Wednesday, August 2, 2017

Poster Session: 9:30 AM - 5:00 PM

TDM/Toxicology/DAU

B-358

Toxicity by Hair Dye in Upper Egypt

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Background: Hair-dye containing paraphenylenediamine is widely used in Middle East and some Asian countries. Many cases of toxicity and mortality either due to accidental or deliberate ingestion of hair dyes were reported. The aim of the present work was the chemical analysis of the black hair dye, to analyze the various aspects of acute poisoning through a retrospective study of fatalities reported in seven governors in Upper in Egypt as a result of its ingestion and if there is a dose-effect relationship. **Method**: the records of acute poisoning cases of seven governors in Upper Egypt investigated by Assiut Forensic Chemical Laboratory in the period from January 2002 to December 2008 were examined as regards type of poison, pattern, incidence, age, sex, geographical distribution and mode of poisoning. The studying of the systemic effects of ingestion of hair dye was conducted by oral administration of hair dye in different doses (500, 200, 100, and 50) to four groups of albino rats. The clinical manifestation was observed and the light microscopic examination of sections of vital organs was done. **The result**: The result revealed that about a fifth of the acute poisoning fatalities investigated by Assiut forensic chemical laboratory were due to ingestion of hair dye. The highest majority of them were suicide cases, particularly in Kenya, Sohag and Aswan Governorates respectively, with a female predominance. The highest percentage was found in the age group (31-40) years, followed by (21-30) years. Death occurred within five minutes in the first group, within ten minutes in the second group and within an hour in the third group. The animals of the fourth group survived until sacrificed after one week. The most common histopathological changes in all studied organs were vascular congestion and lymphocyte infiltration, with degenerative changes in the hepatocytes and the destruction of the renal tubules. **Conclusion**: Deliberate self-poisoning by hair dye is a major problem in Upper Egypt particularly in females. The main toxic effects were directed to the liver and kidneys while the other studied organs were affected to a mild extent. Also, there was a well-established dose-effect relationship.

Keyword: paraphenylenediamine, Upper Egypt, Assiut forensic chemical laboratory

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Evaluation of the semi-automated electrochemiluminescence immunoassay for cyclosporine, tacrolimus, and sirolimus

