, 22,80 DM
④	100165	Schmid, 24,80 DM
⑤	100156	Schmidt-Voigt, 24,80 DM
⑥	100147	Schilling, 24,80 DM
⑦	100144	Lang/Bauer, 25,80 DM
⑧	100145	Rengel, 49,80 DM
⑨	100148	Müller, 25,80 DM
⑩	100170	Maschner, 36,00 DM
⑪	100169	Linden, 24,80 DM

VERLAG KIRCHHEIM MAINZ
Kaiserstraße 41
6500 Mainz

Lab.med. 1/91

In the present work it is shown that adult serum with added bilirubin is also suitable as a calibrator, and that a calibrator bilirubin concentration of about 250 μmol/l (about 15 mg/dl) leads to sufficiently accurate results in the diagnostically relevant range.

Materials and Methods

Serum samples

Samples were obtained from newborns by venipuncture; they were allowed to coagulate completely, then centrifuged and stored at −20 °C.
After thawing and re-centrifugation, the samples were analysed within 2 h. Visibly haemolytic samples, i. e. with a haemoglobin content > 1g/l, were excluded.

All the adult serum samples were from healthy, fasted probands; they contained a starting bilirubin concentration of < 7 μmol/l, and they were visibly neither lipaemic nor haemolytic. Extra bilirubin was added to 30 different adult serum samples, to a final concentration between 70 and 390 μmol/l. These samples, as well as the neonatal serum samples, were measured in bilirubinometer No. 1, and with the reference method (5). To test linearity, a pool of 10 adult sera (bilirubin concentration: 5.1 μmol/l) was equilibrated to pH 7.4 with tonometer gas, followed by addition of bilirubin to a concentration of 400 μmol/l (reference method), then finally diluted with the starting pool.

Addition of bilirubin

Addition of bilirubin to adult samples was performed according to Doumas (5). Thirty milligrams of bilirubin (Fluka) were mixed with 2 ml of dimethyl sulphoxide and 4 ml of Na_2CO_3 solution (0.1 mol/l), and shaken on the vortex mixer until completely dissolved. Appropriate volumes of this solution were then added to the filtered serum pool and to the individual sera.

Controls

The reference method (5) was monitored with the aid of 2 samples (P1, Lot No. 870624, concentration 308.1 μmol/l; P2, Lot No. 870622, concentration 106.1 μmol/l) from the Rijkinstituut voor Volksgezondheid en Milieuhygiene, Bilthoven, Netherlands.

Capillaries

Forty capillaries were weighed empty and filled with water. The average internal diameter was calculated from the weight difference, according

to the formula, $d = \sqrt{\frac{4v}{\pi \cdot h}}$

To assess the unevenness of curvature, 300 capillaries were filled with various serum samples (average bilirubin concentration 265 μmol/l), and the absorbance values noted as each capillary was turned through 360° in the apparatus. The average differences of the largest and smallest values were recorded.

Apparatus and analytical procedure

Five "Moltronic" bilirubinometers (Mochida Pharmaceutical Co., Tokyo, Japan) were used with the capillaries recommended by the manufacturer. One apparatus was new from the factory, while the other four had seen lengthy service in different laboratories for the routine determination of bilirubin.

According to the manufacturer, the apparatus measures at 455 and 575 nm in glass capillaries with an internal diameter of 1.12 to 1.17 mm. All apparatuses were calibrated with a pool of 10 neonatal sera (2) containing a bilirubin concentration of 228 μmol/l.

To test the linearity of the 5 bilirubinometers, pool sera supplemented with added bilirubin were placed in 2 capillaries. Since the capillaries are not perfectly round, they were turned slowly in the apparatus through 360°, and the average of the highest and lowest reading was taken as the analytical value.

For analysis with bilirubinometer No. 1, only one capillary was used for each adult or neonatal serum sample.

To test the effect of the degree of monochromicity of the analytical light beam, 7 supplemented serum samples (100–400 μmol/l) were analysed in 2 spectrophotometers:

a) Spectrophotometer Perkin-Elmer 554, band width 1 nm;
b) Spectrophotometer Zeiss PM4, band width 10 nm and 20 nm.

