UNIVERSITY of WASHINGTON

Undergraduate Research Symposium May 19, 2017 Mary Gates Hall

Online Proceedings

Poster Session 1

MGH 241, Easel 145

11:00 AM to 1:00 PM

Adoption and Evaluation of Abbott HIV-1 Dried Blood Spot (DBS) Assay

Mariah Doty, Senior, Medical Laboratory Science
Mentor: Ming Chang, Laboratory Medicine, UW Medical Center

Dried Blood Spots (DBS) are a promising alternative to plasma monitoring for clinical HIV-1 viral loads in resource-limited settings, however, previous methods for HIV-1 DBS analysis are time consuming with decreased sensitivity and specificity. If a new DBS assay could prove efficient for viral load monitoring it could replace the need for centrifugation and cold shipment of HIV-1 plasma samples from rural areas in resource limited countries. In evaluating the new Abbott Molecular®(Des Plaines, IL) 1.0 mL HIV-1 DBS assay, known viral concentration was used to spike HIV-1 negative blood and create DBS cards. The nominal values were then used to evaluate linearity and precision. In accordance with WHO regulations the limit of detection was 3.0 Log10 (1,000 copies/mL). The assay showed an unexpected increase in calibrator and control values, however, this was likely due to an internal conversion factor in the protocol. Preliminary evaluation of the assay shows ease of use with increased sensitivity and specificity compared to previous in house assays. The Abbott 1.0 mL HIV-1 DBS protocol is easy to adopt.

Session 1S

New Diagnostic Tools for Seeing and Sensing Disease

Session Moderator: Benjamin Freedman, Medicine/Nephrology

JHN 175

12:30 PM to 2:15 PM

* Note: Titles in order of presentation.

The Mechanism of Interference of Hemoglobin and Bilirubin in Heparin Monitoring by Anti-Xa Assay

Alicia Bui, Senior, Medical Laboratory Science
Mentor: Wayne Chandler, Laboratory Medicine, Seattle Children's Hospital

Heparin is given to patients on Extracorporeal Membrane Oxygenation (ECMO) to prevent clot formation in the ECMO circuit. The anti-Xa assay is a chromogenic assay used to monitor heparin therapy by measuring the absorbance of a colored product generated from the reaction between reagent Factor Xa (FXa) and a chromogenic substrate to represent the heparin activity in plasma. Hemolysis and hyperbilirubinemia are mechanical complications of ECMO that interfere with heparin monitoring by anti-Xa assay by reporting falsely lower heparin activity measurements. There are three possible mechanisms for the apparent decrease in heparin activity due to hemoglobin and bilirubin: increased FXa activity, heparin neutralization, or spectrophotometer interference. The objective of this study is to investigate the mechanisms by which hemoglobin and bilirubin interfere with heparin monitoring by anti-Xa assay. The slope of absorbance change, heparin activity, and absorbance values reported between the measurement window were determined on the Stago STA Compact®analyzer for plasma samples spiked with heparin and varying dilutions of hemoglobin or bilirubin. The addition of hemoglobin and bilirubin to the assay resulted in an apparent decrease in the measured heparin activity that was proportional to the amount of hemoglobin and bilirubin added to the sample at 0, 0.3 and 0.6 U/mL heparin levels. The rate of the reaction increased proportionally to the amount of hemoglobin or bilirubin added and the relative increase in apparent reaction rate was independent of the heparin concentration in the reaction. Changes in absorbance values were linear between the measurement window for samples with a range of increasing initial absorbances. We concluded that the apparent decrease in heparin activity was a result of increased FXa activity caused by hemolysis and bilirubin, not heparin neutralization or spectrophotometer interference. This study improves the understanding of interferences in heparin monitoring by the anti-Xa assay in clinical laboratories.

Poster Session 2

MGH 241, Easel 148

1:00 PM to 2:30 PM

Direct Antiglobulin Test Method Comparison (Bio-Rad Vs Immucor)

Guorong (G.R) Lai, Fifth Year, Medical Laboratory Science
Mentor: Monica B. Pagano, Laboratory Medicine

In this method comparison, research was performed to determine if the polyspecific anti-human globulin (AHG) reagent from Immucor®was significantly better than the currently used Bio-Rad. Direct antiglobulin test (DAT) experiments were performed on 11 subject samples with strengths of strong positive, weakly positive, and negative. After reviewing the results obtained, it was observed that the currently used Bio-Rad polyspecific AHG reagent produced stronger results with regards to samples that were weakly positive when compared to Immucor®. Results that were 1+ or higher indicate that the two reagents express relatively consistent results, with Bio-Rad presenting stronger agglutination when compared with Immucor®. A comparison between using 1 drop vs 2 drops of Immucor®(Norcross, GA) polyspecific AHG was also performed to determine if there was a significant difference in results, as the package insert from Immucor®mentioned that 1 or 2 drops may be used, with 2 drops having the risk of diminishing results. The results after testing suggest that there is most likely no significant difference between the numbers of drops used.