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Background: Therapeutic drug monitoring (TDM) of immunosuppressant drugs are used to monitor drug efficacy and toxicity and to prevent organ transplantation rejection. The evaluation of immunoassay-based immunosuppressant drugs methods at a major transplant hospital is important to identify method suitability and limitations. This study evaluates the analytical performance of the recently available semi-automated competitive electrochemiluminescence immunoassay (ECLIA) for immunosuppressant drugs (ISDs) including cyclosporine (CSA), tacrolimus (TAC), and sirolimus (SRL) on the Roche cobas e411 platform. To our knowledge, this is the first report on method evaluation of sirolimus ECLIA method. **Methods:** Residual EDTA whole blood samples that spans the analytical measuring range from patients undergoing immunosuppressant therapy were used to validate the electrochemiluminescence immunoassays for CSA, TAC, and SRL on the Roche cobas e411 platform. Total imprecision was evaluated using manufacturer and third party Bio-Rad whole blood immunosuppressant controls. Linearity was evaluated by measuring CAP linearity material or a series of dilutions spanning the analytical measuring range using a patient sample with concentration above the upper measuring range. Method comparison was assessed by comparing Roche cobas ECLIA ISD assays with Abbott ARCHITECT i2000 chemiluminescent immunoassay (CMIA) ISD assays and an ESI-LC-MS/MS ISD assays using residual patient samples spanning the analytical measuring range. Functional sensitivity and lot-to-lot comparison were also evaluated. **Results:** Total imprecision ranged from 3.3 to 7.1% for CSA, 3.9 to 9.4% for TAC, and 4.6 to 8.2% for SRL (CV goal of ≤7%). Linearity was verified from 30.0 to 960.9 ng/mL for CSA (claimed AMR 30.0-2000.0 ng/mL), from 1.1 to 27.1 ng/mL for TAC (claimed AMR 0.5-40.0 ng/mL), and from 0.5 to 32.3 ng/mL for SRL (claimed AMR 0.5-30.0 ng/mL). The functional sensitivity (CV ≤20%) met the manufacturer's claims and were determined to be <6.5 ng/mL for CSA (claimed LoQ 50.0 ng/mL), 1.1 ng/mL for TAC (claimed LoQ 1.0 ng/mL), and <0.1 ng/mL for SRL (claimed LoQ 1.5 ng/mL). Deming regression analysis of method comparisons of Roche ECLIA with the Abbott CMIA (n=100) yielded the following. For CSA, slope of 0.917 (95%CI: 0.885-0.949), intercept -15.2 (95%CI: -39.4-9.0) and *r* of 0.985. For Tac, slope of 0.938 (95%CI: 0.895-0.981), intercept of 0.2 (95%CI: -0.4-0.8) and *r* of 0.974. For SRL, slope of 0.842 (0.810-1.110), intercept of 0.9 (95%CI: 0.4-1.4) and *r* of 0.982. Deming regression analysis of comparisons of Roche ECLIA with an ESI-LC-MS/MS method (n=20) yielded the following. For CSA, slope of 1.331 (95%CI: 1.167-1.496), intercept of -68.0 (95%CI: -167.7-31.8) and *r* of 0.969. For TAC, slope of 0.924 (95%CI: 0.843-1.005), intercept of -0.2 (95%CI:-2.3-1.9) and *r* of 0.984. For SRL, slope of 0.971 (95%CI: 0.913-1.030), intercept of 2.4 (95%CI:0.5-2.4) and *r* of 0.993. A general positive bias is observed when ECLIA and CMIA are compared to LC-MS/MS assays suggesting analytical interference possibly due to cross-reactivity with drug metabolites. **Conclusions:** The Roche Elecsys ECLIA-based ISD assays have acceptable precision, linearity, and functional sensitivity. The method comparisons demonstrated the method is comparable to Abbott ARCHITECT immunoassays and LC-MS/MS and is fit for purpose for therapeutic drug monitoring of immunosuppressant drugs (CSA, TAC, and SRL).

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Distribution of mephedrone in blood and brain tissue of rabbit after two different sublethal doses

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Introduction: Abuse of mephedrone (4-methylmethcathinone) has been frequently emerged among the last few years. Intoxication and fatality associated with mephedrone have been reported in literature.

Objective: The aim of this study was to investigate the distribution of mephedrone in whole blood and brain tissue after two different sublethal doses.

Methods: Mephedrone was administered to male white Newzeland rabbits orally (40mg/kg) and (100 mg/kg) and then euthanized after three hours. Samples were analyzed by gas chromatography-mass spectrometry (GC-MS). Pretreatment of the sample was involved protein precipitation of the blood (1.0 mL) with acetonitrile and tissue homogenization of the brain with 1.0 N HCl. Isolation of mephedrone from biological sample was required one step derivatization-extraction method by ethyl chloroformate (ECF) as derivatizing reagent and ethyl acetate as extracting solvent. Methamphetamine-d5 was used as internal standard. The method was validated for linearity, sensitivity, precision and accuracy prior to rabbit samples analysis.

Results and discussion: Calibration curves were found to be linear over the concentration ranges of 10-2000 ng/mL (blood) and 25-2000 ng/mL (brain). The precision and accuracy date were within the acceptable limits (< 15%). The average concentrations of mephedrone in blood after 1/10 and 1/14 of the lethal dosage were 41.52 and 353.71 ng/mL, respectively, while the brain was 37.79 and 258.92 ng/mL, respectively. The concentration of mephedrone in blood was nearly closed from the brain.

Keywords: Mephedrone; GC-MS; Validation; Rabbit blood and brain; Concentration levels

B-361

The Effect of Electronic Waste Leachate on Liver Function of Wistar Albino Rats

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Background: Electronic Wastes (E-wastes) Leachate (EWL) releases heavy metals and other persistent toxins into the environment when burnt . As rain water percolates through landfills and dumpsites, toxic substances escape as leachate into soil thereby posing risk to public health. This study was carried out to determine the *invivo* effect of EWL on liver of albino rat.

