Neonatal Total Bilirubin Method Evaluation: Whole Blood on the RAPIDLab 1200 System versus Plasma on the VITROS 950 Chemistry System

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Clinical Background

Bilirubin, the main bile pigment in the liver, is a major end product of hemoglobin decomposition as aged or damaged red blood cells are routinely destroyed. Hemoglobin degradation results in the formation of unconjugated bilirubin. As the unconjugated bilirubin is lipid-soluble, it cannot be excreted until it is bound to albumin and carried to the liver, where it is made water-soluble by conjugation and passed in the urine. For the clinician, bilirubin is considered an index of liver function, as it reflects the liver's ability to take up, process, and secrete bilirubin. Impaired conjugation of bilirubin in the liver can result in elevated bilirubin levels in the blood. An increased level of bilirubin in the blood (hyperbilirubinemia) causes jaundice, resulting in discoloration of body tissues.

Jaundice in newborns is usually harmless, a consequence of immature hepatic function and the normal breakdown of fetal hemoglobin as it is replaced with adult hemoglobin. Severe neonatal jaundice, however, may indicate more serious conditions, including erythrocyte hemolysis (erythroblastosis fetalis) generally caused by blood incompatibilities between baby and mother. Newborn bilirubin levels should be closely monitored, as extremely high levels of bilirubin in infants may cause a form of brain damage (bilirubin encephalopathy or kernicterus).

When it comes to hyperbilirubinemia in newborns, the cost of treatment is low compared to the potentially high expense of missing the condition. Infants with elevated bilirubin levels are often treated successfully with phototherapy, while those who are untreated run the risk of requiring a blood transfusion, or worse, develop the bilirubin-induced brain damage known as kernicterus. As a result, hospitals typically test any newborn appearing jaundiced within 24 hours of birth.

In the United States, the Centers for Disease Control (CDC) counted more than four million births in 2007 and 2008; the World Fact Book estimates a world birthrate of roughly 135 million.[1] With more than half of all newborns showing some evidence of jaundice, this equates to a large potential number of bilirubin tests performed.

Although the actual testing is not difficult, obtaining an adequate quality sample from very small infants can be challenging. Analyzing the sample as quickly as possible is critical to ensure optimal therapeutic action. For this reason, offering the ability to analyze these patient samples in the neonatal intensive care unit (NICU) has become standard practice in many hospitals. This is where systems such as the RAPIDLab® 1200 blood gas analyzer are viewed as a valuable solution for the detection of hyperbilirubinemia as well as other critical conditions.

Principles of Operation on the RAPIDLab 1200 System

The Siemens RAPIDLab 1200 blood gas systems are designed to measure pH, pO_2, pCO_2, Na^+, K^+, Ca^{++}, Cl^-, glucose and lactate on unhemolyzed whole blood. Direct multiple wavelength spectrophotometry technology measuring light transmission through the same specimen is also applied to determine concentrations of total hemoglobin and its derivatives (tHb, FO_2Hb, FCOHb, FMetHb, FHHb).

The option to report total bilirubin on neonatal whole blood specimens (nBili) is currently available on both the RAPIDLab 1245 and RAPIDLab 1265 models of the RAPIDLab 1200 blood gas series systems.

1. Central Intelligence Agency. World Factbook: People; Available at https://www.cis.gov/library/publications/the-world-factbook/geos/xx.html

RAPIDLab 1200 Method versus VITROS 950 Method

The RAPIDLab 1245 and RAPIDLab 1265 analyzers assess neonatal bilirubin concurrently with total hemoglobin and CO-oximetry on whole blood using direct spectrophotometry. Raw bilirubin values are determined by iterative least-squares analysis and further adjustments made to produce the reported nBili results. The Ortho-Clinical Diagnostics VITROS® 950 analyzer (hospital reference method), on the other hand, first employs a caffeine and sodium benzoate reactive chemistry step on plasma (or serum) matrix. After a fixed incubation period, endpoint colorimetric dual-wavelength analysis is made to determine the concentrations of unconjugated and conjugated bilirubin fractions which, when added together, are used to derive the reported total neonatal bilirubin (NBIL) value.

