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Operational impact of using a vanadate oxidase method for direct bilirubin measurements at an academic medical center clinical laboratory

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ABSTRACT

Objectives: The aim of this study was to compare the operational impact of using vanadate oxidase versus diazo direct bilirubin assays for an academic medical center patient population.
Design and methods: Retrospective study was done over an approximately 3.5 year period. The main automated chemistry instrumentation was a Roche Diagnostics cobas 8000 line. The Roche Direct Bilirubin assay was compared to Diazyme Laboratories Direct Bilirubin Assay and Randox Laboratories Direct Bilirubin assay using manufacturer's guidelines for hemolysis index, lipemia index, and analytical measurement range (AMR).
Results: Retrospective data was analyzed for 47,333 serum/plasma specimens that had clinical orders for direct bilirubin. A total of 5943 specimens (12.6%) exceeded the hemolysis index limit for the Roche method compared to only 0.2% and 0.05% of specimens for the Diazyme and Randox methods, respectively. The impact was particularly large on patients less than 2 years old, for which 51.3% of specimens exceeded the hemolysis index for the Roche method. A total of 1671 specimens (3.5%) exceeded the lipemia index limit for the Roche method compared to less than 0.1% for the Randox method. Lastly, 988 (2.1%) of specimens had direct bilirubin concentrations exceeding the upper AMR limit of 10 mg/dL [171 μmol/L] for the Roche assay compared to less than 1% of specimens for the vanadate oxidase methods.
Conclusions: Vanadate oxidase direct bilirubin methods offer advantages over diazo methods in terms of less interference by hemolysis and lipemia, as well as wider AMR. The advantages are particularly evident for neonatal and infant populations.

1. Introduction

Bilirubin is the major metabolite of heme, a key component of hemoglobin, myoglobin, and cytochromes [1]. Measurement of direct bilirubin (DBIL, also known as conjugated bilirubin) and total bilirubin (TBIL) is widely used in clinical medicine, especially in the diagnosis and management of disorders affecting the hematologic and hepatobiliary systems [1–3]. Bilirubin can be measured in body fluids by a variety of analytical methods including chromatography, electrophoresis, and spectrophotometry [1]. Given that DBIL and TBIL are among the most commonly ordered laboratory tests, clinical laboratories typically utilize automated clinical chemistry assays. The most widely used methods for measurement of different forms of bilirubin employ the diazo method, originally developed by Ehrlich in 1883 [4,5] and modified multiple times over the years [1,6–9]. Conjugated bilirubin reacts directly with the diazo reagent (thus the designation of conjugated bilirubin as “directly reacting” or “direct” bilirubin) [1].

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In a typical automated diazo method, serum/plasma is incubated with diazo reagent at approximately pH 1.7–2.0 to form a diazonium salt [8]. The resulting product then reacts with bilirubin to form isomers of azobilirubin. In DBIL assays, the conjugated bilirubin is the predominant form converted by the diazotized sulfanilic acid (approximately 5% of unconjugated bilirubin may react as well). The intensity of the red color of azobilirubin is measured photometrically at approximately 600 nm and is proportional to DBIL concentration. Unconjugated bilirubin reacts with diazo reagents following the addition of accelerants (e.g., caffeine, sodium benzoate, or methanol), thus allowing for the determination of the concentration of TBIL. The difference between TBIL and DBIL measurements allows for the estimation of the concentration of unconjugated bilirubin (hence the designation of this difference as "indirect" bilirubin).

While the diazo DBIL methods are inexpensive and readily automated, they have some key limitations, including interference by low levels of hemolysis and less commonly by lipemia or paraproteins [1,10–12]. The interference by hemolysis can be especially problematic in the neonatal and infant populations, where sample collection may be challenging and lead to suboptimal quality of specimens. While TBIL is the laboratory test primarily used for assessment and management of neonatal hyperbilirubinemia, DBIL measurements can be very useful in the differential diagnosis and clinical management of neonates whose hyperbilirubinemia is due to a cause other than physiologic jaundice of the newborn [3,13,14]. A recent study has shown that hemolysis interference with a diazo DBIL method can lead to unpredictable bias even at low levels of hemolysis [15].