Methods: EWL was obtained from Oke-padre, Ibadan, Nigeria dump site and simulated using the American society for testing and materials (ASTM) method. Forty (40) male strain albino rats were randomly assigned into 8 groups of 5 rats each. Rats were fed on pellets and water ad-libitum. Group one - Control Group (CG) were given deionized water while the Experimental Groups (EG) two to six were treated with ascending concentrations (20%, 40%, 60%, 80% and 100%) of the leachates respectively and groups seven and eight were given 20mg/kg of $PbCl_2$ and 40mg/kg of $CuCl_2$ per body weight respectively, orally for 14 days. The rats were sacrificed 24 hours after the last administration of treatment. Blood was collected for biochemical analysis of Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT) and Alkaline phosphatase (ALP) using International Federation for Clinical Chemistry method. Total protein and Albumin were analysed using biuret and bromocresol green methods respectively. The results were analysed using descriptive statistics, t-test and ANOVA at p=0.05.

Results: Mean concentration of AST (158.4±24.1iu/l) and ALT (62.6±9.7iu/l) were significantly higher in EG2 when compared to the AST (99.0±41.6iu/l) and ALT (46.2±12.7iu/l) in CG. While a significant decrease was observed for ALP in EG3 (234.20±120.4iu/l) and EG4 (138±40.7iu/l) when compared with the CG (422.00±111.7iu/l). There was a significant increase in the total protein (8.32±0.4g/dl) and albumin (4.60±0.2g/dl) in EG4 when compared with CG (7.18±0.4g/dl and 3.86±0.4g/dl) respectively.

Conclusion: Leachate from the electronic waste dumpsite from Oke Padre Ibadan, Nigeria induced liver dysfunction in rats. Proper treatment of electronic waste is imperative to prevent possible health risks to humans.

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Determination of posaconazole in plasma/serum by high-performance liquid chromatography with fluorescence detection

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Background: The objective of this study was to develop a sensitive high-performance liquid chromatographic (HPLC) method with fluorescence (FL) detection for the determination of posaconazole in human plasma/serum. Posaconazole is an extended-spectrum triazole antifungal agent with enhanced activity against major microorganisms. It is FDA-approved for the prophylaxis of invasive Aspergillus and Candida infections in patients who are at high risk of developing these infections due to being immunocompromised, such as hematopoietic stem cell transplantation recipients with graft versus host disease or those with hematologic malignancies with prolonged neutropenia from chemotherapy. **Methods:** Plasma/serum samples were deproteinized using methanol as extraction solvent in a single dilution step. A methanolic solution of ketoconazole was served as the internal standard for the assay. Following protein precipitation supernatant was transferred to an autosampler vial, the methanol extract was direct injected onto the HPLC system. The mobile phase consisted of a mixture of 0.1 M ammonium acetate, acetonitrile, and trifluoroacetic acid (440:560:1, v/v/v). The isocratic run was carried out at a flow rate of 1.1 mL/min. Separation of internal standard and posaconazole were achieved within 8 min by using a reversed-phase C18 column (250 x 4.6 mm, 5-µm) and column temperature at 45°C. Fluorescent measurements were performed at an excitation wavelength of 245 nm and emission wavelength of 380 nm. **Results:** The method achieved a linear concentration range of 0.1-10 mg/L, which adequately covered the therapeutic range for appropriate patient monitoring. The limit of detection was 0.04 mg/L. Both the within-run and between-run precisions were lower than 5%. A single dilution step produced mean recoveries of 92.4±1.3% and 91.6±1.3% for posaconazole and ketoconazole, respectively. No interferences with other substances in plasma/serum were observed. The method's limit of quantitation, linearity, imprecision, and accuracy met all criteria required by the Guidance for Industry Bioanalytical Method Validation.**Conclusion:** This method is sensitive, simple and easy to perform with excellent reproducibility, only requires a single dilution step and one centrifugation step prior to the HPLC analysis. The instrument time is less than 8 min per injection, an improvement over most published HPLC/FL and HPLC/UV methods using tedious and labor-intensive preparation and lengthy chromatography.