Assessments of neonatal total bilirubin were conducted on the RAPIDLab 1245 and the VITROS 950 to determine the comparability of the two methods which utilize different sample matrixes.

Materials and Methods
All samples were obtained from clinical patients and evaluated in the core chemistry lab at the hospital site during three separate studies across 28 test days, incorporating four different RAPIDLab 1245 units and two VITROS 950 instruments.

As bilirubin is light sensitive, care was taken to keep the test samples protected from light exposure prior to testing. The majority of samples tested were from neonatal patients. Gender was equally distributed, and the neonate ethnic profile was reflective of the demographic mixture of the region's population. The neonatal patients' ages ranged from less than 1 through 14 days, with the majority being ≤5 days old.

In addition, remnant arterial and/or venous whole blood taken immediately from umbilical cords was tested. A small number of remnant whole blood samples from adult patients were also evaluated as described below.

Neonatal samples were collected as whole blood in amber-colored microtainers (plasma separator tubes with heparin; Becton Dickinson). For each neonate test sample, a small volume was removed via glass capillary and measured on a RAPIDLab 1245 blood gas analyzer. The volume remaining in the microtainer was spun in a centrifuge. The resulting plasma was poured off into plastic cups and measured using the NBIL assay on either of two VITROS 950 chemistry analyzers resident in the core chemistry lab. For each neonate test specimen, the whole blood RAPIDLab 1245 nBili value was compared to the corresponding plasma VITROS 950 NBIL result, in mg/dL.

In similar fashion, portions of remnant adult and cord whole blood samples were measured on a RAPIDLab 1245 and compared to the corresponding plasma matrix measured on the VITROS 950. Prior to testing, some of these remnant specimens were doped with varying amounts of concentrated bilirubin spiking solution (100 mg/dL unconjugated in 0.85 percent HSA, pH adjusted) to artificially elevate the bilirubin concentration detailed later in this report.

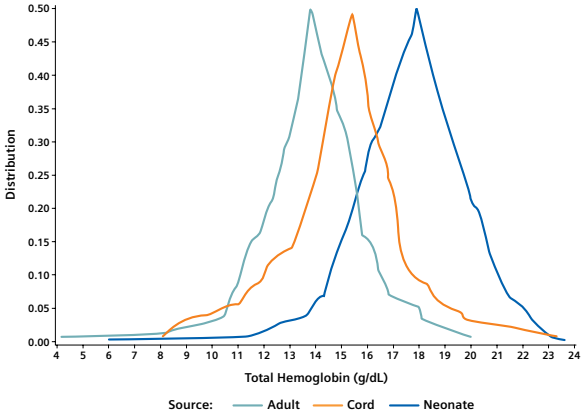
Results

Described below is a neonatal bilirubin performance evaluation of the new RAPIDLab 1200 blood gas system bilirubin measurement, using whole blood, compared to the VITROS 950 as the plasma chemistry reference method.

Early development of the whole blood nBili measurement on the RAPIDLab 1200 series indicated a pronounced inverse relationship of total hemoglobin/hematocrit versus raw bilirubin results; as total hemoglobin increases, the raw bilirubin results decreases. To compensate for this relationship, a mathematical hematocrit

