Background: We previously described the clinical validation of a second-generation rapid dilute-and-shoot ultra-performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS) assay to quantify and semi-quantify over 30 prescription drugs, drugs of abuse, and related glucuronides and other metabolites in human urine. Unlike our institution’s first-generation format, this second-generation assay does not include enzymatic glucuronide hydrolysis. Based on clinician feedback and an analysis of 12 months of immunoassay (IA) and first- and second-generation UPLC-MS/MS clinical pain management testing at our institution, three additional drug metabolites (hydromorphone, lorazepam, and oxazepam glucuronides) were added to the UPLC-MS/MS pain management assay while maintaining facile sample preparation and acceptable analytical performance.

Methods: Clinical testing results from 6 months of IA plus our first-generation UPLC-MS/MS assay were compared to test results from the first 6 months of the recently validated second-generation UPLC-MS/MS assay. Along with soliciting clinician feedback, data were analyzed for clinical performance, including positivity rates, to determine the clinical need for additional analyte testing. Human urine samples were diluted with water and spiked with deuterated internal standards without any proceeding enzymatic hydrolysis, analyte extraction, or sample purification. Analytes were separated by reverse-phase UPLC and then quantified by positive-mode electro-spray ionization and collision-induced dissociation MS. Assay performance was validated per FDA bioanalytical guidelines.

Results: Analysis of clinical testing between the first- and second-generation UPLC-MS/MS pain management assays showed appreciable decreases in positivity rates for diazepam (and metabolites such as oxazepam), hydromorphone, and lorazepam, presumably due to the fact that the second-generation assay did not include analysis of the glucuronide metabolites. Three glucuronide metabolites were added to the newest version of the UPLC-MS/MS assay for pain management. Total analytical runtime was maintained at 5 minutes. All analytes, including the additional glucuronide metabolites, demonstrated acceptable linearity, analytical limits of detection, accuracy, precision, stability, and matrix effects, based on FDA bioanalytical guidelines and clinical specifications.

Conclusions: The additional glucuronide metabolites were successfully incorporated into an existing clinical UPLC-MS/MS assay for simultaneously monitoring 39 clinically relevant analytes in human urine for use in chronic pain management. Quantification of these three additional glucuronides should enhance clinical decision making by providing additional information about drug metabolism and enabling the detection of exogenous addition of analytes.

Performance Evaluation of BRAHMS Procalcitonin Assay on Abbott Architect

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Background: Procalcitonin (PCT) is the precursor of calcitonin (CT), a hormone synthesized by the parafollicular C cells of the thyroid and involved in calcium homeostasis. However, PCT has a different biological function and regulation of induction than CT. PCT is released mainly from nonthyroid tissues as an acute-phase reactant in response to inflammatory stimuli, especially bacterial infection. Increased serum PCT is associated with bacterial endotoxin and inflammatory cytokines but not followed by an increase of CT or serum calcium levels. Therefore, PCT is widely used as a biomarker for bacterial infection and sepsis. Clinically, PCT greater than 2 ng/mL is associated with high risk of sepsis, and PCT less than 0.5 ng/mL is associated with low risk. The objective of this study was to assess the analytical performance of BRAHMS PCT on the Abbott Architect iSystem.

Methods: PCT was quantitatively determined by the BRAHMS PCT assay on the Architect iSystem, which is a two-step chemiluminescent microparticle immunoassay (CMIA). The evaluation was performed following CLSI guidelines. The performance was evaluated for linearity, sensitivity, carryover, reference range, and precision. The within-run and between-run precisions were assessed by analyzing QC material at low and high levels of concentrations. Since currently there is no agreed-on reference method for PCT available, accuracy was assessed by comparison between PCT on Architect and BioMérieux Vidas, which has been in clinical use at our institution for the past 4 years.

Results: The analytical measurement range was determined to be linear between 0.00 and 98.55 ng/mL with a slope of 0.999 and intercept of 0.002. The limit of blank was determined as 0.00 ng/mL, and the limit of detection was 0.02 ng/mL. The carryover was 0.00 ng/mL. The reference range was verified as less than 0.07 ng/mL. The within-run CVs for PCT were 2.6% at the low level of 0.196 ng/mL and 1.7% at the high level of 66.0 ng/mL. The between-run CVs at low and high levels were 8.2% and 4.1%, respectively. Comparison of PCT on the Abbott Architect with PCT on BioMérieux Vidas showed the slope was 0.631 (95% CI, 0.574–0.687) with an intercept of 0.376 and correlation coefficient of 0.9643 (Deming). The mean bias between Architect and Vidas was –5.937. Using samples with PCT less than 5 ng/mL, the comparison between the two analyzers showed the slope was 0.674 (95% CI, 0.642–0.706) with an intercept of 0.052 and correlation coefficient of 0.9942 (Deming), and the mean bias was –0.289.

Conclusion: Our data demonstrate that the BRAHMS PCT on Abbott Architect analyzer has good linearity, precision, and no carryover. There is a good correlation.
Increased Incidence of Acquired Protein S Deficiency in Sickle Cell Disease

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Patients with sickle cell disease (SCD) show various manifestations of arterial and venous thrombosis, especially around sickle cell crises. There are numerous pathophysiologic factors that could explain hypercoagulability in SCD (vessel injury, enhanced platelet function, impaired fibrinolysis, activated coagulation cascade, etc). The aim of this study is to demonstrate the association of acquired protein S deficiency (PSD) with SCD, elucidate the mechanism in vitro, and correlate it with timing of sickle cell crises in our patient population. This study includes every patient diagnosed with sickle cell trait (SCT) and SCD in a large center of SCD excellence between 2000 and 2017. The data are gathered using a user-friendly interactive software developed in-house for the evaluation of health care quality, efficiency, and effectiveness. The EMR database (EPIC) we use consists of laboratory, clinical, pathological, and radiological reports allowing us to use different search criteria and to outline cutoff values for continuous and categorical variables while permitting us to define a time frame for the different observations. It is a useful resource to compare and contrast how two or more populations are affected by a particular disease and to separate out patients with a particular diagnosis. The initial data we extracted looked at patients with a diagnosis of SCD between 2000 and 2017 who had acquired PSD (<55%) and compared it to patients who have SCT with acquired PSD (<55%) in the same time period. We observed that out of 5,799 patients who had a diagnosis of SCD, 74 patients (1.27%) had a concomitant PSD, and out of 11,551 patients with SCT, 110 patients (0.95%) had a concomitant PSD. These preliminary results suggest a strong association of acquired PSD with SCD when compared to the general population (incidence of 0.2%) and, more specifically, the role of sickled red blood cells in acquired PSD. A chi-square analysis of SCD vs SCT ($P = .04$) and SCD vs general population ($P = .03$) showed a statistically significant increase in the incidence of PSD in SCD > SCT > general population. The higher incidence of PSD in SCD compared to SCT and the general population suggests that PSD is directly dependent on the extent of sickled cells. We hypothesize that acquired PSD in SCD is due to direct binding of protein S to sickling red cells that express increased surface phosphatidylserine. We are currently evaluating this hypothesis by in vitro flow cytometry studies. We are also studying how acquired PSD correlates with the timing of sickle cell crises in our patient population. In summary, acquired PSD may be another risk factor that contributes to thrombosis in SCD and thus may be a useful clinical marker to predict and/or monitor thrombosis in SCD.

In Situ Multiplex Immunofluorescence Analysis of Plasma Cell Myeloma Tissue Sections

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Plasma cell myeloma (PCM), with a highly heterogeneous clinical behavior and response to therapy, accounts for approximately 10% of hematologic malignancies. Current diagnostic and prognostic tests include serum-based assays, multiparametric flow cytometric analysis of bone marrow aspirates, morphologic and immunohistochemical analysis of bone marrow sections, cytogenetic analysis, and in vivo imaging. None of these tools allow for determination of expression of large numbers of antigens on a single myeloma cell in spatial context, which may be useful in order to better understand disease progression and therapy response. In a pilot study, we sought to evaluate the performance of a new in situ hyperplex immunofluorescence platform, Cell-DIVE, that allows spatial and subcellular localization of over 60 markers on three bone marrow samples (two non-decalcified clot sections and one decalcified core biopsy) from PCM cases. The platform utilizes a signal cycling process wherein repeated cycles of staining with fluorophore-conjugated antibodies, imaging, and signal removal are performed on the same tissue sections. Postcycling, images from multiple cycles are registered, corrected for tissue auto-fluorescence, and segmented into single cells and subcellular compartments using compartment-specific markers. To demonstrate feasibility, a panel of five markers that included plasma cell markers (CD138, CD45), markers of clonality (kappa and lambda light chains), and the proliferation marker Ki-67 were evaluated on a single histologic section. A virtual H&E image was also generated from the same tissue section. Both clot and core biopsy samples showed staining patterns similar to immunohistochemistry—the PCM cells expressed CD138 and either kappa or lambda light chains but lacked CD45. The cellular localization of staining was also as expected, with CD138 displaying surface staining, light chains displaying cytoplasmic staining, and Ki-67 displaying nuclear staining. This new immunofluorescence platform blends the strengths of immunohistochemistry (preservation of tissue architecture) with those of flow cytometry (analysis of many antigens on the same cell). Our preliminary data suggest that the in situ hyperplex immunofluorescence platform is a powerful new approach to study PCM biology, which allows for preservation of the spatial context.