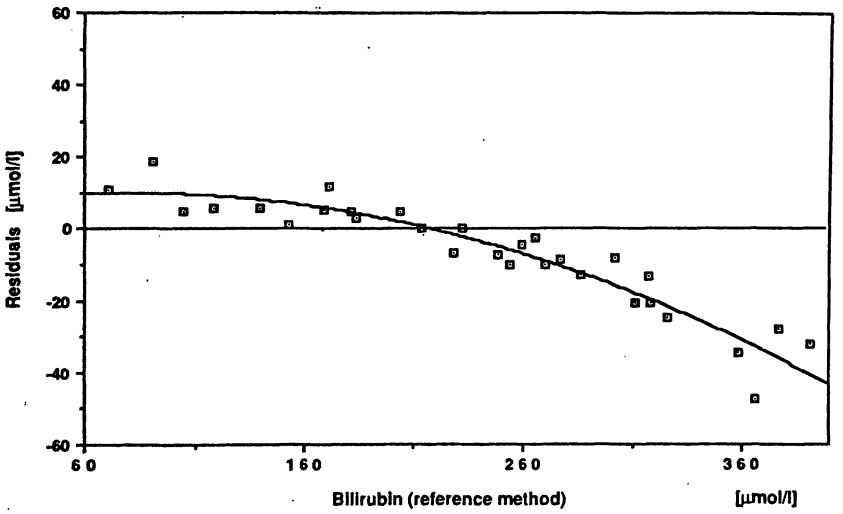


Fig. 1: Bilirubin concentration of 30 adult sera containing added bilirubin
y-axis: Difference between bilirubinometer values and reference method values
x-axis: Reference method values

The samples were placed in a cuvette of 0.9 mm light path (manufacturer: Hellma, short light path cuvette) and measured at 455 and 575 nm.

The wavelength accuracy of the photometers was tested with the emission line of the deuterium lamp (λ = 565.1 nm), and the absorption accuracy was tested with cyanohaemiglobin solutions, with assigned values determined by the Physical-Technical Federal Institute, Berlin.

Total bilirubin in serum samples was determined by the reference method of Doumas (5). All volumes in the original method were decreased ten-fold, and all other procedures were unchanged; precision and accuracy were the same as reported for the original method (6). The spectrophotometer Perkin-Elmer 554 was used for these reference value measurements, and results were calculated using the molar absorption coefficient for azobilirubin, $\varepsilon = 75500\ L \times mol^{-1} \times cm^{-1}$

Statistics

The regression function through the scattered points for adult and neonatal sera was calculated as a second order polynome (7)

Results and Discussion

Bilirubinometers are used not only in the laboratories of women's and children's hospitals, but also for the direct determination of bilirubin at maternity stations. The results are often used as a basis for therapeutic decisions (e. g. phototherapy). A multicentre study on „critical" bilirubin concentrations in premature babies was also based on the use of bilirubinometers (8). At first, however, little attention was paid to the problem of accuracy. In particular, the question of the correct calibration of bilirubinometers, which is now attracting increasing attention, was not addressed until recently (1, 2, 4).

If the proposal of Blijenberg (4) is followed, i. e. if bilirubinometers are calibrated with a pool of neonatal sera, for which a reference method value has been determined, the accuracy of the results is improved (1, 2). For most laboratories, however, this procedure is not practicable, since only capillary blood is often used for analysis, and even with venous samples, the collection of „residues" presents many problems (e. g. homogeneity of the pool, sufficiently high bilirubin concentrations). On the other hand, a pool of adult sera supplemented with added bilirubin is relatively easy to prepare.

Fig. 1 shows the results for 30 supplemented adult sera, while Fig. 2 shows the results for 80 neonatal sera. All samples were measured with the reference method, and in bilirubinometer No. 1. The difference between the bilirubinometer value and the reference method value (y-axis) is plotted against the reference method concentration (x-axis).

For adult sera, the regression functions for the x- and y-values determined in this way are (Fig. 1): $y = 6.736 + 0.0837x - 0.000524x^2$. For neonatal sera (Fig. 2), $y = 24.932 + 0.359x - 0.0011x^2$.