POSTER SESSION 3

MGH 241, Easel 144

2:30 PM to 4:00 PM

Ceftazidime E-test Antimicrobial Susceptibility Validation and Rescue of Susceptibility with Ceftazidime-Avibactam

Sihan (Bronte) Li, Senior, Medical Laboratory Science
Mentor: Andrew Bryan
Mentor: Heather Berger

Ceftazidime as a cephalosporin antibiotic has become much less effective against Gram-negative bacterial infections because of the appearance of multi-drug resistant, β-lactamase-producing strains. One of the newly FDA-approved drug inhibitor combinations, ceftazidime-avibactam, is a promising solution to rapidly decreasing susceptibilities to ceftazidime-resistant bacteria. E-test is a well-known manual antimicrobial susceptibility testing method widely used in clinical laboratories as an alternative for the gold standard. This study aims to validate ceftazidime E-tests with a selected panel of non-fastidious Gram negatives consisting of Escherichia coli, Stenotrophomonas maltophilia , Acinetobacter baumannii calcoaceticus complex, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi, Citrobacter spp., Enterobacter cloacae, Klebsiella oxytoca and Proteus mirabillis, against gold standards including TREK Sensitre®micro-broth dilution and agar dilution while performing ceftazidime-avibactam E-tests to generate more data for future investigations on the novel antibacterial drug and its resistance profiles at University of Washington Medical Center. Ceftazidime and ceftazidime-avibactam E-tests were done on all selected patient isolates, and agar dilution was performed only on Stenotrophomonas maltophilia as the reference method instead of TREK Sensitre®. Both the accuracy and precision study results support ceftazidime E-test as a valid susceptibility test, and ceftazidime-avibactam was found to restore sensitivity in most of the resistant strains that were tested. We conclude that ceftazidime E-test is verified and the comparison of ceftazidime and ceftazidime-avibactam yields consistent outcomes as previous research.

POSTER SESSION 3

MGH 241, Easel 128

2:30 PM to 4:00 PM

Determination of the Clinical Efficacy of Film Array Testing for Detection of Gastrointestinal Pathogens

Roberto Morales, Fifth Year, Medical Laboratory Science
Mentor: Allen Bateman, Laboratory Medicine

Traditional gastrointestinal pathogen detection includes many bacterial, viral, and parasite tests that require clinicians to order many different tests that have various sensitivity and turn-around-time (TAT). As a result, pathogen detection can be delayed or missed, if the correct tests are not ordered. This ongoing study is evaluating the time-to-diagnosis of gastrointestinal infections using the FilmArray Gastrointestinal (GI) Panel test (BioFire Diagnostics, Salt Lake City, UT, USA) compared to conventional methods. The Filmarray GI test is a multiplex PCR test with targets for 22 gastrointestinal bacteria, viruses, and parasites. The study is also comparing the FilmArray GI test to conventional stool cultures, to evaluate the impact of the FilmArray GI test on clinical decision-making. In this interim analysis, the sample population was composed of 167 outpatient or recently admitted ($<$3 days) patients who had stool specimens submitted for the FilmArray GI test. The time from collection to result for the FilmArray GI test was compared to stool culture. Results from our interim analysis found that the mean time from collection to a FilmArray GI result was 9.8 hours, while the mean time from collection to first actionable stool culture result (if positive) was 62.9. The time from collection to final stool culture result was even longer (77.2 hours). We also found that the FilmArray GI test identified many more pathogens than stool culture: 38% of the samples were positive by FilmArray GI, while only 5% were positive by stool culture. Thus far, the FilmArray test has demonstrated more rapid results and higher sensitivity than stool culture. Ongoing work is evaluating the clinical impact of the FilmArray GI test, but this interim analysis shows that it is more rapid and sensitive than traditional stool culture. As such, it appears to be a promising

test for promptly detecting gastrointestinal pathogens.

POSTER SESSION 3

Balcony, Easel 89

2:30 PM to 4:00 PM

Salinity and pH Comparisons Between Shallow and Deep Sites in the Possession Sound

Andrew Nielsen, Freshman, Medical Laboratory Science, Biochemistry, Pre-Medical, Everett Community College
Kelsey Bassett, Freshman, Biology, Marine Biology, Everett Community College
Mark Yamane, Freshman, Marine Biology, Everett Community College
Aidan Emmons

Mentor: Ardi Kveven, Ocean Research College Academy, Everett Community College
Mentor: Robin Araniva, Everett Community College
Mentor: Katherine Dye, Everett Community College