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Automated solid phase extraction LC-MS/MS procedure for measuring fentanyl in clinical and medical legal blood specimens.

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Background: Fentanyl is a synthetic µ-opioid agonist with potency up to 50-100 times greater than that of morphine. It has a short duration of action and is consequently used therapeutically for the induction and maintenance of surgical anaesthesia and analgesia. It is available as an injectable solution but other formulations of the drug include tablets, lozenges, nasal sprays and transdermal patches for the management of chronic pain. Subsequently, fentanyl is not an uncommon finding following general drug screening. Low therapeutic blood concentrations and often limited blood specimen can compromise the analysis of this drug. We present a rapid LC-MS/MS method for the measurement of fentanyl in blood samples prepared for analysis using an automated solid phase extraction (SPE) technique. **Methods**: Using an HTS-PAL autosampler robot and disposable ITSP C18 SPE cartridges preconditioned sequentially with 150 µL 100% methanol, 150 µL deionised water and 150 µL ammonium acetate, extraction of fentanyl from 400 µL of blood serum was achieved using 200 µL of 100% methanol. LC-MS/MS-ESI of fentanyl [m/z 337.25>188.13 (quan ion), 337.25>132.03 (qual ion)] and fentanyl-d5 [m/z 342.29>221.17], using a Waters Acquity BEH C8 column (1.7 µm, 50 x 2.1 mm i.d.) held at 30°C and gradient mobile phase of 0.1% formic acid in water (A) and 0.1% formic acid in methanol (B) [(ratio A:B): 0.25 min 85/15, 1.75 min 55/45, 0.5 min 50/50, 1.5 min 0/100] at 0.35 mL/min, was used for chromatographic separation and quantification. **Results:** The assay was linear (*curve fit: $y = mx + c$*) over the analytical range 0.5-1,000 ng/mL. Lower limits of detection (LLOD) and quantification (LLOQ) of fentanyl were 0.12 ng/mL and 0.21 ng/mL, respectively. Intra-assay (n = 5) and inter-assay (n = 5) imprecision of fentanyl in all samples were 0.81% relative standard deviation (RSD) (r^2 for slope of calibration curve 0.9905) and 0.80% RSD (r^2 for slope of calibration curve 0.9934), respectively. The analytical recovery of fentanyl spiked into blood (QC levels 0.5 and 10 ng/mL) was >95%. Matrix effect in blood was -7.8% and extracted samples were stable for at least 14 days at 10°C. **Conclusion:** The described validated LC-MS/MS method for the detection of fentanyl in small volumes of SPE-prepared blood is a quick and easy procedure for the measurement of this drug in samples taken for toxicological purposes.

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Analytical Evaluation of a New Biochip Array for the Simultaneous Screening of 20 Drugs of Abuse in Urine on the New Random Access Fully Automated Analyser Evidence Evolution

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Background

Maximising the reliable result output in the screening step during the drug testing process is advantageous. Biochip array technology enables the detection of multiple analytes from a single sample. With the aim of consolidate testing and increase the screening capacity of clinical laboratories, this study reports the analytical evaluation of the new DOA Ultra Urine biochip array for the simultaneous testing of 20 drugs of abuse on the new analyser Evidence Evolution. This system is a fully automated, high throughput, random access with STAT capability system. The time to first result is 36 minutes, 60 samples can be loaded per hour and 20 different biochip arrays can be on-board at any one time.

Methodology

Competitive semi-quantitative chemiluminescent biochip-based immunoassays applied to the Evidence Evolution analyser were employed. The signal output was is inversely proportional to the concentration of drug in the sample. The system incorporated dedicated software to process, report and archive the multiple data produced. Ligands were immobilized and stabilized to the biochip surface defining an array of twenty discrete test sites including: amphetamine, methamphetamine, barbiturates, benzodiazepine class 1, benzodiazepine class 2, cannabinoids (THC), buprenorphine, cocaine/benzoylecgonine, dextromethorphan, fentanyl, ketamine, meprobamate, methadone, opiates, oxycodone 1, oxycodone 2, phencyclidine, tramadol, tricyclic antidepressants and zolpidem. The sample volume required is 6 µL of neat urine.

Results

The 20 simultaneous immunoassays presented the following cut-off values in urine: amphetamine 200ng/mL, methamphetamine 200ng/mL, barbiturate 200ng/ml, benzodiazepine class 1 100ng/mL benzodiazepine class 2 100ng/mL, buprenorphine 5ng/mL, cannabinoids 50ng/mL, cocaine/benzoylecgonine 150ng/mL, dextromethorphan 20ng/mL, fentanyl 2ng/mL, ketamine 750ng/mL, meprobamate 500ng/mL, methadone 300ng/mL, opiates 200ng/mL, oxycodone 1 100ng/mL, oxycodone 2 100ng/mL, phencyclidine 25ng/mL, tramadol 5ng/mL, tricyclic antidepressants 100ng/mL and zolpidem 10ng/mL. Limit of detection was also determined by running 20 negative urine samples, the resultant mean concentrations plus three standard deviations were less than 50% of the cut-offs required. The cut-off values were further validated by assessing inter-assay precision. Urine samples were spiked with the appropriate drug compound 50% below, at the cut-off, and 50% above the recommended cut-off. Three replicates were assessed over 5 separate runs and the inter-assay precision calculated to be less than 20% for all levels across all assays. Intra-assay precision was also assessed with 20 replicates of a low, mid and high concentration sample within one run; resultant intra-assay precision was also calculated to be less than 20% for all levels across all assays.

Conclusion

The results indicate optimal analytical performance of the twenty immunoassays on the DOA ULTRA biochip array and applied to the new Evidence Evolution analyser for the simultaneous comprehensive semi-quantitative screening of drugs in urine samples. For this application no sample preparation is required and the use of the Evidence Evolution analyser allows high through put testing, random access and STAT capability. Toxicology tests can be run alongside various clinical tests making this system a new reliable multi-analytical tool for test consolidation.

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Multicenter Evaluation of new ONLINE TDM Vancomycin Gen.3 (VANC3) assay on Roche Clinical Chemistry Analyzers

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Objectives:

The new ONLINE TDM Vancomycin Gen.3 (VANC3) assay of Roche Diagnostics was developed to increase comparability to other vancomycin assays and to improve calibration stability. The analytical performance of the assay was tested in three laboratories.

Medical Background:

Vancomycin is an antibiotic recommended for intravenous administration as a first-line treatment for complicated skin infections, bloodstream infections, endocarditis, bone and joint infections, and meningitis caused by methicillin-resistant Staphylococcus aureus. Blood levels have to be monitored closely due to several side effects.

Assay principle:

The kinetic interaction of microparticles in solutions (KIMS) is induced by binding of drugconjugate to the antibody on the microparticles. The competitive reaction is inhibited by the presence of Vancomycin in the sample. The resulting kinetic interaction of microparticles is indirectly proportional to the amount of drug present in the sample.

Study Design:

The analytical performance of the new VANC3 assay was evaluated in four independent laboratories using **cobas c** 702, **cobas c** 502 and **cobas c** 501 instruments.

Study program: Recovery of Roche TDM Control set, recovery of Ring Trial samples from RfB and Instand e.V., within-run precision of human sample pools, precision according to CLSI EP5-A3, method comparisons VANC2 vs. VANC3, instrument-to-instrument comparability (**cobas c** 701 vs **cobas c** 502), and calibration stability.

Methods and Results:

Repeatability and intermediate precision were measured according to the CLSI EP5-A3 protocol using three Roche controls and five human serum pools in the concentration range from 6.8 - 61 µg/mL. For the repeatability the coefficients of variation (CVs) were determined to be less than 3.5 % and for intermediate precision yielded CVs ranging between 2.0 and 5.3 % (two runs/day, 21 days). The recovery of three controls (Roche Diagnostics) was determined in three independent runs measuring 3 aliquots. The recovery of target values ranged from 95.9 to 105.5 %.

More than 115 samples in the concentration range from 2.8 to 72.6 µg/mL were used for method comparison experiments measured according to CLSI EP09-A3 protocol. Passing-Bablok regression analysis of between VANC2 (x) and VANC3 (y) assay resulted in slopes in a range of 0.92 to 1.03, intercepts of -0.4 to 1.0 µg/mL, and Pearson correlation coefficients ≥ 0.985. Interchangeable values were measured in method comparisons of **cobas c** 502 (x) versus **cobas c** 701 (y) analyzers using VANC3 reagent. Passing-Bablok regression resulted in slopes of 0.98 and 1.04, intercepts of +0.1 and +0.3 µg/mL, and Pearson correlation coefficients ≥ 0.992. At least 14 days calibration stability was shown on Roche analyzers.

Conclusions:

The results of the multicenter evaluation study prove a good analytical performance of the new VANC3 assay as well as an increased calibration stability. The assay is well-suitable for routine use.

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Development and Validation of a LC-MS/MS Method for Measuring Erythrocyte Thiopurine Metabolites in Patients on Thiopurine Drug Therapy

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Background: Thiopurine drugs including azathioprine, mercaptopurine, and thioguanine, are commonly used in post-organ transplantation regiments, to treat inflammatory bowel disease (IBD) and many other autoimmune diseases, acute lymphoblastic leukemia. Thiopurines are metabolized to 6-thioguanine nucleotides (6-TGNs) and 6-methyl-mercaptopurine nucleotides (6-MMPNs). Elevated levels of 6-TGN and 6-MMPN are associated with myelosuppression and hepatotoxicity, respectively. Clinical trials and practice guidelines suggest that thiopurine metabolites testing can assist physicians in optimizing clinical efficacy, minimizing drug toxicity, and modifying clinical management. In order to facilitate patient care, we developed and validated a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the quantification of thiopurine metabolites, 6-TGN and 6-MMPN, in erythrocytes. **Methods:** 6-TGNs and 6-MMPNs were extracted from 50 µL washed erythrocytes with 70% perchloric acid in a 300 µL mixed solution of DTT and the internal standards (IS) 6-TG-$^{13}C^{215}N$ and 6-MMP-d3. The supernatants were heated for 60 min at 98°C and converted to 6-thioguanine (6-TG) and a 6-methylmercaptopurine (6-MMP) derivative respectively. The cooled supernatant was diluted with water and injected (5 µL) for analysis on a Shimadzu Prominence HPLC coupled to a Sciex 4000 QTRAP mass spectrometer. The separation of the analytes was achieved using an Allure PFP propyl 5µm (50 x 2.1 mm) analytical column at 40°C by gradient elution with a 0.6 ml/min flow rate. The mass spectrometer was operated in positive polarity using an electrospray ionization (ESI) source. Sample analysis was performed in the multiple-reaction monitoring mode with the transitions *m/z* 168/151(quantifier) vs 168/134 (qualifier) for 6-TG, *m/z* 171/154 for 6-TG IS, *m/z* 158/110 (quantifier) vs 158/82 (qualifier) for the 6-MMP derivative, and *m/z* 161 > 110 for the 6-MMP derivative IS. This method was evaluated for precision, limit of quantification, linearity, and ion suppression. Forty-seven samples from IBD patients receiving thiopurine drug therapy were assayed and compared with an established HPLC method. The results were standardized to pmol/8 x 10^8 RBCs. **Results:** Total run time was 2.8 min. The intra- and inter-assay precisions for two levels of quality control were less than 10 % for both the 6-TG and 6-MMP derivative. The limits of quantification for 6-TG and 6-MMP derivative were 0.1 µmol/L and 1.0 µmol/L respectively. The calibration curves exhibited linearity and reproducibility in the range 0.1-25 µmol/L for 6-TG and 1.0-250 µmol/L for the 6-MMP derivative. No significant ion suppression effects were noted. The 47 clinical sample comparison with an HPLC method demonstrated good agreement, for 6-TG (LC/MS/MS = 1.26 HPLC - 6.88, R^2 = 0.975; mean difference 16.5%) and 6-MMP derivative (LC/MS/MS = 1.07 HPLC - 121, R^2 = 0.982; mean difference -2.4%). **Conclusion:** This rapid and reliable LC-MS/MS method is suitable for routine monitoring of 6-TGN and 6-MMPN concentrations in erythrocytes samples from patients receiving thiopurine drug therapy.

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Evaluation of quantitative microsampling for immunosuppressant drug monitoring

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Background: Immunosuppressant drugs (sirolimus, everolimus, tacrolimus, and cyclosporine A) are used during chemotherapy, pre- and post- organ transplant, and other conditions. They are characterized by narrow therapeutic window and serious implications with hypo- therapeutic (tissue rejection or remission) and hyper-therapeutic doses (toxicity, immunodeficiency). A commercial volumetric absorptive microsampling device (VAMD) for fixed-volume blood collection is available. It enables quantitative analysis while minimizing sample volume requirements. VAMDs provide the potential for significant cost saving by elimination of phlebotomy and simplification of transport. Our objective was to evaluate a method for quantifying four immunosuppressant drugs in whole blood using VAMD by LC-MS/MS.

Methods: Calibrators, controls, and samples were equilibrated at room temperature while mixing on a rocker before collection by VAMD. Before drying 20 µL of whole blood EDTA samples were collected by contacting VAMD to 100 µL of sample spotted on parafilm. Samples were then extracted using 200 µL of MeOH/ water mixture containing the internal standard. Subsequently, they were sonicated and vortexed followed by evaporation. Samples were then reconstituted with 100µL of 5mM ammonium acetate containing 0.2% Formic acid in ACN/MeOH/water, followed by centrifugation, and transfer of 80 µL to a vial for injection (35 µL) into the LC system. Samples were chromatographically separated using a C18 analytical column on Agilent 1200 series HPLC system and detected by AB Sciex API 4000 mass spectrometer operating in positive MRM mode. This method was compared to a validated in-house LC-MS/MS method. Accuracy experiments were performed using residual de-identified patient whole blood samples for tacrolimus. Spiked samples were used to perform sensitivity and linearity experiments. Low (L) and High (H) quality control samples containing all four analytes were analyzed five times on a single run to evaluate imprecision.

Results: Tacrolimus VAMD sample extraction method compared to our in-house conventional extraction with Deming regression and 95% confidence intervals (±): tacrolimus VAMD= 1.153 ±0.121(in-house) + 0.743 ±1.5 ng/mL, R=0.99, (n=11). Other analytes will be evaluated. Limits of quantitation for tacrolimus (2.6 ng/mL), everolimus (2.6 ng/mL), cyclosporine A (27 ng/mL), and sirolimus (1.7 ng/mL) were achieved, which are comparable to conventional extraction. Calibrators sampled and extracted through VAMD showed linearity within the specified AMR for tacrolimus (up to 37 ng/mL), everolimus (up to 42 ng/mL), cyclosporine A (up to 896 ng/mL), and sirolimus (up to 48 ng/mL) with correlation coefficients greater than 0.995 for all analytes (quantitative and qualitative fragment ions). Coefficients of variation for tacrolimus (L: 4% H: 3%), everolimus (L: 5% H: 1%), cyclosporine A (L: 5% H: 3%), and sirolimus (L: 6% H: 3%) were obtained, indicating acceptable reproducibility. In addition, conventional sample processing was compared to VAMD sample collection and extraction for all four analytes.

Conclusion: This sample preparation method was able to achieve comparable limits of quantitation, linearity, and precision to the conventional sample preparation method. This sample collection and processing approach will require studies comparing VAMD sampling methodology in patient populations but represents an applicable method for simplified and more patient-friendly approach to therapeutic drug management.

