Validation of the Procalcitonin Assay on the Abbott Architect i1000

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Background: Procalcitonin (PCT) is an emerging biomarker for detecting sepsis. Recently, the US Food and Drug Administration cleared the expanded use of this biomarker for guiding clinicians regarding antibiotic treatment. To our knowledge, there are no published method validations for the Abbott Architect PCT assay. This article will discuss the process of method validation of the B·R·A·H·M·S PCT assay on the Abbott Architect platform.

Methods: We studied the precision, accuracy, and linearity of the Architect method following the guidance of the Clinical and Laboratory Standards Institute EP5-A2 document. Furthermore, we also tested the impact of major sources of interference from hemolysate, lipoproteins, and bilirubin. To validate the Architect method, we compared patients’ serum PCT measurements with our previously established Mini VIDAS (bioMerieux) PCT assay.

Results: Statistical analysis showed that the 2 assays have good correlation ($r > 0.99$), slope of 1.023, and intercept of $-0.760$. The calculated bias is $-7.435\%$. The Architect method showed good precision with $%CV < 3.5\%$ for both interassay and intraassay compared with $%CV < 6.5\%$ for Mini VIDAS, which was previously determined at our institution. No bias >$10\%$ was observed with the Architect method when pooled serum samples were spiked with interferants. The turnaround time for both platforms was the same (20 min); however, in contrast with Mini VIDAS, the Architect system has automated pipetting of samples and can perform multiple assays simultaneously.

Conclusion: These results showed that the Architect B·R·A·H·M·S PCT assay has analytical characteristics conducive for diagnostic use in clinical laboratories. Our method validation report will be beneficial for other institutions to adapt this assay on existing Abbott Architect i1000 immunoassay analyzers.

IMPACT STATEMENT

This study is the first report on method validation for an Architect procalcitonin (PCT) assay. Our results showed that the PCT assay validated on the Abbott Architect i1000 was not substantially affected by grossly hemolyzed, lipemic, or icteric samples. This is beneficial for pediatric and critically ill patients such as those under extracorporeal membrane oxygenation.

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3Nonstandard abbreviations: PCT, procalcitonin; AMR, analytical measureable range; CCR, clinical reportable range; ECMO, extracorporeal membrane oxygenation.

4Human Genes: CALC-1, calcitonin I.
Sepsis, characterized by marked systemic inflammation and infection, is the 10th leading cause of death in the US (1). When sepsis is not treated early, severe septic conditions may occur, with a reported mortality rate of about 29% (2). However, the symptoms of sepsis are not specific, making it difficult to obtain an early diagnosis, which can result in delay of proper therapy. Procalcitonin (PCT) and C-reactive protein are used as clinical biomarkers for sepsis, but PCT has been shown to be more sensitive and an early marker for monitoring septic shock (3, 4). PCT is specific for bacterial infection, which causes upregulation of the CALC-1 gene and results in higher levels of PCT. The regulation of PCT expression is tissue specific, and in the absence of infection, PCT is suppressed in non-neuroendocrine cells except in the parafollicular cells of the thyroid glands (5). The specificity of increase in PCT to bacterial infection has been suggested by the observed PCT increase within 3 h after a single dose of the endotoxin lipopolysaccharide in animal and human studies (6, 7).

In 2006, the Food and Drug Administration cleared the use of the B·R·A·H·M·S PCT assay (bioMerieux) to help providers predict the likelihood of a patient dying or whether the patient’s condition is worsening because of sepsis. In early 2017, the Food and Drug Administration cleared the expanded use of this assay to aid clinicians with identifying when antibiotic treatment should be initiated and halted. We anticipate that the expanded use of PCT would result in an increase in the utilization of this test; therefore, validation of the B·R·A·H·M·S PCT assay on an automated, high-throughput platform would be beneficial.

**MATERIALS AND METHODS**

Precision and reportable ranges on the Abbott Architect i1000 were performed. The manufacturer’s instructions were followed, including quality control, calibration, calibration validation, and related functions (10). Reagents, fluids, and disposable materials met the manufacturer’s specifications.

**Precision studies**

The guidelines stated in the Clinical and Laboratory Standards Institute EP5-A3 document were adopted to conduct repeatability and reproducibility studies. Using Abbott quality control materials, repeatability was determined by analyzing low (0.2 ng/mL), medium (1.97 ng/mL), and high (69 ng/mL) controls in 10 replicates each within the run. Reproducibility was determined by analyzing low, medium, and high controls once each day for 10 days.