In the concentration range 150–350 μmol/l, the graphs of the regression functions are almost identical, showing a difference of less than 5 μmol/l.

As early as 1972, Hertz and Dybkaer showed that the absorbance curves of unconjugated bilirubin in supplemented adult and neonatal sera were practically identical, if the samples were diluted with borate buffer (pH 9.3) (9). The present data (Figs. 1 and 2) show that adult sera supplemented with bilirubin have similar properties to neonatal sera when analysed in the bilirubinometer. Carotenoids, which are often quoted as an interfering factor (10–13), generally have little effect, even in adult sera (9).

According to Henry (14), the concentration of carotenoids in healthy fasting serum is only 2–3 μmol/l.

Adult sera from jaundiced patients, however, show a different type of spectral absorbance (4, 9, 15). In contrast to neonatal sera, adult sera with bilirubin concentrations greater than 150 μmol/l always contain bilirubin glucuronide, and under certain circumstances also δ-bilirubin. The spectral properties of these latter two fractions, generally known as „direct" bilirubin, differ from those of the „indirect" bilirubin of neonatal serum. In methods involving the reaction of bilirubin with a diazonium salt prior to the spectrophotometric measurement, these differences have little or no effect. In direct spectrophotometric methods, however, they can lead to rather large errors.

Our analytical results show that the bilirubinometer may be calibrated not only with a pool of neonatal sera, but also with a pool of non-icteric adult sera supplemented with added bilirubin, provided its bilirubin concentration is determined with a reference method. It should therefore be possible to prepare a lyophilized standard containing adult serum as the protein matrix, which is suitable for the calibration of bilirubinometers. To test whether

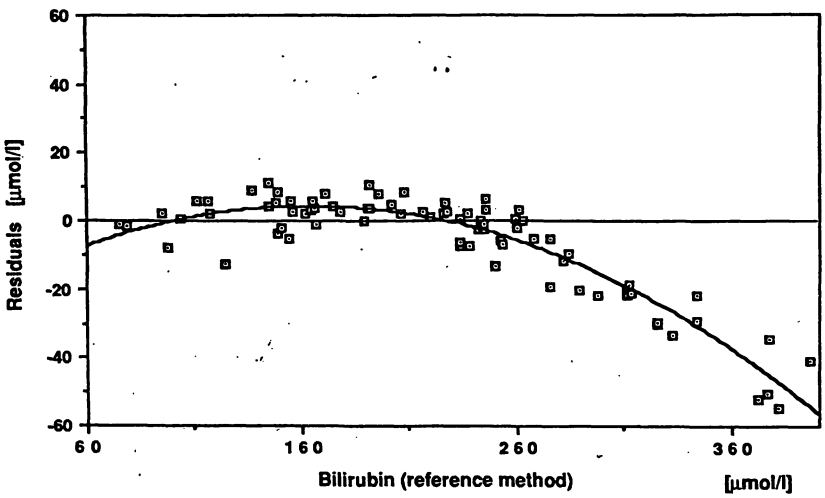


Fig. 2: Bilirubin concentration of 80 neonatal sera
y-axis: Difference between bilirubinometer values and reference method values
x-axis: Reference method values

Fig. 3: Calibration curves of 5 bilirubinometers
y-axis: Difference between bilirubinometer values and reference method values
x-axis: Reference method values
Bilirubinometers: 1 ⊡—⊡ 2 ◇—◇ 3 ◇—◇ 4 □—□ 5 ▣—▣