The mechanism by which hemolysis interferes with diazo DBIL assays is complex and incompletely understood [15]. The interference is influenced by composition of DBIL assay reagents, primary/sub-wavelength used, type of chemical reaction (e.g, rate vs. end-point), assay pH, serum/plasma albumin concentration, medications, and presence of low molecular weight molecules. A detailed analysis of hemolysis interference is described by Devgun and Richardson, who utilized hemolysis thresholds to better define the extent of interference on diazo DBIL analysis [15]. Depending on patient population, a sizable number of specimens may have hemolysis that exceeds the recommended hemolysis limit for diazo DBIL assays given that hemolysis is the most common endogenous interference [16–20]. Although the mechanisms of hemolysis interference with DBIL are incompletely understood, several studies have shown predominantly negative interference [15,21]. The sensitivity of diazo DBIL assays to even low degrees of hemolysis places the clinical laboratory in a difficult position - either cancel the testing if hemolysis is present or, alternatively, report with a disclaimer that the results may be impacted by hemolysis. The former approach can lead to clinician and patient dissatisfaction, while the latter approach risks reporting inaccurate results.

Paraproteins (especially IgM monoclonal proteins) have also been reported to interfere with the diazo DBIL and TBIL methods, producing either positive or negative interferences [10–12,22]. Paraprotein interference is method specific, with significant variability between assays even for the same specimen. The concentration and isotype of the paraprotein does not correlate well with presence or degree of interference. Paraprotein interference may be detected by unusual results such as DBIL concentration much greater than the TBIL concentration, unusual reaction kinetics or absorbance readings (sometimes resulting in error flags on instruments), or a significant discrepancy between serum icterus index and either DBIL or TBIL concentration. Proposed mechanisms for paraprotein interference include increased turbidity, interference with reaction kinetics, and/or alterations in spectrophotometric readings [10–12,22].

An alternative approach to measurement of DBIL are enzymatic methods utilizing bilirubin oxidase, an enzyme that catalyzes the oxidation of bilirubin to biliverdin [23–28]. The vanadate oxidase method for determination of DBIL utilizes vanadate as the oxidizing agent for the conversion of bilirubin (yellow colored) to biliverdin, which may then be further oxidized to colorless products. Although the vanadate oxidase method for determination of DBIL has been known for several decades, there is relatively little published literature on the application of this method to clinical samples except for the recent study referenced above [15] and a veterinary publication analyzing samples from dog, monkey, and rat [29]. For the vanadate oxidase methods, specimen is mixed with reagent at approximately pH 3, converting DBIL to biliverdin, thereby decreasing the absorbance of yellow (main wavelength 450 nm, sub-wavelength 546 nm) [27,28]. There is very little interference from hemolysis for the vanadate oxidase DBIL methods [15]. There are several vanadate oxidase DBIL methods available for automated clinical chemistry analyzers, although these usually need to be run on open analyzer channels given that the major clinical chemistry instrumentation vendors generally offer their own diazo DBIL methods as the default choice [1].

The aim of the present study was to estimate the operational impact of using a vanadate oxidase DBIL method at an academic medical center. The initial impetus for the study was clinician complaints from cancellation of testing for hemolyzed specimens, especially in the neonatal and infant population. The primary aim was therefore to estimate how often the vanadate oxidase assays allowed for analysis of specimens that exceeded the hemolysis limit specified by the manufacturer of the diazo DBIL method. We also examined the impact of vanadate oxidase DBIL assays on lipemia interference and analytical measurement range (AMR). We utilized a 3.5 year retrospective time period where we had complete data on hemolysis index (HI), lipemia index, and patient demographics for all samples for which DBIL was ordered clinically.

2. Materials and methods

2.1. Study setting and population

The study was conducted at the University of Iowa Hospitals and Clinics (UIHC), a 734-bed pediatric and adult tertiary/quaternary care academic medical center with level one trauma center, inpatient units (including multiple intensive care units), and outpatient clinics. The electronic health record is currently Epic (Epic Systems, Inc., Madison, WI, USA) which contains historic data covering the entire period of retrospective analysis. The data in this study is from the UIHC core laboratory and was collected as part