The Possession Sound is formed where freshwater from the Snohomish River and the salt water from Puget Sound creates an estuarine habitat that supports biodiversity within this ecosystem. Decreases in pH make it difficult for calcifiers to produce their calcium-carbonate shells, thus decreasing their survival rate in a higher acidity environment. Ocean Research College Academy (ORCA) has been sampling water chemistry at multiple sites in Possession Sound for 11 years. This study utilized ORCA data to compare two sites, one near shore and a second in a deep, central location. The purpose of this investigation was to study the pH and salinity trends from 2008 to 2016, considering influence of surface runoff, temperature, river discharge, and climate variations. It was hypothesized that the pH would decrease, becoming more acidic over the eight year period, and the salinity would remain within normal limits. Salinity and pH data were collected in 1-meter increments using a YSI 650 instrument. Initial data demonstrates an inverse relationship between salinity and pH. In 2013, salinity ranged with depth from 22.2-29.5 ppt near shore and 19.2-30.2 ppt at the deep site, correlating with higher surface pH that decreased with depth of 6.8-7.3 near shore and 6.5-7.1 at the deep site. This relationship was consistently demonstrated over the eight-year period, with the exceptions of 2011 and 2015. Further investigations include exploring the impact of climate patterns on the Snohomish River discharge and its influence on pH and salinity trends, as well as looking for seasonal trends in primary productivity of phytoplankton and their impact on temporal and spatial trends.

POSTER SESSION 3

MGH 241, Easel 127

2:30 PM to 4:00 PM

Modulating the Nuclear Factor-kappa B Inflammatory Pathway with Natural Products Derived from Fungi

Sharon Papagayo, Senior, Medical Laboratory Science
Mentor: Stephen Polyak, Laboratory Medicine

Research has shown that chronic inflammation can facilitate the progression of certain diseases, including cancer and diabetes. One way to prevent inflammatory-linked diseases is to reduce the occurrences of inflammation. Since much of society uses nature-derived approaches of healing and disease prevention, we focus on how natural products (i.e. compounds from nature) alter cellular inflammatory status. Fungi and fungal extracts provide a rich source of novel natural products. Fungi have been shown to have beneficial health properties, exemplified by the antibiotic penicillin, derived from the fungus *Penicillium chrysogenum.* By studying many different fungal extracts and chemically separated fractions of fungal extracts, we hope to find novel anti-inflammatory compounds. In this study, novel fungal extracts and fractions were tested for anti-inflammatory activity against the cellular transcription factor, nuclear factor kappa B (NF-kB), a major mediator of cellular inflammation. Prior single-dose screening resulted in extracts and fractions that were shown to inhibit NF-kB activity. From these, eight were tested in dose-response assays that measured NF-kB activity and cytotoxicity. Human hepatoma Huh7.5.1 cells were transfected with a luciferase reporter gene under control of the NF-kB promoter. Twenty-four hours later, transfected cells were incubated with either extracts or fractions for 30 minutes prior to activation of inflammation by tumor necrosis factor-alpha, (TNF-α). NF-kB activity and cellular ATP were measured by luminescence 3.5 hours later. The ultimate goal is to identify extracts, fractions, and pure compounds that inhibit NF-kB without causing cytotoxicity. Such compounds will be advanced for further study and possible application in chronic inflammatory disease states.

POSTER SESSION 3

MGH 241, Easel 131

2:30 PM to 4:00 PM

Let's Talk About RBP: Pre-Validation of Retinol Binding Protein Quantification Assay by LC-MS/MS

Vincent Babasa, Senior, Medical Laboratory Science
Mentor: Andrew Hoofnagle, Laboratory Medicine
Mentor: Thomas Laha, Laboratory Medicine
Mentor: Hannah Pflaum
Mentor: Anna Merrill

Retinol Binding Protein (RBP) is a protein used by the body

to transport Vitamin A and is often used to monitor short-term nutritional changes. Traditionally, RBP has been measured via a nephelometric immunoassay with a high sample requirement. In an attempt to reduce turnaround time and to reduce the volume of sample needed for testing, an in-house assay using liquid chromatography tandem-mass spectrometry (LC-MS/MS) technology is being developed.The method being developed is based on the Vitamin D Binding Protein assay which sets a precedent for LC-MS/MS methods targeted toward high-abundance globulins. This method consists of denaturation of RBP using heat and denaturation agents, then digested by trypsin into peptides which are detected by LC-MS/MS. Currently there are three peptides with multiple transitions being monitored for each (FSGTWYAMAK, YWGVASFLQK, and LIVHNGYC[+57]DGR).The assay is being developed and validated according to the document CLSI C-62A and the assay is currently in its pre-validation phase. Experiments done include standard curve verification and comparison to development data, simple repeatability 5x5, carryover and various troubleshooting experiments.Data analysis has exposed high control and internal standard variability in the assay which are being resolved, but progress looks promising.The RBP assay is still far from completion and further testing and troubleshooting needs to be done to reduce variability before it can enter the validation phase. Once optimized, the LC-MS/MS assay, while less automated than nephelometric platforms, should allow UW Medical Center to have better control over an in-house assay in terms of turnaround time, while also using much smaller volumes of sample. LC-MS/MS also has improved sensitivity when compared to the nephelometric assay. This increased performance may lead to assay utility with other conditions such as diabetes, liver disease and kidney disease.