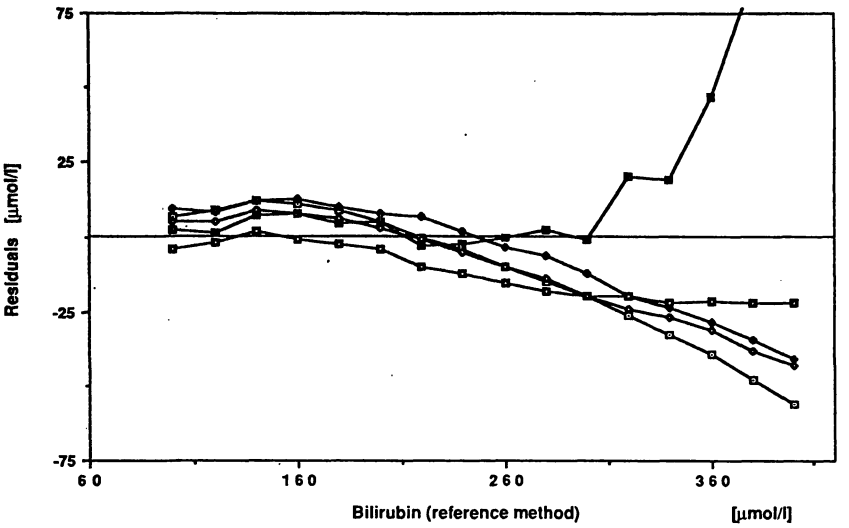
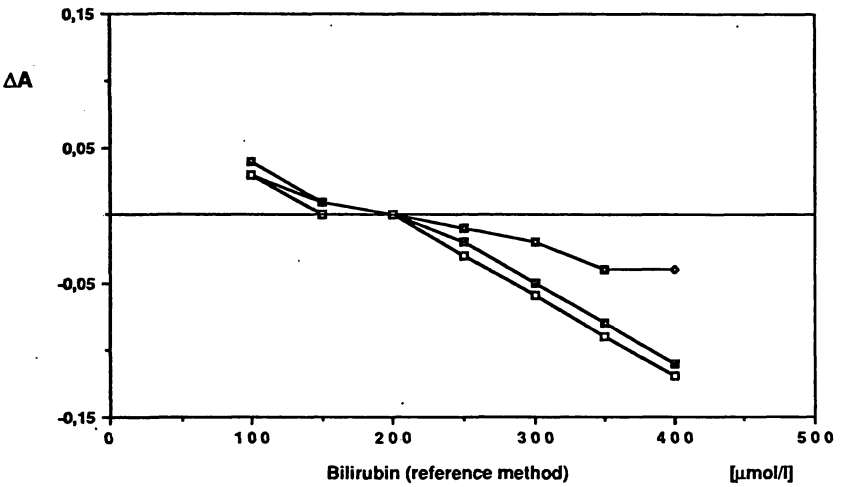


Fig. 4: Measurement of 7 pool sera at 455/575 nm with light beams of different band widths.
(1nm [⊡—⊡], 10 nm [2 ◇—◇] und 20 nm [□—□])
y-axis: Difference between the theoretically calculated and the measured absorbance
x-axis: Bilirubin concentration of the samples



this principle is generally applicable, five bilirubinometers were calibrated with an identical pool of neonatal sera, containing 228 µmol/l bilirubin (reference method value).

In the five apparatuses, a pool of 10 adult sera containing 220 µmol/l bilirubin gave values between 210 and 227 µmol/l (average 219 µmol/l). A pool containing 240 µmol/l bilirubin gave values between 228 and 242 µmol/l (average 236 µmol/l).

These differences between the results from different machines may also be due partly to variations in the internal diameter of the capillaries. The manufacturer quotes the internal diameter as 1.12–1.17 mm, i. e. a tolerance of ± 2.2%. Our own measurements of 40 capillaries gave an average internal diameter of 1.126 mm with a scatter of ± 1.04% (2 standard deviations), i. e. for a bilirubin concentration of 240 µmol/l, the maximal difference (± 2s) is about 5 µmol/l.

The uneven curvature of the capillaries can have an even greater influence on the results.

In the present investigation, 300 filled capillaries in 5 bilirubinometers were turned through 360°. The average difference between the largest and smallest recorded value was 6.3 µmol/l for an average bilirubin concentration per sample of 265 µmol/l. The greatest difference was 15 µmol/l, the smallest 2 µmol/l. No significant differences were found between the different bilirubinometers.

Figs. 1 and 2 show a non-linear relationship between the reference method values and the bilirubinometer results.

From Fig. 3 it is clear that this is not a problem of one particular machine, and that none of the investigated bilirubinometers produced a linear calibration.

