M213

UNBOUND BILIRUBIN DETERMINATION IN NEWBORNS: DEVELOPMENT OF AN AUTOMATED ASSAY ON THE INDIKO THERMO SCIENTIFIC

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BACKGROUND

The unbound bilirubin (UBB) concentration is probably the most critical parameter in establishing the risk for bilirubin encephalopathy in neonates. This parameter takes in consideration 3 biological risk factors for kernicterus in newborns: hyperbilirubinaemia, hypoalbuminaemia and competitors of the bilirubin-albumin bond. It thereby allows to identify risk situations of bilirubin encephalopathy which cannot be detected with individual testing of either bilirubin and albumin. In our laboratory, the UBB analysis has been routinely performed since 1987 on a dedicated instrument, the UB Analyser (Arrows, Co, Ltd.Osaka, Japan, non-automated assay) with peroxidase method. The principle of this assay is a rapid deterioration of the UBB into a leuco-derived compound by the action of a peroxidase in the presence of hydrogen peroxide. The UBB concentration is calculated from the oxidation kinetics. Since 2006 we had transfered the UBB assay to open biochemistry systems: the CX4-CE and DxC 800 Beckman-Coulter.

The aim of this study is the transfer of the UBB assay to a new open biochemistry system: Indiko Thermo Scientific.

UBB TESTING BY PEROXYDASE METHOD
MECHANISM

UBB + Albumin ⇌ UB Bilirubin - Albumin

H_2O_2 + Peroxydase → O• + H_20 → *Dye*

UBB is quickly degraded in the presence of peroxidase and H_2O_2 as a source of oxygen radicals

Measurement of the peroxydase reaction kinetic allows to calculate the UBB concentration (by knowing the peroxydase reaction factor).

METHODS

	UB analyser	CX4-CE	DxC 800	INDIKO
Plasma sample	25 µl	7 µl	7 µl	7 µl
Phosphate buffer pH 7.4 + H_2O_2	1 ml	200 µl	200 µl	200 µl
Peroxydasis	25 µl	6 µl	6 µl	6 µl
Reaction type		CINETIC1 decreasing	CINETIC1 decreasing	CINETIC decreasing
Primary wavelength Secondary wavelength	460 nm	470 nm 650 nm	470 nm 650 nm	450 nm 660 nm
Reaction temperature	37°C	37°C	37°C	37 °C
Incubation buffer + sample time	variable	48 s	48 s	360 s
Blank reading	Black box	50 s	40s	
First reading after peroxydase addition Reading time	Black box	3 s 30s ,16 points	10 s 30s	4s 30s

REAGENTS and PARAMETERS

2 Point Calibration
Point 0: distilled water
Point 1: UBB Cal prepared in CNRHP
Target value for UBB: 0.46 µg/dl

Quality Control
Précibil (level 1) Roche
Liquicheck Pediatric control (level 2) BioRad

Reagent stability :
1 day at 4°C in the system

RESULTS (1)
INDIKO ANALYTICAL PERFORMANCES

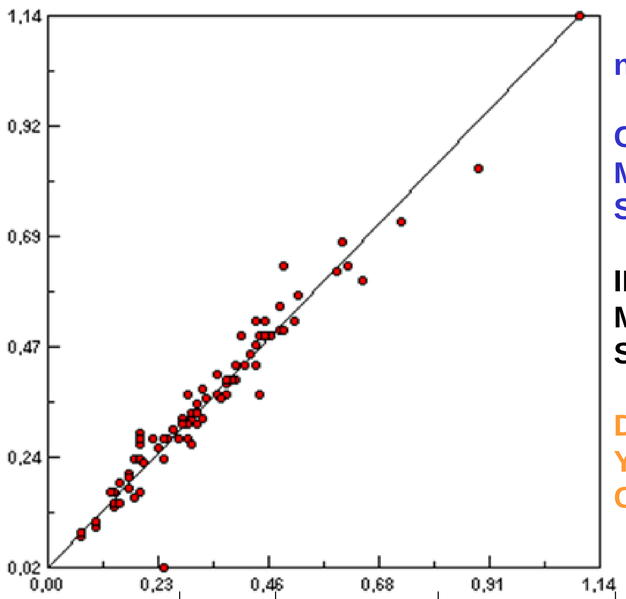
IMPRECISION
Within run

	N	Mean [µg/dl]	*Standard error*	CV [%]
LEVEL1	30	0.671	0.014	2.1
LEVEL 2	30	1.257	0.02	1.1

Between run

	N	Mean [µg/dl]	*Standard error*	CV [%]
LEVEL1	20	0.798	0.030	3.8
LEVEL 2	20	1.288	0.051	4.0

RESULTS (2)
INDIKO/ CX4-CE INSTRUMENT CORRELATION



n=87

CX4 (X)
Mean : 0.335
Standard deviation : 0.175

INDIKO(Y)
Mean : 0.359
Standard deviation : 0.178

Deming Regression
Y = 1.02 X + 0.02
Correlation : 0.97

BNL level µmol/l	X	Y calculated	Inaccuracies acceptable	Inaccuracies calculated	Conclusion
Low	0.20	0.22	0.03	0.02	Acceptable
Middle	0.50	0.53	0.09	0.03	Acceptable
High	1.50	1.55	0.26	0.05	Acceptable

CONCLUSION

Beyond the interest of having an automated assay for UBB dosage (lower sample volumes, better reliability of assays, data export…), this work may contribute to larger diffusion of UBB determination among laboratories equipped with different instruments. Diffusion of this method would be of great help for pediatricians in order to assess severity of jaundice especially in newborns who have risk factors for bilirubin toxicity (hemolysis, acidosis, dehydration and prematurity).