**Linearity study**

Six different concentrations (0, 0.1, 0.5, 12.1, 20.5, and 100 ng/mL) of the Abbott Architect PCT calibrators were analyzed in duplicate to verify the analytical measurement range. Three high (100 ng/mL) concentrations using these platforms. The Architect B·R·A·H·M·S PCT assay uses the chemiluminescent microparticle immunoassay particle, whereas the Mini VIDAS B·R·A·H·M·S PCT assay uses enzyme-linked fluorescence detection. At our institution, the Mini VIDAS is used exclusively for PCT testing. Therefore, shifting the test to the Abbott Architect will help streamline the process. In addition, our Mini VIDAS instrument was not interfaced with the laboratory information system, whereas our Architect is already interfaced with the laboratory information system. Moving our PCT assay to the Architect system will also eliminate preanalytical errors such as manual pipetting of samples into reaction wells, manual labeling of reaction wells in Mini VIDAS, and manual entry of test results.
ng/mL) PCT concentration calibrators run in duplicate were used for on-board ×10 dilution. The clinical reportable range and analytical measurement range were determined from these results.

### Sensitivity

Ten replicates of zero concentration calibrator and 3 replicates of low (0.1 ng/mL) concentrations were performed.

### Sample carryover

Low and high controls were measured in the order of 3 low controls (0.20 ng/mL), 1 high control (71.30 ng/mL), and 1 low control. The percent carryover (k) was calculated as described in Eq. 1:

$$k = \left( \frac{\text{position 5 result} - \text{average of position 1, 2 and 3 results}}{\text{position 4 result}} \right) \times 100\%$$

where positions 1, 2, 3, and 5 are low controls and position 4 corresponds to the high control. A carryover <1% is acceptable.

### Interference studies

Common interferences used in validation studies are free hemoglobin, lipids, and bilirubin (17). Previously analyzed random patient samples were pooled together into 2 groups: >2 ng/mL PCT and <2 ng/mL PCT. Previously, our institution identified that a PCT >2 ng/mL is highly suggestive of bacterial infection (12); therefore, we wanted to know how the interferants will affect PCT values <2 ng/mL or >2 ng/mL. Aliquots of the pooled serum were either spiked with an interferant (free hemoglobin, triglyceride-rich lipoproteins, and bilirubin) from a kit (INT-01 Routine Interferents ASSURANCE™ Interference Test Kit by Sun Diagnostics®) or with 1× PBS. The concentrations of the interferants were 12 g/L free hemoglobin, 11 g/L triglyceride-rich lipoproteins, or 0.29 g/L bilirubin to mimic hemolyzed, lipemic, and icterus samples, respectively. Throughout the rest of this report, the terms hemolyzed, lipemic, and icterus will be used to reference these conditions.

All blanks were spiked with an appropriate volume of 1× PBS. In addition, 200 μL of the spiked samples was first manually aliquoted for Mini VIDAS, and the remainder of the same solution was immediately run in Architect for comparison. Samples were run in singlicate.

### Method comparison

The Mini VIDAS B·R·A·H·M·S PCT assay was previously validated (12) at Texas Children’s Hospital and was used as the comparative method when the Abbott Architect B·R·A·H·M·S PCT was the test method. Thirty-two samples were analyzed in both platforms, and Deming regression was used to examine the results.

### Reference range

Twenty-four serum/plasma samples from random pediatric patients without a known medical history of bacteremia were analyzed for PCT to confirm whether we could adapt our previously established reference range (0.05–2.0 ng/mL) (12).

### Statistical analyses

EP Evaluator® software was used to generate the plots, mean, SD, Deming regression, and %CV.

### RESULTS

#### Linearity and precision studies

The Architect B·R·A·H·M·S PCT assay showed good precision at all 3 control concentrations with intraassay and interassay %CVs <3.5%. Table 1 summarizes the mean, SD, and %CV results. The obtained analytical measurement range for Architect is 0.02 to 100 ng/mL, which verifies the manufacturer’s claim (see Fig. 1). Dilution studies extended the clinical reportable range to 0.02 to 1000 ng/mL.
**Sensitivity and carryover**

The manufacturer claims the limit of blank to be 0.02 ng/mL. Analysis of the zero concentration calibrator showed 2 SD limit of the blank to be 0.009 ng/mL, which passed the acceptance criteria based on the EP Evaluator. Also, no significant sample carryover was observed. The calculated k was −0.019%, which is below the acceptable 1% carryover. Table 2 summarizes the carryover study results.