Three of the apparatuses produced slightly parabolic calibration curves. The degree of curvature of the various calibration lines differed, sometimes markedly, from machine to machine. (The results in Figs. 1 and 2 were obtained with the bilirubinometer showing the most strongly curved calibration lines). In just one inexplicable case, the calibration curve was totally different for bilirubin concentrations above 280 µmol/l.

For the other apparatuses, the curvature of the calibration lines could be largely explained by the laws of spectrophotometry: the Bougouet-Lambert-Beer law, which describes the linear relationship between absorbance and concentration, is only valid for dilute solutions and monochromatic light.

Figure 4 shows the results of analysing adult pool sera containing bilirubin concentrations between 100 and 400 µmol/l, by measuring absorbance at 455 and 575 nm

with light beams of different band widths. In order to show the small deviation from linearity, the absorbance (A_{St}) of the 200 μmol/l sample (C_{St}) was chosen as the reference value. The theoretical absorbance of 6 other samples of different concentrations can then be calculated with the aid of the formula:

$$A_i = \frac{A_{St}}{C_{St}} \times C_i, \text{ where } i = 100\ \mu\text{mol/l} - - - 400\ \mu\text{mol/l}$$

The difference between the theoretically calculated and the measured absorbance was then plotted against concentration. Even with a highly monochromatic light beam (1 nm band width), the photometer does not show a linear relationship between absorbance and concentration. If the sample containing 200 μmol/l is used as the standard, then the recorded value for the sample containing 100 μmol/l is 7 μmol/l too high, and the value for the sample containing 400 μmol/l is 9 μmol/l too low. The absorbance of this latter sample is $\Delta A = A_{455} - A_{575} = 2.190$.

As expected, the non-linearity of the absorbance/concentration curve increases when the light beam becomes less monochromatic (16). At 10 nm band width the value for the sample containing 400 μmol/l is 17 μmol/l too low, and at 20 nm band width it is 22 μmol/l too low.

The manufacturers give the half band width of the filters used in the bilirubinometer as ≤ 20 nm, so that a corresponding non-linearity must be expected.

In the spectrophotometric measurements a cuvette of light path 0.9 mm was used, whereas the internal diameter of the bilirubin capillaries was about 1.12 mm. However, the light paths of the cuvette and the capillary cannot be compared directly, because the „effective" light path of the capillary is dependent on the width of the light beam. Only exactly in the centre of a round cuvette is the light path the same as the internal diameter; further to the sides, the light path becomes shorter. The width of the light beam in the bilirubinometer is determined by a slit in the capillary housing. This varies between 0.4 and 0.55 mm, depending on the particular machine, giving an „effective" light path between 1.05 and 1.07 mm. Absorbances in the bilirubinometer are therefore about 15% greater than those in the photometer, which leads to a rather higher degree of non-linearity.

Detailed investigations of the linearity of bilirubinometers have so far been published only for the American-Optical-Bilirubinometer No. 10200 (17). The single model investigated showed a non-linearity similar to that of four of our machines. From spectrophotometric theory alone, a curved calibration line can be expected, so that the analytical range of 0-500 μmol/l quoted by the manufacturer is hardly realistic.

With curved calibration lines, and calibration with a single standard, correct results can be obtained only in a limited analytical range. Sera with bilirubin concentrations near to the calibrator concentration naturally show the smallest deviations from the true value. Recorded values will be too low or too high, depending on whether the true concentration is respectively higher or lower than that of the calibrator. Four of our apparatuses showed in principle the same type of curve. From these four curves an average calibration curve was calculated, which was used for the calculations shown in Table 1.

The data show that with a calibrator concentration of 340 μmol/l, the recorded values for samples with bilirubin concentrations < 240 μmol/l are at least 15 μmol/l too high. On the other hand, with a calibrator concentration of 220 μmol/l, the values for samples containing > 280 μmol/l are more than 15 μmol/l too low.

Thus, according to the Table, the best compromise seems to be a calibrator concentration of 250 μmol/l. It is then possible to determine concentrations up to 320 μmol/l with sufficient accuracy (i. e. systematic variation ≤ ± 15 μmol/l [~ ± 1mg/dl]).

