

Interlaboratory comparison of the Doumas bilirubin reference method

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Abstract

Objectives: To assess the performance of the Doumas bilirubin reference method.

Design and methods: Ring trials using pooled patient specimens, a calibrator and human sera enriched with unconjugated bilirubin were analyzed in five laboratories using the Doumas bilirubin reference method.

Results: The coefficient of variation for the linear measurement range between laboratories ranged from 1–3%.

Conclusions: The Doumas bilirubin reference method is robust and reproducible. Bilirubin results using this method may be used in the development of more accurate and reliable calibrators.

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Introduction

In 2000, the Bureau International des Poids et Mesures (BIPM), the International Federation of Clinical Chemistry (IFCC) and the Interlaboratory Accreditation Cooperation (ILAC) established the Joint Committee for Traceability in Laboratory Medicine (JCTLM) to promote equivalence in measurements in laboratory medicine internationally. The role of the JCTLM is to promote traceability of routine laboratory methods to internationally accepted standards by designating reference measurement procedures and identifying laboratories for reference measurement services. Since 2003, reference laboratories have been conducting “ring trials” for a variety of serum constituents; the results from ring trials are published once a year.

For total bilirubin, results from the two laboratories (the University of Hannover, Germany, and the Children's Hospital of

Wisconsin, USA) participating in the ring trial differed consistently by 6% to 14%, a discrepancy unacceptable for laboratories using the reference method. To investigate this discrepancy a ring trial with five laboratories participating was initiated to identify and possibly eliminate the source of the discrepancy.

Materials and methods

Materials

Bilirubin, Standard Reference Material (SRM) 916a was obtained from the National Institute of Standards and Technology (NIST).

Specimens

Bilirubin (SRM 916a) stock standard solutions in 4 g/dL aqueous BSA, having concentrations near 20 mg/dL (342 $\mu\text{mol/L}$), were prepared as described in the reference method [1]. Dilutions of the stock solution were made with the same BSA solution.

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Jaundiced human sera were used to prepare five pools to obtain bilirubin concentrations from 0.5 to 6.5 mg/dL (8.6 to 111 $\mu\text{mol/L}$). Another jaundiced specimen from a single patient, and a Roche bilirubin calibrator (Cfas), consisting of human sera enriched with unconjugated bilirubin and ditaurobilirubin, were also used. All samples were stored at -80°C until shipped in dry ice to participating laboratories for analysis.

Methods

All specimens were analyzed in duplicate by the reference method of Doumas et al. [1], which is based on the Jendrassik-Grof principle [2]. In the ring trial conducted in January 2007, two of the participating laboratories prepared bilirubin calibrators (from SRM 916a) and calculated the bilirubin concentration of the specimens from the regression equation of the calibration curve. The other three laboratories calculated the bilirubin concentration using the absorbance and a molar absorptivity (ϵ) of $75,500\text{ L mol}^{-1}\text{ cm}^{-1}$ for the azopigment at 598 nm. This value, reported in [1], is low because it had not been corrected for the non-bilirubin impurities (1%) in the SRM 916, (the first bilirubin SRM issued by NIST). Following the issuing of SRM 916a, molar absorptivities for bilirubin in caffeine reagent at 432 nm and 457 nm, and for the neutral and alkaline azopigments at 530 and 598 nm, respectively were established in two Round Robins held in 1988 [3] and 1998 [4]. The ϵ values, corrected for 1.7% non-bilirubin impurities in SRM 916a, were 76 500 and 76 640 for the 1988 and 1998 Round Robins, respectively. At least one laboratory used the correct ϵ value at 598 nm for preparing calibrators. The following spectrophotometers were used for absorbance measurements: Beckman DU640, Kontron Uvikon 930, Perkin-Elmer Lambda 25 UV/VIS and Lambda 35 UV/VIS, Varian Cary 1E and Cary 300 SCAN.

Results and discussion

Data from the January 2007 ring trial are shown in Table 1. Laboratories A and C calculated the bilirubin values of the specimens from the regression equation of calibrators prepared in their laboratories. Values for the specimen with a nominal bilirubin concentration of 20 mg/dL (342 $\mu\text{mol/L}$) varied from 20.12 to 20.60 mg/dL (344 to 352 $\mu\text{mol/L}$). Coefficients of

Table 2

Human serum pools analyzed for total bilirubin by the reference method: results (mg/dL^a) and statistics.