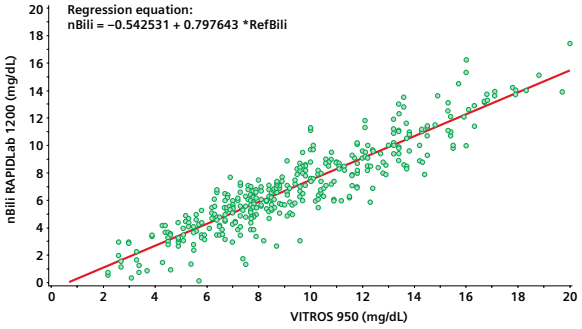
correction was applied to the raw nBili results. An effective hematocrit correction is of importance as the total hemoglobin/hematocrit distribution may be different and/or more diverse among various sample source populations. Figure 1 illustrates the total hemoglobin distribution of the test samples at the clinical site differentiated by sample source (adult, cord, and neonate). The bilirubin determination for the neonatal population (in blue), overall displaying a wide spread and overall larger total hemoglobin values (mean = 18.2 g/dL), would be inversely affected by a poor or no hematocrit correction. The adult population (in light green), overall has a lower average total hemoglobin (mean = 13.8 g/dL). The total hemoglobin distribution for cord specimens (in orange) falls slightly less than midway (mean = 15.5 g/dL) between the neonatal and adult peaks.

Figure 1. Mountain plot showing distribution of total (native) hemoglobin in adults (light green), neonates (blue), and cord blood (orange).



Despite the application of a RAPIDLab 1200 nBili hematocrit correction, significant bilirubin under-recovery and imprecision was observed on the RAPIDLab 1200 versus the VITROS 950 reference on neonatal samples (Figure 2).

Figure 2. Interim method comparison of bilirubin measurement on the RAPIDLab 1200 versus the VITROS 950 on neonatal specimens (with RAPIDLab 1200 nBili software using only hematocrit correction).

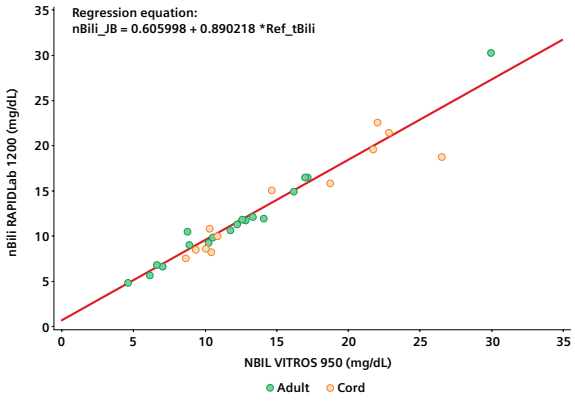


This under-recovery was not observed during internal developmental testing where adult whole blood samples adjusted for total hemoglobin and doped with various amounts of prepared unconjugated bilirubin spiking solution were used. An adjustment for the presence of fetal hemoglobin was already being applied in determining the concentration of total hemoglobin and the CO-oximetry fractions on the RAPIDLab 1200. However, the original fetal hemoglobin compensation was determined to be inadequate for bilirubin determination.

Whole blood from adult and cord sources was spiked with varying concentrations of the same lot of prepared bilirubin. Cord whole blood, unlike adult, contains elevated levels of native fetal hemoglobin. Therefore, the significant difference in the specimens is not the bilirubin source (native versus artificial), but the presence of fetal hemoglobin. Figure 3 exhibits method comparison of RAPIDLab 1200 bilirubin results versus the VITROS 950 for spiked adult and cord samples using the refined fetal hemoglobin correction vector.

RAPIDLab 1200 systems employ effective compensation algorithms to account for hematocrit and fetal hemoglobin concentrations.

Figure 3. Bilirubin recovery of spiked adult and cord samples: RAPIDLab 1200 vs. VITROS 950.



On the basis of t-test analysis of the RAPIDLab 1200 mean bilirubin bias against the VITROS 950 for spiked cord and spiked adult samples, the two populations are statistically equivalent (i.e., there is not enough evidence to conclude with 95 percent confidence that the two populations are different; Table 1). This supports the use of the additional fetal hemoglobin compensation and minimizes the difference seen between contrived and natural specimens.