Since the introduction of phototherapy, exchange transfusion for the treatment of neonatal jaundice is performed far less frequently. Nowadays, bilirubin concentrations of 340 μmol/l or more are seldom encountered, and the decision limit for phototherapy for most normal-weight newborns is placed at 220–270 μmol/l (18). In this concentration range, measurements should be as accurate as possible. It therefore seems expedient to calibrate the bilirubinometer with a standard containing about 250 μmol/l bilirubin.

If there are indications that the calibration curve of an individual apparatus differs from the „normal" calibration curve described here (see apparatus No. 5 in Fig. 3), then this is easily tested with the described dilution series. Because of the matrix dependency of the bilirubinometer results, however, the dilution must not be performed with physiological saline or similar diluents, but only with a (practically) bilirubin-free serum pool.

Table 1: Influence of the calibrator concentration on the accuracy of bilirubinometer results

	Calibrator concentration							
	220 μmol/l		250 μmol/l		280 μmol/l		340 μmol/l	
Serum concentration (μmol/l)	Calculated conc. (μmol/l)	Difference (μmol/l)	Calculated conc. (μmol/l)	Difference (μmol/l)	Calculated conc. (μmol/l)	Difference (μmol/l)	Calculated conc. (μmol/l)	Difference (μmol/l)
120	125	+5	129	+9	132	+12	135	+15
160	168	+8	174	+14	177	+17	182	+22
200	203	+3	210	+10	214	+14	220	+20
240	235	−5	243	+3	247	+7	254	+14
280	266	−14	276	−4	280	0	288	+8
320	298	−22	308	−12	314	−6	323	+3
360	330	−30	341	−19	347	−17	357	−3
400	359	−41	372	−28	378	−22	389	−11

References:

1. BRÜGMANN, G.: Calibration of direct reading photometers for the determination of neonatal bilirubin. J. Clin. Chem. Clin. Biochem. 28, 83–89 (1990).
2. SCHLEBUSCH, H., LIAPPIS, N., RÖHLE, G., SCHNEIDER, Ch.: Zur Kalibration von Bilirubinometern. Lab.med. 13, 364–368 (1989).
3. RÖHLE, G., SCHLEBUSCH, H., GEILENKEUSER, W. J., KRUSE, R.: External quality control in the determination of neonatal bilirubin – an approach to the improvement of results. J. Clin. Chem. Clin. Biochem. 26, 441–446 (1988).
4. BLIJENBERG, B. G., ROETERING, H. A., DE VOS, A., LEIJNSE, B.: Further studies on the standardization of neonatal bilirubin. J. Clin. Chem. Clin. Biochem. 25, 737–741 (1987).
5. DOUMAS, B. T., KWOK-CHEUNG, P. P., PERRY, B. W., JENDRZEJCZAK, B., MC COMB, R. B., SCHAFFER, R., HAUSE, L. L.: Candidate reference method for determination of total bilirubin in serum: Development and validation. Clin. Chem. 31, 1779–1789 (1985).

