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 transferred 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.

METHODS

UBB TESTING BY PEROXYDASE METHOD

MECHANISM

UBB + Albumin \rightarrow UB Bilirubin - Albumin

H₂O₂
Peroxidase

Ub is quickly degraded in the presence of peroxidase and H₂O₂ as a source of oxygen radicals.

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

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
Liquichcek Pediatric control (level 2) BioRad

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

RESULTS (1)
INDIKO ANALYTICAL PERFORMANCES

IMPRECISION

Within run

<table>
<thead>
<tr>
<th>N</th>
<th>Mean [µg/dl]</th>
<th>Standard error</th>
<th>CV [%]</th>
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<tbody>
<tr>
<td>30</td>
<td>0.671</td>
<td>0.014</td>
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<tr>
<td>30</td>
<td>1.257</td>
<td>0.020</td>
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Between run

<table>
<thead>
<tr>
<th>N</th>
<th>Mean [µg/dl]</th>
<th>Standard error</th>
<th>CV [%]</th>
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<tbody>
<tr>
<td>20</td>
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<td>0.030</td>
<td>3.8</td>
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<tr>
<td>20</td>
<td>1.288</td>
<td>0.051</td>
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</tbody>
</table>

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

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).