For the automated assay, streptavidin-coated beads and biotinylated antibody were removed from the reagent cartridge, mixed, and incubated 4.5 hours. The mixture containing biotin-tagged antibody bound to streptavidin beads was added back to the cartridge and placed on the analyzer to evaluate spiked specimens. Lastly, we compared the pre-incubation method to a standard biotin-stripping protocol in the ELISAs to compare the effectiveness of mitigating biotin interference.

Results: We observed biotin interference across the three ELISAs, where 400 μg/L biotin spiked in serum pools reduced analyte detection to between 10 – 15% of the total activity using the standard assay format. Our time-course studies showed 84 – 95% recovery of the total activity when the biotinylated antibody was pre-incubated in the streptavidin-coated wells for 1 hour prior to addition of serum specimens, compared to 69 – 99% by a standard biotin stripping protocol. We extended this concept to the automated immunoassay where at 1000 μg/L biotin in the specimen, only 9 ±0.01% of the total analyte was measured by the conventional method. However, pre-mixing biotinylated antibody and streptavidin beads resulted in 97 ±0.01% of the total analyte recovery in the presence of 1000 μg/L biotin.

Conclusion: We have demonstrated that pre-conjugating the biotin antibody to streptavidin is as effective as biotin-stripping methods to avoid biotin interference in sandwich immunoassays that utilize the biotin-streptavidin system, with the additional benefit of optimizing turnaround times, cost, and labor. A simple change in manufacturer assay design could make immunoassays more robust against biotin interference in patient samples.

Evaluation of cefiderocol activity for Gram-negative bacteria and performance of cefiderocol disk diffusion testing using multiple brands of Mueller Hinton Agar

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Cefidericol is a cephalosporin-siderophore antibiotic for the treatment of multidrug resistant Gram-negative bacteria. Similar to other cephalosporin antibiotics, the lethal mechanism of action is due to inhibition of penicillin binding proteins leading to lysis of the bacteria. However, unlike previously developed antibiotics, the siderophore portion of cefidericol is able to bind iron and then be actively transported into the periplasmic space. To ascertain the feasibility of cefidericol antibiotic susceptibility testing in the Barnes-Jewish Clinical Microbiology Laboratory, we collected a cohort of multidrug Enterobacteriacae (5 *Enterobacter cloace*, 8 *Escherichia coli*, 12 *Klebsiella pneumoniae*), *Pseudomonas aeruginosa* (n=23), *Stenotrophomonas maltophila* (n=24), and *Acinetobacter baumannii* (n=25). We evaluated activity of cefidericol on these strains, and the performance of disk diffusion using three different brands of Mueller-Hinton Agar (BD, Hardy, and Remel). The reference method for comparison was an FDA-cleared broth microdilution panel containing cefidericol (ThermoFisher Scientific). Using CLSI breakpoints, we found that disk diffusion with BD agar had 96% categorical agreement for Enterobacterales, 100% for *P. aeruginosa*, 92% for *A. baumannii*, 96% for *S. maltophila*. We found that Hardy had 96% categorical agreement for Enterobacterales, 92% for *P. aeruginosa*, 92% for *A. baumannii*, 96% for *S. maltophila*. Finally, we found that Hardy had 96% categorical agreement for Enterobacterales, 92% for *P. aeruginosa*, 92% for *A. baumannii*, 96% for *S. maltophila*. Minor errors on any media never exceed 4% and there were no very major errors. Resistance to cefidericol within our cohort of selected antibiotic resistant bacteria was rare, one *E. coli* isolate and two *P. aeruginosa* isolates had minimal inhibitory concentrations (MICs) > 32 μg/mL. The highest MICs for one isolate of *A. baumannii* and one isolate *S. maltophila* was 8 μg/mL and 4 μg/mL, respectively, both of which were intermediate. There was no difference in the distribution of zone disk diffusion diameter for *A. baumannii* or Enterobacterales. However, there was a significant difference in the distribution of zone disk diffusion diameters for *P. aeruginosa* and *S. maltophila* on BD vs Hardy agar. The median for *P. aeruginosa* on BD is 25 mm while it is 29 mm on Hardy. The trend for *S. maltophila* is the opposite as the median for BD was 31.5 mm and 28.5 mm for Hardy. Use of FDA vs CLSI vs EUCAST breakpoints significantly changes outcome of susceptibility testing for broth microdilution and disk diffusion. As one example for broth microdilution of *A. baumannii*, we had one isolate intermediate using CLSI breakpoints, 4 resistant using EUCAST breakpoints, and 4 resistant and 3 intermediate isolates using FDA breakpoints. Our work demonstrates that cefidericol testing can be performed in a routine format, with certain organismal differences on Mueller-Hinton agar, and that different interpretative criteria significantly change outcomes.

