Bilirubin Standardization in the Netherlands: Alignment within and between Manufacturers

To the Editor:

In 2008, two manufacturers lowered the values of their bilirubin calibrators: Abbott Laboratories by 18% and Roche by 10%–17% (different values per instrument). Beckman Coulter, Siemens/Bayer, and Siemens/Dade Behring did not restandardize their methods. In 2009, the Dutch External Quality Assessment Organization in Medical Laboratories [Stichting Kwaliteitsbewaking Medische Laboratoria (SKML)1] investigated the impact on the mean measured concentration and the interlaboratory variation of assays for total bilirubin.

Pooled human serum was supplemented with unconjugated bilirubin (>99%; Bilirubin, Mixed isomers; Sigma-Aldrich). Serum samples were dispensed, frozen below −70 °C, and shipped on dry ice to the participating laboratories in the regular external quality-assessment program of February 2009. Mean target values were 26.7 μmol/L (95% CI, 26.1–27.3 μmol/L) and 68.7 μmol/L (95% CI, 67.2–70.2 μmol/L), as assigned with the Doumas reference measurement procedure (1–3) in the Joint Committee for Traceability in Laboratory Medicine (JCTLM)-listed reference laboratory in Hanover, Germany. The manufacturers also analyzed the samples in house with their respective routine methods. This design allowed a direct comparison of the results from routine laboratories and manufacturers’ in-house results with the target values assigned by the reference method.

Fig. 1 shows the results submitted by 183 routine laboratories, of which 99 laboratories used a Roche method, 42 used a Beckman Coulter method, 19 used an Abbott method, 9 used an Ortho Clinical Diagnostics VITROS method, 7 used a Siemens/Dade Behring method, and 7 used a Siemens/Bayer method. The Beckman Coulter group included users of the LX20, Synchron, and UniCel DxC 800 instruments; the Abbott group included users of the Aerset and ARCHITECT instruments; and the Roche group included users of the Cobas Integra, Cobas 6000, Modular Analytics, and Hitachi instruments. Because we found no relationship between results and instrument type, we present the data by manufacturer. The interlaboratory CVs at the high bilirubin concentration were approximately 3-fold higher for the Abbott (11%) and Roche (8%) instruments than for the Beckman Coulter (3%), Siemens/Dade Behring (2%), and Siemens/Bayer (3%) instruments.

The mean recovery of the spiked unconjugated bilirubin (expressed as a percentage of the target set by the reference laboratory for the high bilirubin concentration, excluding in-house results of man-

Fig. 1. Total bilirubin measured by routine laboratories, manufacturers, and a reference laboratory.

Results are plotted with the high-concentration sample on the x axis and the low-concentration sample on the y axis (17.1 μmol/L = 1 mg/dL). Colored squares represent mean bilirubin concentrations ±1 SD in both samples, as measured by the routine laboratories in the external quality-assessment program. Stars represent bilirubin concentrations measured in house by the respective manufacturers. The red dot is the target set in the JCTLM-listed reference laboratory of Professor Gerhard Schumann in Hannover, Germany (Institut für Klinische Chemie, Medizinische Hochschule Hannover, Hannover, Germany). OCD, Ortho Clinical Diagnostics; BC, Beckman Coulter.

1 Nonstandard abbreviations: SKML, Stichting Kwaliteitsbewaking Medische Laboratoria; JCTLM, Joint Committee for Traceability in Laboratory Medicine.
able from the JCTLM, have not
ment procedures
ries with listed reference measure-
assignment by reference laborato-
reference materials and/or value
tivation traceability with the certified
procedure, suggesting that calibra-
pared with the target value of the
Bayer methods were high, com-
the Beckman Coulter and Siemens/
atter laboratory variation. Results for
which caused undesirably high in-
for the Roche and Abbott methods,
manufacturer’s in-house result was close
but clearly higher than the target
value. For the Roche and Abbott
methods, the in-house results from
the manufacturers were close to the
target, but the peer group means
of their customers were higher. For the Ortho VITROS
method, the LABORATORYs obtained lower values, and the manufac-
turer’s in-house result was somewhere between the target value and the
customers’ values. We cannot ex-
clude the possibility that some lab-
oratories reported unconjugated
bilirubin (“Bu”) and the sum of bilirubin mono-
and digluco-
rondide (“Bc”), although we explicit-
itly asked VITROS users to report
total bilirubin (“TBIL”). We were unable to clarify the method used
upon retrospective inquiry of
VITROS users.

