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# Adaptation of a Non-Albumin-Bound Bilirubin test in the serum of newborns to the DxC 800® Beckman Coulter

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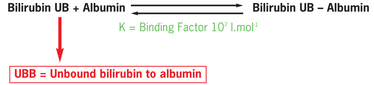
## INTRODUCTION

The unbound bilirubin (UBB) concentration is probably the most critical parameter in establishing the risk for bilirubin encephalopathy[1]. This parameter takes in consideration 3 biological risk factors for kernicterus in newborns: hyperbilirubinaemia, hypoalbuminaemia and competitors of bilirubin-albumin bound. It also identifies risk situations which cannot be detected with individual testing of either bilirubin and albumin.

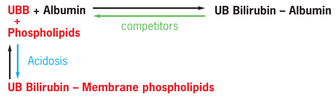
### BILIRUBIN-ALBUMIN BOUND – CHARACTERISTICS




K very strong: very low concentration of UBB in plasma = 0 – 3 µg/dl or 0 – 50 nmol/l

- One main binding site (secondary site with less affinity)
- The sites are non specific and accessible to many endogeneous and exogeneous competitors: in vivo UBB cannot be substracted of the ratio Bilirubin UB /Albumin

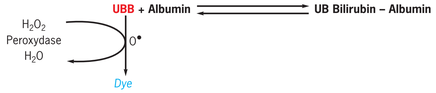
### BILIRUBIN-ALBUMIN BOUND – INTEREST OF EXPLORATION




- Takes in consideration 3 biological risk factors for kernicterus: hyperbilirubinaemia, hypoalbuminaemia, competitors
- Allows to identify risk situations which cannot be detected individual testing of bilirubin and albumin

## PRINCIPLE OF THE TEST

### UBB TESTING BY PEROXYDASE METHOD – MECHANISM




- 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).

## AIM OF THE STUDY

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 the peroxidase method[2]. The aim of this study is the transfer of the UBB assay to open biochemistry systems: the CX4-CE® and DxC 800® Beckman Coulter.

## METHOD

Validation of this transfer was conducted by reducing the volumes of reaction (6 µl of plasma instead of 25 µl, 200 µl of phosphate buffer instead of 1 ml, 6 µl of peroxydase instead of 25 µl). We have chosen a Cinet1 decreasing mode as reaction type with a primary wavelength at 470 nm and a secondary wavelength at 650 nm. We have adjusted the parameters of reading in order to calculate the initial kinetic of the reaction: incubation time of buffer with sample = 48s, blank reading = 40s, first reading after peroxydase addition = 10s and reading time = 30s.

**Table 1 ▸ REAGENTS and PARAMETERS**

| | UB analyser | CX4-CE | DxC 800 |
|---|---|---|---|
| Plasma Sample | 25 µl | 7 µl | 7 µl |
| Phosphate buffer pH 7.4 + $H_2O_2$ | 1 ml | 200 µl | 200 µl |
| Peroxydasis | 25 µl | 6 µl | 6 µl |
| Reaction Type | | CINET1 decreasing | CINET1 decreasing |
| Primary wavelength | 460 nm | 470 nm | 470 nm |
| Secondary wavelength | | 650 nm | 650 nm |
| Reaction temperature | 37°C | 37°C | 37°C |
| Incubation buffer + sample time | variable | 48 s | 48 s |
| Blank reading | Black box | 50 s | 40 s |
| First reading after peroxydase addition | Black box | 3 s | 10 s |
| Reading time | | 30 s, 16 points | 30 s |

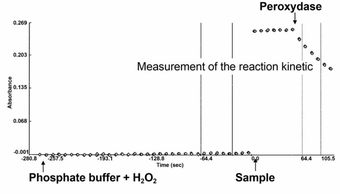
**2 Point Calibration**
Point 0: distilled water
Point 1: UBB Cal prepared in CNRHP
Target value for UBB: 0.70 µg/dl

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

**Quality Control**
Précibil Roche (medium level): LEVEL 1
BioRad (high level): LEVEL 2

## RESULT (1)

**Figure 1 ▸ REACTION KINETIC**




## RESULT (2)

### DxC 800 ANALYTICAL PERFORMANCES

**Table 2 ▸ Within run**

| | N | Mean [µg/dl] | *Standard error* | CV [%] |
|---|---|---|---|---|
| Level 1 | 20 | 0.77 | 0.02 | 2.78 |
| Level 2 | 20 | 1.01 | 0.02 | 1.90 |

**Table 3 ▸ Between run**

| | N | Mean [µg/dl] | *Standard error* | CV [%] |
|---|---|---|---|---|
| Level 1 | 20 | 0.76 | 0.02 | 3.19 |
| Level 2 | 20 | 1.00 | 0.03 | 2.15 |

## RESULT (3)

An inter-instrument correlation (Deming linear regression) has been made with a hundred clinical samples.

**Figure 2 ▸ CORRELATION UBB UB ANALYZER / CX4-CE**

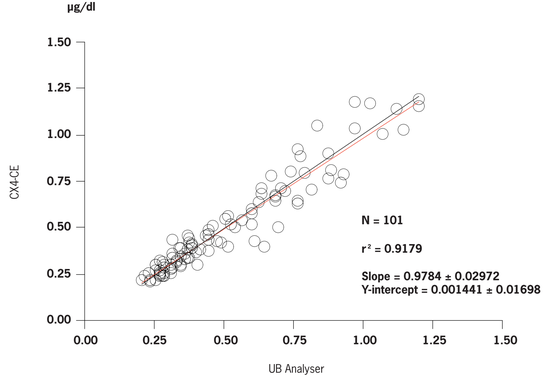
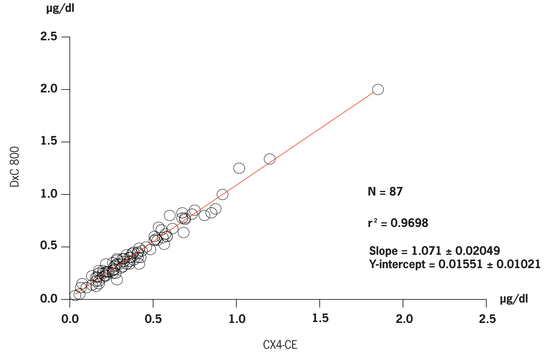



**Figure 3 ▸ CORRELATION UBB CX4-CE / DxC 800**




## CONCLUSION

Beyond the interest of having an automated assay (lower sample volumes, better reliability of assays, data export, …), this work will contribute to the large diffusion of UBB analysis in laboratories that had no analytical capability for UBB measurement. The method will help pediatricians to assess severity of neonatal jaundice especially in the presence of high risk factors for kernicterus (hemolysis, acidosis, dehydratation and prematurity).

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### *References*

*(1) – Wennberg RP, Ahlfors CE, Vinod K. Bhutani, Johnson LH, and Shapiro SM. Toward Understanding Kernicterus: A Challenge to Improve the Management of Jaundiced Newborns. Pediatrics. 2006;117(2 ):474-485*

*(2) – Jacobsen J, Wennberg RP. Determination of unbound bilirubin in the serum of newborns. Clin Chem. 1974;33 :783 –789*