STANDARDIZATION OF SERUM TOTAL BILIRUBIN MEASUREMENT FOR IMPROVED DIAGNOSIS AND MANAGEMENT OF NEONATAL JAUNDICE

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BACKGROUND

In pediatrics, accurate measurement of total serum bilirubin is of major importance for reliable diagnosis and appropriate management of neonatal jaundice. However, several studies evidenced poor comparability of results obtained with the different available methods. This situation is partly due to the lack of Reference Materials, especially for high bilirubin concentrations. In this study, we produced different candidate calibrators of proven commutability that were used to perform an *in silico* recalibration of the most popular routine assays.

METHODS

Commutability of 1 EQA material consisting in lyophilized serum and of 4 candidate materials consisting in frozen primary standard or serum pools with or without PEG was assessed through a split sample study. The same 30 clinical specimens and the candidate reference materials were measured in triplicate with 8 different methods (reagent / analyzer combinations). Statistical analysis was performed using the difference in bias approach. The materials exhibiting sufficient commutability were then value assigned with a reference method. Raw results were then compared with those obtained after virtual recalibration using different strategies: single point and 2 point recalibration using different combinations of calibrators.

Laboratories	**SAINT-ANTOINE Hospital**
	Lab 1 : CNRHP
	Lab 2 : LBU
	Lab 3 : Biochemistry lab
	TROUSSEAU Hospital
	Lab 4 : Biochemistry lab
	TENON Hospital
	Lab 5 : Biochemistry lab

Analyzers	Indiko Thermo-scientific
	AU640 Beckman-Coulter
	DCX 800 Beckman-Coulter
	Architect ABBOTT
	Modular Roche

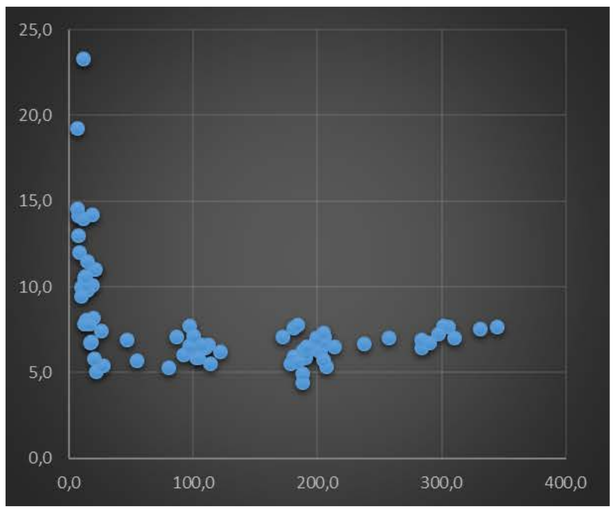
Methods	Method 1: Diazo Beckman / Indiko (Lab 1)
	Method 2 : Spectral / Indiko (Lab 1)
	Method 3 : Diazo Beckman / DXC 800 (Lab 2)
	Method 4 : Spectral /DXC 800 (Lab 2)
	Method 5 : Diazo / AU640 (labo3)
	Method 6 : Diazo Abbott / Architect (Lab 5)
	Method 7 : DPD / Roche Modular (Lab 4)
	Method 8 : Synermed / Roche Modular (Lab 4)