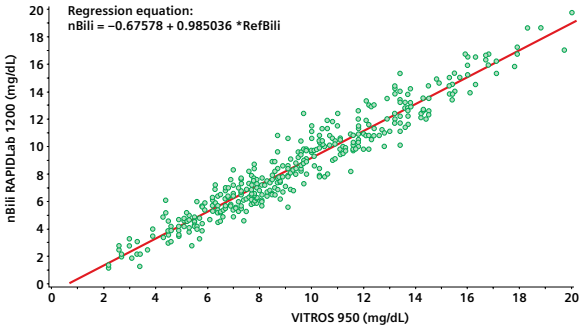
Table 1. Statistical comparison of bias (RAPIDLab 1200 – VITROS 950) for bilirubin-spiked samples.

Spiked Cord Samples			Spiked Adult Samples			t-Test		Reject
N	mean bias	SD	N	mean bias	SD	t Stat	Pr>t	Null?
12	–1.55	2.26	18	–0.44	0.83	1.62	0.13	No

Despite the difference between technologies and sample matrix, measurements of neonatal total bilirubin using spectrophotometry with the RAPIDLab 1200 on whole blood are comparable and show excellent correlation to that of the reagent-based VITROS 950 chemistry system on plasma.

Once this correction for native fetal hemoglobin was applied to the clinical neonatal data set utilized in Figure 2, the final method comparison of bilirubin on the RAPIDLab 1200 versus the VITROS 950 chemistry system demonstrated good correlation. Both bias and precision showed observable improvement (Figure 4).

Figure 4. Final method comparison of RAPIDLab 1200 versus VITROS 950 on neonatal specimens (with final RAPIDLab 1200 nBili software using hematocrit and fetal hemoglobin corrections).



Despite the differences between the technology and the sample matrix, RAPIDLab 1200 nBili versus VITROS 950 NBIL shows excellent correlation. Linear regression comparison of the clinical neonate specimens using pre- versus post-fetal hemoglobin correction (RMSE = root mean square, r^2 = correlation coefficient) is outlined in Table 2.

Table 2. Comparison of linear regression analysis of method comparison of RAPIDLab 1200 pre- or post-fetal hemoglobin correction vs. VITROS 950.

Figure	RAPIDLab 1200 Software	N	Intercept	Slope	RMSE	r2
2	Interim (no fetalHb correction)	379	–0.54	0.80	1.258	0.832
4	Final (with fetalHb correction)	378	–0.68	0.99	0.966	0.928

Conclusions

A feature of the RAPIDLab 1200 series of blood gas analyzers is the ability to quantify and report patient results for blood gases, pH, electrolytes, metabolites and CO-oximetry. Neonatal total bilirubin is now available on the same analyzers from the same single whole blood sample, eliminating the need for an additional draw.

The testing concludes that the accuracy of the RAPIDLab 1200 whole blood bilirubin method is comparable to that of the VITROS 950 plasma chemistry method. The absence of a statistically significant difference in bias between spiked cord and spiked adult samples indicates the effectiveness of the fetal hemoglobin compensation algorithm.

The new RAPIDLab 1200 neonatal total bilirubin measurement provides an alternative method to conventional chemistry analyzers. Using direct spectrophotometry, it provides results in only 60 seconds on whole blood neonatal specimens.

The RAPIDLab 1245 system requires a small sample volume of only 140µL; the RAPIDLab 1265 system, only 175µL. The unique microsample capability ensures the maximum number of tests to be run on the smallest of patient samples.

Tiny Patients—Big Demands

Siemens RAPIDLab 1200 systems offer a comprehensive test menu, fast turnaround, and processes that speed delivery of patient test results and improve quality of care to newborns. Designed for use in a busy laboratory or NICU, the system operation is quick and easy, and clinicians will find:

- A full test panel, including blood gas, electrolytes, metabolites, and CO-oximetry, complete with neonatal total bilirubin.
- Minimal sample volumes required with fewer patient re-draws.
- Results available in just 60 seconds, offering quick diagnosis and treatment and, ultimately, better patient care.
- All with the accuracy and precision required and expected.

This neonatal total bilirubin method evaluation was a collaborative study performed at Northwest Community Hospital, Arlington Heights, IL. USA

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Order No. A91DX-0701383-GC2-4A00 | 09-2011 |