**Interference studies**

Table 3 shows that icterus samples produced the highest bias observed in both platforms. The highest observed bias for the Architect system was 6.6% compared with 28.6% in the Mini VIDAS. Table 3 summarizes the interference results.

**Method comparison**

Comparison of results (shown in Fig. 2) obtained for the Architect with the reference method, Mini VIDAS, by Deming regression yields good correlation as indicated by a slope of 1.023 and an intercept of −0.760. The calculated bias is −7.435% with a correlation coefficient of 0.9981.

**Reference range**

Based on the 24 “healthy” patient samples, the proposed reference interval of 0.02 to 2.00 ng/mL PCT passed after EP Evaluator reference range analysis.

### Table 1. Precision results of low, medium, and high controls.

<table>
<thead>
<tr>
<th></th>
<th>Low</th>
<th>Medium</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean, ng/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Repeatability</td>
<td>0.20</td>
<td>2.01</td>
<td>70.15</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>%CV</td>
<td>3.3%</td>
<td>1.6%</td>
</tr>
<tr>
<td>Reproducibility</td>
<td>0.21</td>
<td>2.00</td>
<td>69.08</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>%CV</td>
<td>3.0%</td>
<td>1.6%</td>
</tr>
</tbody>
</table>

### Table 2. Carryover study results.

<table>
<thead>
<tr>
<th>Position</th>
<th>Concentration, ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.21</td>
</tr>
<tr>
<td>2</td>
<td>0.21</td>
</tr>
<tr>
<td>3</td>
<td>0.22</td>
</tr>
<tr>
<td>4</td>
<td>71.32</td>
</tr>
<tr>
<td>5</td>
<td>0.20</td>
</tr>
</tbody>
</table>

**Fig. 1. Linearity plot.**

Linear plot of manufacturer-provided calibrators (n = 6; 0.00–100.00 μg/mL) run in duplicates. y = 1.014x + 0.000.
DISCUSSION

PCT is an important biomarker for bacterial sepsis and antibiotic treatment management. To our knowledge, our study is the first published that validates the Architect B·R·A·H·M·S PCT. Our validation studies showed that the Architect B·R·A·H·M·S PCT exhibited good precision with a %CV <3.5% for both interassay and intraassay and has excellent linearity over a wide analytical range; also, the Architect system was susceptible to fewer errors because of autodilution. The analytical measureable range (AMR) for the Mini VIDAS B·R·A·H·M·S PCT from our previous studies was 0.05 to 200 ng/mL, with a clinical reportable range (CRR) of 0.05 to 2000 ng/mL. Our verified Architect B·R·A·H·M·S PCT AMR and CRR are 0.02 to 100 ng/mL and 0.02 to 1000 ng/mL, respectively. Although the AMR and CRR are lower for the Architect, the range covers the decision limits previously established in our institution, for which 2 ng/mL has been determined to be highly suggestive of bacterial sepsis (12). Also, method comparison between Mini VIDAS and the Architect system yielded excellent

### Table 3. Comparison of interference study in Mini VIDAS and Architect.

<table>
<thead>
<tr>
<th>Low PCT, ng/mL</th>
<th>High PCT, ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mini VIDAS</td>
</tr>
<tr>
<td>Hemolyzed (12 g/L)</td>
<td>1.05</td>
</tr>
<tr>
<td>1× PBS</td>
<td>1.19</td>
</tr>
<tr>
<td>% Bias</td>
<td>−11.76</td>
</tr>
<tr>
<td>Lipemic (11 g/L)</td>
<td>1.19</td>
</tr>
<tr>
<td>1× PBS</td>
<td>1.21</td>
</tr>
<tr>
<td>% Bias</td>
<td>−1.65</td>
</tr>
<tr>
<td>Icteric (0.29 g/L)</td>
<td>0.85</td>
</tr>
<tr>
<td>1× PBS</td>
<td>1.19</td>
</tr>
<tr>
<td>% Bias</td>
<td>−28.57</td>
</tr>
</tbody>
</table>