From the external quality-
assessment survey performed in
2009, it appears that recalibration
of total bilirubin was “in progress”
for the Roche and Abbott methods,
which caused undesirably high in-
terlaboratory variation. Results for
the Beckman Coulter and Siemens/
Bayer methods were high, com-
pared with the target value of the
Doumas reference measurement
procedure, suggesting that calibra-
tion traceability with the certified
reference materials and/or value
assignment by reference laborato-
ries with listed reference measure-
ment procedures (4), both avail-
able from the JCTLM, have not
been achieved. From a clinical
point of view, the effect of restan-
dardization by –10% to –20% is
not very dramatic at commonly
observed “adult” bilirubin concen-
trations of 20–80 μmol/L; how-
ever, restandardization of total
bilirubin will affect the clinical
decision to start or stop treatment at
concentrations usually seen in neo-
nates (100–600 μmol/L), owing to the
specific treatment thresholds or
decision limits for phototherapy and
blood-exchange transfusion (5).

We conclude that notwith-
standing the appearance of Euro-
pean In Vitro Diagnostic Directive
98/79/EC in 1998 and the founda-
tion of the JCTLM in 2002, stan-
dardization has not been achieved.
In addition, standardization of to-
total bilirubin is complex, because the measurand is not unequiv-
cally defined and the matrix may
contain a mixture of bilirubin spec-
ies (unconjugated, mono- and
diconjugated, albumin-bound),
which pose additional challenges.

Finally, because bilirubin rest-
andardization has clinical conse-
quences for the treatment of jaundiced neonates, the Chemistry
Section of the SKML, in close col-
laboration with Dutch neonatol-
gists, specifically aims to focus in
2010 on the analytical and clinical
performance of commutable high-
concentration neonatal bilirubin
samples.

Author Contributions: All authors con-
irmed they have contributed to the intellec-
tual content of this paper and have met the
following 3 requirements: (a) significant con-
tributions to the conception and design, ac-
quision of data, or analysis and interpreta-
tion of data; (b) drafting or revising the article
for intellectual content; and (c) final approval
of the published article.

Authors’ Disclosures of Potential Con-
flicts of Interest: No authors declared any
potential conflicts of interest.

Role of Sponsor: The funding organiza-
tions played no role in the design of study,
choice of enrolled patients, review and in-
terpretation of data, or preparation or ap-
proval of manuscript.

References
1. Lo S, Kytzia HJ, Schumann G, Swartzentruber M,
Vader HL, Weber F, Doumas BT. Interlaboratory
comparison of the Doumas bilirubin reference
2. Doumas BT, Wu TW. The measurement of bili-
3. Doumas BT, Kwok-Cheung PP, Berry BW, Jendr-
zezak B, McComb RB, Schaffer R, Hause LL.
Candidate reference method for determination of
total bilirubin in serum: development and
4. JCTLM. JCTLM database: laboratory medicine
jctlm/ (Accessed March 2010).
5. American Academy of Pediatrics Subcommit-
tee on Hyperbilirubinemia. Management of
hyperbilirubinemia in the newborn infant 35
or more weeks of gestation. Pediatrics 2004;
114:297–316.

Christa Cobbaert2*
Cas Weykamp3
Christian V. Hulzebos4

2 Department of Clinical Chemistry
Leiden University Medical Center (LUMC)
Leiden, the Netherlands
3 MCA Laboratory
Queen Beatrix Hospital
Winterswijk, the Netherlands
4 Department of Pediatrics
Division of Neonatology
Beatrix Children’s Hospital
University Medical Center Groningen
(UMCG)
Groningen, the Netherlands

* Address correspondence to this author at:
Leiden University Medical Center
(LUMC)
Department of Clinical Chemistry E2-P
P.O. Box 9600
2300 RC Leiden
the Netherlands
Fax +31-(0)84-7461316
E-mail c.m.cobbaert@lumc.nl.

Previously published online at
DOI: 10.1373/clinchem.2009.142059