RESULTS AND DISCUSSION

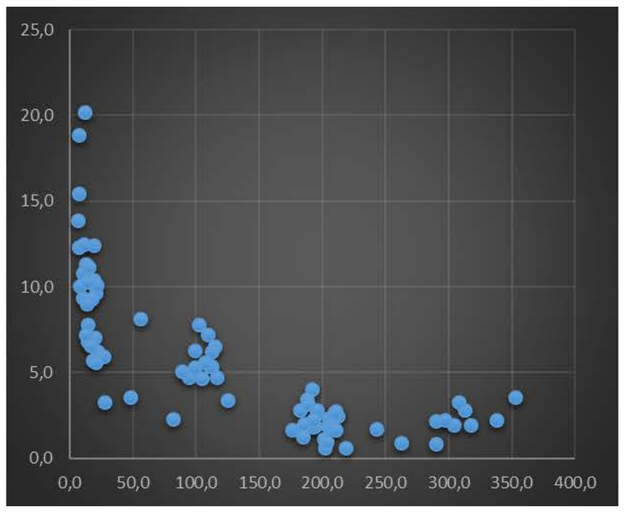
The lyophilized EQA material PBQ was found non-commutable for almost all methods and was considered inappropriate to assess methods trueness. In contrary, the 4 frozen candidate reference materials (BNLSEG, BNLAEG, HANL, HANH) were found highly commutable for almost all methods except some spectral methods. "C" stands for Commutable, "NC" for non-commutable. "I" means that the statistical analysis was inconclusive and/or that the commutability is doubtful.

	BNLSEG	BNLAEG	HANL	HANH	PBQ
Method 1: Diazo Beckman / Indiko (Lab 1)	C	C	C	C	C
Method 2 : Spectral / Indiko (Lab 1)	NC	NC	C	C	NC
Method 3 : Diazo Beckman / DXC 800 (Lab 2)	C	NC	I	C	NC
Method 4 : Spectral /DXC 800 (Lab 2)	NC	C	C	NC	I
Method 5 : Diazo / AU640 (labo3)	C	C	C	C	NC
Method 6 : Diazo Abbott / Architect (Lab 5)	C	C	C	C	NC
Method 7 : DPD / Roche Modular (Lab 4)	C	C	C	C	NC
Method 8 : Synermed / Roche Modular (Lab 4)	C	C	C	C	NC

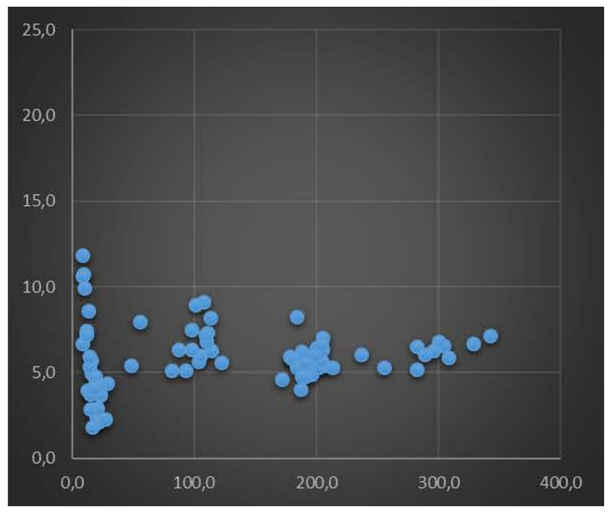
Inter-laboratory variation – CV% = f(concentration - µmol/L)



Raw data
Mean CV = 5,5%



1-point recalibration
Mean CV = 2,0%



2-point recalibration
Mean CV = 5,0%

After discarding spectral methods, between-method CV measured with 30 clinical specimens was 5.5% without recalibration, 2% after single point and 5% after 2 point virtual recalibration. Using a single calibrator with high bilirubin concentration results in a great improvement of results comparability for medium and high bilirubin concentrations but not for low bilirubin concentrations. Adding an second calibrator with low bilirubin concentration improved comparability of results at low bilirubin concentrations but results comparability at medium and high bilirubin concentration was not as much improved as with a single point calibration. This result can be explained by differences and/or limitations of routine assays to measure low bilirubin concentrations (<60 µmol/L).

CONCLUSION

Recalibration of assays for serum bilirubin with commutable calibrators can improve comparability and accuracy of the different methods. However, concentration of calibrators should be carefully chosen depending on the field of application. In pediatrics, single point calibration with high bilirubin concentration was shown to be the most appropriate. On this basis, a more extensive study will be conducted with French biological society (SFBC) to progress in harmonization process for neonatal bilirubin.