**Fig. 2. Method comparison study.**
Thirty-two patient serum samples were analyzed in Architect and Mini VIDAS for comparison. (A), Deming regression plot showed good correlation, $y = 1.023x - 0.760, R = 0.9981$. (B), Bias plot. (C), Percent bias plot.
correlation. The negative bias indicates that Architect measurement is about 7% lower than Mini VIDAS. The negative bias for Abbott Architect compared with Mini VIDAS is also observed in the recently released College of American Pathologists Participant Summary PCT-A 2018 (13). The survey reported the consensus mean to be at least 9% lower in the Abbott Architect “i” system than in the bioMerieux VIDAS/Mini VIDAS. Our interference studies also showed that the Architect system was less affected by common interferants compared with the Mini VIDAS.

An assay that is not affected by hemolysis is particularly beneficial for pediatric patients. Hemolysis is a common interferant especially in pediatric patients because of the blood collection techniques used for this population, such as the small syringe gauge often used. Furthermore, critically ill patients such as those undergoing extracorporeal membrane oxygenation (ECMO) have risk of obtaining samples that have mechanically induced hemolysis. Also, ECMO increases the risk of contracting nosocomial infections; therefore, a PCT assay that is not interfered with by hemolysis would be extremely beneficial. A few months after implementing the Architect B·R·A·H·M·S PCT assay, we reviewed PCT values from the Architect in ECMO patients who had not yet received antibiotic therapy at Texas Children’s Hospital. These results were correlated with microbiology and virology studies on samples taken at the same time in 5 patients. The review showed that 4 patients with high PCT values (>2 ng/mL) were positive for organism growth in culture studies. Not surprisingly, 1 patient with a viral infection had a PCT value of 0.24 ng/mL. These findings agree with the manufacturer’s guideline that PCT >2 ng/mL has a high risk of progressing to bacterial infection/sepsis. However, because of the exposure of blood to synthetic materials, ECMO patients may require a different PCT cutoff owing to the systemic inflammatory response syndrome commonly observed in this setting (14). Prospective study of pediatric patients undergoing veno-arterial ECMO reported that although a high PCT concentration is a good predictor of multiple organ dysfunction, the biomarker is not a dependable marker of infection (15). Interestingly, these authors also reported that patients with infection had lower plasma PCT concentration than those without infection. On the contrary, a retrospective study on pediatric ECMO patients suggested that PCT at 0.5 ng/mL has 92% sensitivity and 43% specificity in determining bacterial infection (16). Another prospective study of PCT and C-reactive protein in uninfected term and preterm neonates revealed that these markers fluctuate after birth; therefore, utilization of these markers in the early onset of infection should be based on age (17). Given the paucity of PCT studies regarding utility in pediatric populations on ECMO, properly powered, multiinstitutional studies must be conducted to address this question.

Cholestasis and liver disease are conditions that result in increased bilirubin in patient samples. Icterus samples exhibited the highest bias in both assays as shown by our interference studies. However, it is important to note that the 2 platforms use different types of detection methods. The Architect system uses chemiluminescence, whereas the Mini VIDAS uses fluorescence measurement at 450 nm. Bilirubin has been reported to have an absorbance peak of approximately 456 nm (18). Therefore, a high bilirubin could result in fluorescence quenching, resulting in a lower value as observed in the Mini VIDAS with a negative bias of 28.6%. On the other hand, the Architect PCT assay uses acridinium-conjugate antibody reaction with hydrogen peroxide. A possible explanation for the slight positive bias (6.6%) in the icterus sample with high PCT values is that ultra-weak chemiluminescence has been reported in bilirubin when reacted with activated oxygen species (19). Overall, our interference studies have shown that the chemiluminescence detection by the Architect system was less affected by common high concentrations of
interferants compared with Mini VIDAS with fluorescence detection.

Moving the PCT assay to the Abbott Architect system allowed us to do away with manual pipetting of samples and manual input of results (which are required when using the Mini VIDAS platform), eliminating sources of preanalytical error and improving turnaround time. Our method validation report will be beneficial for other institutions considering implementation of this assay on the Architect immunoassay analyzer.

**Author Contributions:** All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.

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**REFERENCES**