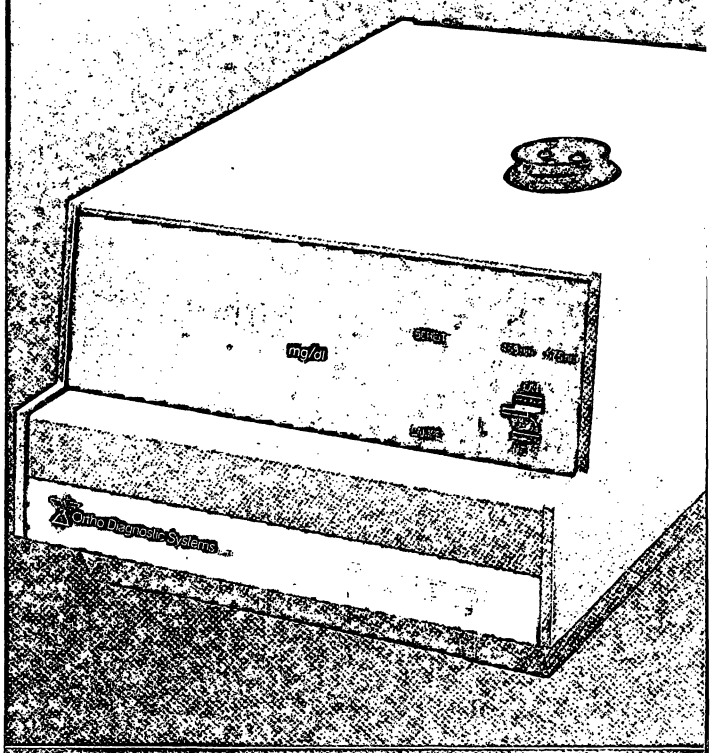
6. SCHLEBUSCH, H., AXER, K., SCHNEIDER, Ch., LIAPPIS, N., RÖHLE, G.: Comparison of five routine methods with the candidate reference method for the determination of bilirubin in neonatal serum. J. Clin. Chem. Clin. Biochem. **28**, 203–210 (1990).
7. BORTZ, J.: Statistik für Sozialwissenschaften. Springer Verlag, Berlin, Kap. 6.3.1, 3. Auflage (1989).
8. VAN DE BOR, M., VAN ZEBEN–VAN DER AA, T. M., VERLOOVE-VANHORICK, S. P., BRAND, R., RUYS, J. H.: Hyperbilirubinemia in preterm infants and neurodevelopmental outcome at 2 years of age: Results of national collaborative survey. Pediatrics **83**, 915–920 (1989).
9. HERTZ, H., DYBKAER, R.: Molar absorption coefficients for bilirubin in adult and infant serum with determination of an isosbestic point. Scand. J. Clin. Lab. Invest. **29**, 217–230 (1972).
10. GREILING, H., GRESSNER, A. M.: Lehrbuch der Klinischen Chemie und Pathobiochemie. Schattauer-Verlag, Stuttgart, p. 1066 (1987).
11. THOMAS, L.: Labor und Diagnose. Medizinische Verlagsgesellschaft, Marburg, 3. Auflage, p. 226 (1988).
12. RICK, W.: Klinische Chemie und Mikroskopie, Springer-Verlag, Berlin, 6. Auflage, p. 221 (1990).
13. KAPLAN, A., SZABO, L. L., OPHEIM, K. E.: Clinical Chemistry, Interpretation and Techniques. Lea & Febiger, Philadelphia, 3rd edition, p. 221 (1988).
14. HENRY, R. J., CANNON, D. C., WINKELMANN, J. W.: Clinical Chemistry, Principles and Techniques, Harper & Row Publ., Hagerstown, 2nd edition, p. 1070 (1974).
15. HERTZ, H., DYBKAER, R., LAURITZEN, M.: Direct spectrometric determination of the concentration of bilirubins in serum. Scand. J. Clin. Lab. Invest. **33**, 215–230.
16. RAND, R. N.: Practical spectrophotometric standards. Clin. Chem. **15**, 839–863 (1969).
17. STEIN, S. M., KEAY, A. J., HORN, D. B.: A critical assessment of a new instrument designed for rapid measurement of bilirubin in microsamples if neonatal sera. Ann. Clin. Biochem. **8**, 8–12 (1971).
18. MAISELS, M. J.: Neonatal jaundice. In: AVERY, G. B. (ed): Neonatology, pathophysiology and management of the newborn. J. B. Lippincott Co., Philadelphia 1987, pp. 592–593 (1987).

Anschrift der Verfasser:

Dr. H. Schlebusch
Zentrum für Geburtshilfe
und Frauenheilkunde
der Universität Bonn
Sigmund-Freud-Straße 25
5300 Bonn 1

BILIMETER

Photometer für die Bestimmung des Gesamtbilirubins bei Neugeborenen



1 Tropfen Fersenblut
Zentrifugieren
Messen

○ Schonend für den Säugling
○ Einfach für das Personal

Ortho Diagnostic Systems GmbH
DR. MOLTER GMBH
D - 6903 Neckargemünd · ☎ (06223) 77-0

© MO 3/90

Info-Gutschein BILIMETER
DR. MOLTER GMBH · Postfach 1320 · 6903 Neckargemünd

Name:
Krankenhaus/
Labor:
Straße:
PLZ/Ort: