gaps were comparatively more common in the NICU population, with 983 patients having a negative anion gap [mkrasows2] (0.289 %). NICU patients constituted 91% of all blood gas analyses with a negative anion gap, demonstrating that negative anion gaps are much more prevalent in this patient population than they are in other pediatric and adult populations. Negative anion gaps are relatively rare occurrences in the adult outpatient population; however, they can be seen in association with certain underlying disease states such as malignancy. By comparison, negative anion gaps are much more common in critically-ill populations, most notably NICU patients.

Comparative Evaluation of Three Serologic Assays for the Identification of SARS-CoV-2 Antibodies.

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Serologic assays for the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibodies continue to be developed and approved rapidly with limited external validation. Accurate diagnostics are an essential component of pandemic management and public health. Residual serum samples (N=113) from patients who were evaluated for SARS-CoV-2 infection status by polymerase chain reaction (PCR) were retrospectively tested in parallel across three automated SARS-CoV-2 serologic assays: Liaison SARS-CoV-2 S1/S2 IgG, Elecsys anti-SARS-CoV-2 total antibody, and Access SARS-CoV-2 IgG. Testing of 51 PCR-positive and 62 PCR-negative patients demonstrated qualitative inter-test agreement of 96% overall, 100% in PCR-negative patients, 88% in early positive samples (0-13 days post positive PCR), and 100% in convalescent samples (14+ days post positive PCR). Calculated kappa values for paired inter-test agreement ranged 0.93-0.96. Compared to PCR, overall percent positive agreement ranged from 82-86% (100% for convalescent samples) and percent negative agreement was 100% for each assay. This study demonstrates high diagnostic accuracy and inter-test agreement for three automated SARS-CoV-2 serologic assays. External validation of serologic assays is critical to ensure diagnostic accuracy and appropriate utilization of critical resources.

Quantitation of 1 α ,25-Dihydroxyvitamin D by Liquid Chromatography-Tandem Mass Spectrometry without immunoextraction

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Objective: The objective is to develop a LC-MS/MS method to quantify 1 α ,25-dihydroxyvitamin D without using antibody-based extraction procedure.

Methods: Vitamin D is a lipid-soluble micronutrient that regulates calcium and phosphate absorption. 25-Hydroxyvitamin D is the primary form in circulation and 1 α ,25-dihydroxyvitamin D (DHVD) is the biologically active form. DHVD assay is challenging because of very low concentrations (picomolar) and numerous isomers or epimers present in serum. Solid phase extraction and Cookson-type triazolinedione derivatization of vitamin D increases the analytical sensitivity of DHVD assay. However, presence of isomers and epimers remain to be a challenge. One approach is adding antibody-based purification steps prior to PTAD derivatization. However, antibody-based extraction increases sample preparation time, test turn-around time, and the cost per reportable. In this study, we developed a liquid chromatography method to separate DHVD peak from major interference without using affinity purification. Specimens were added acetonitrile to precipitate serum proteins. After centrifugation and solid phase extraction, analytes were derivatized with PTAD reagent at ambient temperature. Analytical column was a Kinetex Phenyl-hexyl column, 2.1X100 mm, 1.7 μ m particle size. AB Sciex 5500 QTrap mass spectrometer was operated in triple quadrupole mode. MRM transitions and ion source parameters were optimized according to previous report. Quantitation was performed using MultiQuant software with a six-point calibration curve.

Results: Previous reports showed that 4 β ,25-dihydroxyvitamin D3 and 3-epi-1 α ,25-dihydroxyvitamin D3 may be coeluted with 1 α ,25-dihydroxyvitamin D3. In a method comparison study, we found that this interference could result in a 70% positive bias if using a short LC separation method on a C18 column. On the phenyl-hexyl column, we found that PTAD derivatized 1 α ,25-dihydroxyvitamin D3 has two epimer peaks and the peak area ratio was 1 to 2. Neat 4 β ,25-dihydroxyvitamin D3 has only one peak following derivatization. We did a mixing study and found that 4 β ,25-dihydroxyvitamin D3 peak was eluted between the two epimer peaks of 1 α ,25-dihydroxyvitamin D3. This is a direct evidence proving 4 β ,25-dihydroxyvitamin D3 causes positive bias in DHVD3 quantitation. DHVD3 assay based on the dominant epimer peak is the optimal strategy to quantify DHVD3 accurately in serum. The results were within 15% of expected values. We observed similar interference for DHVD2 quantitation, which was likely due to the presence of 4 β ,25-dihydroxyvitamin D2. Our LC separation method was 9 minute, which is slightly long and but acceptable as a clinical method. The analysis time can be reduced by using a multiplex LC system.

Conclusions: We developed a LC-MS/MS method for the quantitation of DHVD in serum without using immunoextraction procedure. Results demonstrated that 4 β ,25-dihydroxyvitamin D3 is a major interference. DHVD assay based on the dominant epimer peak is an optimal method to quantify DHVD3 and DHVD2 levels.