Lab	Total bilirubin, mg/dL						Statistics		
	A	B		C	D	E	Mean ^b	SD ^b	CV% ^b
		Run #1	Run #2						
<i>Specimen</i>									
1	0.39	0.56	0.43	0.44	0.40	0.45	0.42	0.03	6.13
2	2.10	2.44	2.14	2.14	2.06	2.17	2.12	0.04	2.01
3	3.45	3.96	3.57	3.48	3.36	3.53	3.48	0.08	2.31
4	4.74	5.72	4.87	4.80	4.65	4.90	4.79	0.10	2.10
5	6.50	7.38	6.63	6.47	6.36	6.69	6.53	0.13	2.01
6	2.11	2.39	2.21	2.18	2.22	2.24	2.19	0.05	2.31
7 ^c	4.23	4.18	4.12	4.22	4.19	4.36	4.22	0.09	2.07

^a To convert mg/dL to mmol/L, multiply by 17.1.

^b Data from laboratory B, Run #1, were not included in the statistical calculations.

^c Specimen #7 is a calibrator for Roche instruments.

variation are close to 1% (except those for the low concentrations). If bilirubin values from the 3 laboratories, which used for calculations the ϵ value of the azopigment, are multiplied by 0.983 to correct for the non-bilirubin impurities, the results vary from 20.12 to 20.25 mg/dL (344 to 346 $\mu\text{mol/L}$). The tight range of the bilirubin values for the 20 mg/dL (342 $\mu\text{mol/L}$) specimen is similar to those obtained in the 1988 and 1998 Round Robins indicating the robustness and stability of the reference method and the reproducibility of the color yield (absorbance/concentration relationship).

In the second ring trial (Table 2) three laboratories calculated bilirubin results from a calibration curve while the other two used the ϵ value. Results for the human serum specimens from laboratory B were consistently higher than those from of other four, while the value for the Cfas was within the range reported by the other four laboratories (Run #1). Because of these discrepant results, another set of the same 7 specimens was sent to laboratory B in July 2007 for analysis. Results from this set shown in the second column under laboratory B (Run #2) are well within the range of the other laboratories. The cause for the higher values in human sera reported by laboratory B in May 2007 has not been identified. There is no explanation why the results for the second set were much closer to the mean value of the other 4 laboratories, and why the values reported for the specimens containing only unconjugated bilirubin were no different than those of the other laboratories. The presence, most likely, of bilirubin conjugates in the human specimens (specimens 1–6 were prepared from adult sera) does not explain the discrepant results because it has been well documented by NMR studies that the reference method measures accurately both total and direct bilirubin [5], and because in the final reaction mixture the conjugated azopigment is de-esterified by the alkali to that of unconjugated bilirubin [1].

Conclusions

The results of the present ring trials confirmed the robustness of the reference method and the reproducibility of the color yield (absorbance/concentration relationship). The results reported by the laboratories, which chose to use the ϵ value instead of a standard curve for calculations, are impressive. The fact that

Table 1

Ring trial #1. Specimens of unconjugated bilirubin in bovine serum albumin analyzed for total bilirubin by the reference method: results (mg/dL^a) and statistics.

Lab	Total bilirubin, mg/dL					Statistics		
	A	B	C	D	E	Mean	SD	CV%
<i>Specimen</i>								
1	0.98	1.01	1.00	1.05	1.05	1.02	0.03	3.06
2	1.96	2.06	2.07	2.07	2.12	2.06	0.06	2.85
3	4.12	4.14	4.13	4.09	4.22	4.14	0.05	1.17
4	10.19	10.29	10.29	10.39	10.54	10.34	0.13	1.28
5	20.12	20.52	20.26	20.60	20.51	20.40	0.20	0.99

^a To convert mg/dL to mmol/L, multiply by 17.1.

these data were obtained from 6 different spectrophotometers in 5 geographical areas demonstrates that the reference method is transferable and should be used to standardize the measurement of total bilirubin. The expectation that the availability of the reference method and the SRM 916 would improve the accuracy of bilirubin measurements in clinical laboratories has not been realized. A recent report on laboratory performance in neonatal bilirubin testing reveals that in the past four years there has been no improvement in the accuracy of bilirubin determinations [6]. The lack of reliable calibrators appears to be the cause of persisting inaccuracy. The recent decision of the Chemistry Resource Committee of the College of American Pathologists (CAP) to educationally grade the performance of laboratories participating in the CAP Neonatal Bilirubin Surveys on the basis of results obtained by the reference method could provide the impetus for more reliable calibrators.

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