Assessment of Coagulation Tests in Hospitalized COVID19 Patients; Challenging Coagulopathies

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COVID-19 has caused a worldwide illness and New York has become the epicenter of COVID-19 in the United States. During the last year, The Bronx, one of the five boroughs of New York City, had the highest prevalence per capita in New York making it the epicenter of the pandemic. During the first wave of the pandemic, almost every

labratory received tremendous amount of tests, and here we examined demographic and laboratory data, as well as trajectories of laboratory results, in order to determine the relation between these laboratory parameters, in particular tests of coagulation, to illness severity and mortality. **Methods**: This is a retrospective study of all positive COVID cases who were admitted between 2/22/2020-4/20/2020 at Montefiore Health System (MHS), a large tertiary care center in the Bronx. Together the ambulatory and hospital networks care for 2.8 million visits a year. All adults with positive COVID tests performed by MHS and who were admitted between 2/22/2020-4/20/2020 are considered. All hospitalized COVID positive cases were queried from the electronic medical record system. Physiological, demographic (age, sex, socioeconomic status and self-reported race and/or ethnicity) and laboratory data was captured. A subset of cases were chart-reviewed for accuracy and additional information. Statistical analysis was performed using R studio. **Results**: Discharge from hospital and mortality were the primary measured outcomes. 7096 patients tested positive for COVID, of which 2897 had an associated inpatient admission and 845 patients were seen in the ER and then discharged. A total of 767 COVID positive patients died during hospitalization. A multivariable logistic regression analysis shows increased odds ratio for mortality by age, gender(males > females), BMI, neutrophil to lymphocyte ratio, Charlson Score, and D-Dimer. The receiver operating characteristic curve (ROC) of D-Dimer combined with age showed an area under the curve (AUC) of 0.77. The optimal cut-point, calculated using Youden's index, for the initial D-Dimer to predict mortality was found to be 2.43ug/ml. D-Dimer trajectories between survivors and non-survivors showed a clear separation for non-survivors since admission. **Conclusions:** In this study we comprehensively studied demographic, physiological and laboratory parameters of COVID19+ minority patients in the Bronx, NYC, USA. This study confirms laboratory and clinical observations made by Wuhan studies of COVID19 infected patients. In particular the association of initial D-Dimer and its trajectory during hospitalization with mortality.

Development and Validation of a Liquid Chromatography Mass Spectrometry Method for Simultaneous Measurement of 25-OH D3, epi-25-OH D3, 25-OH D2, Vitamin A, α-Tocopherol, and γ-Tocopherol

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Background: Circulatory fat-soluble vitamin levels are commonly measured to identify deficiencies that may lead to rickets, osteomalacia, night blindness, and reversible motor and sensory neuropathies. We developed and validated a rapid and robust LC-MS/MS method that simultaneously measures 25-OH D3, epi-25-OH D3, 25-OH D2, vitamin A, α-tocopherol, and γ-tocopherol for clinical use.
Method: 100 µL of serum was mixed with isotope-labeled internal standard and extracted using a 96-well supported-liquid extraction plate with 1.5 mL of hexanes/isopropanol (90/10) (v/v). Dried eluate was reconstituted with 100 µL of methanol/water (90/10) (v/v) and analyzed by LC-MS/MS with a 10-minute gradient. Accuracy was assessed using NIST Standard Reference Materials SRM972a and SRM968f, patient comparison analysis with a LC-MS/MS method at a reference lab, and spike-recovery studies using patient sera and vitamin D-depleted serum. Analytical measurement range (AMR) was determined by spiking 6 analytes into vitamin D-depleted serum to give 7 specimens at varying concentrations. The lower limit of the measuring interval (LLMI) was assessed using 6 pooled specimens with varying low concentrations of each analyte over 20 days. Precision (repeatability and reproducibility) was assessed using quality control materials. Interference studies were performed using pooled patient specimens spiked with varying concentrations of hemoglobin, bilirubin, or intralipid. Matrix effect was assessed by post-column infusion and by matrix dilution with saline.
Results: The method was linear covering physiological concentrations with $r^2 > 0.99$. Repeatability and reproducibility were <15% CV at all QC levels. LLMI for 25-OH D3, epi-25-OH D3, 25-OH D2, vitamin A, α-tocopherol, and γ-tocopherol were 4 ng/mL (15% CV), 4 ng/mL (15% CV), 4 ng/mL (18% CV), 1 µg/dL (20% CV), 0.2 µg/mL (20% CV), and 0.2 µg/mL (8% CV). Recoveries for NIST Standard Reference Materials were between 92 - 111% and between 81 - 122% for spike-recovery studies. Passing-Bablok analyses for vitamin D total, vitamin A, and α-tocopherol demonstrated slopes between 1.04 and 1.11 and r^2 between 0.94 and 0.96. Minimal matrix effect was observed.
Conclusions: We have developed and validated a comprehensive and rapid LC-MS/MS method for the simultaneous measurement of 25-OH D3, epi-25-OH D3, 25-OH D2, vitamin A, α-tocopherol, and γ-tocopherol for clinical use.

Low ADAMTS13 Activity Correlates with Increased Mortality in COVID-19 Patients

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Systemic inflammation and coagulopathy are characteristic hallmarks of COVID19. "COVID coagulopathy"