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Aluminum toxicity: Evaluation of 16-year trend among 14,919 patients

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Background: Chronic exposure to aluminum from contaminated dialysis water and the use of aluminum-containing binders has historically been a major problem in patients undergoing dialysis. Aluminum exposure has been linked to iron-refractory microcytic anemia, osteomalacia, and even fatal encephalopathic disorders. Recognition of this problem has led to successful preventative measures by reverse osmosis and aluminum-level monitoring, which has led to aluminum toxicity becoming uncommon. Despite this trend, guidelines from the National Kidney foundation continue to recommend annual serum aluminum testing for ongoing surveillance. Aluminum is a ubiquitous ultratrace element and exogenous sources may lead to false elevations due to contamination during collection, processing or analysis. As the rate of true aluminum toxicity declines, the relative incidence of false elevations due to contamination would be expected to increase. The objective of this study was to investigate long-term trends in serum aluminum levels in a large Veteran population and estimate the frequency of falsely elevated values presumably due to contamination.

Methods: A retrospective observational study covering a 16-year period through October 2016 was conducted with information extracted from the Veterans Affairs corporate data warehouse. Serum aluminum concentrations >60 g/L were considered false positives, presumably due to contamination, if another specimen retested within 45 days was <20 g/L. Serum aluminum concentrations less than 20 g/L were considered normal. **Results:** A total of 45,480 serum aluminum results involving 14,919 patients and 119 Veteran Affairs 16 facilities over a 16-year period ending in October 2016 were evaluated. The percentage of elevated (>2017 g/L) serum aluminum results declined from 31.5% in 2000 to 2.0% in 2015. Average testing intervals changed from every 159 days in 2000 to every 238 days in 2015. Of 529 patients with serum aluminum concentrations >60 g/L, 216 (40.8%) were retested within 45 days (average=21 days) of which 83 (38.4%) were below 20 g/L after repeat measurements. **Conclusion:** Aluminum toxicity, as assessed by serum levels, has substantially declined over time and is now rare. In addition, the frequency of serum aluminum testing has declined in accordance with guidelines for routine surveillance. Our study revealed, that despite NKF guidelines recommending annual screening, testing is still performed more frequently (1.5 times per year). Further, testing intervals have remained relatively constant since 2011 despite declining overall rate in abnormal serum aluminum levels. Our study also revealed that a relatively high frequency of elevated serum aluminum concentrations were false positives and most likely the result of contamination. As a consequence, the incidence of aluminum toxicity is likely frequently overestimated. Therefore, patients with elevated serum aluminum concentrations should be retested before undergoing treatment or more investigation into sources of exposure should be performed to exclude false elevations.

B-369

Modeling nicotine in oral fluid: a possible tool to assess nicotine use and exposure

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Background: Oral fluid collection is non-invasive and easy to handle making it an ideal matrix for determining nicotine and hence smoking status. Various smoking related compounds including nicotine and cotinine have been used as markers for smoking status assessment. In the present study, we develop a transformed and normalized mathematic model of nicotine historical data for a quick assessment of nicotine exposure.

Methods: A historical distribution "model" of nicotine in oral fluid was developed using data collected from quantitative liquid chromatography tandem mass spectrometry (LC/MS/MS) analysis. A data set of 6158 independent nicotine positive saliva specimens (10 ng/mL cutoff) collected over a 2 year period (2015-2017) is the foundation of this near Gaussian model. Demographic data were used to calculate patient specific parameters (i.e., calculated blood volume (CBV), lean body weight (LBW), and body surface area (BSA)) for the transformation and normalization of the oral fluid nicotine data. An earlier study indicated that using 10 ng/mL cutoff for nicotine identified 88% of self-reported smokers in a controlled clinical setting (Scheidweiler et al. *Ther Drug Monit.* 2011; 33(5): 609-618). Hence, this model is likely specific for active smokers.

Results: After transformation and normalization, the resulting model derived from positive nicotine test results shows a near Gaussian distribution. A separate population of patients who used the nicotine transdermal patch was used to test whether this mode of nicotine administration matches active smoking. This assessment found 96% of nicotine patch users fall within two standard deviations from the mean of the distribution consistent with a true Gaussian distribution. Transformation and normalization of nicotine results below 10 ng/mL exhibit a separate distribution from the overall nicotine positive consistent with results from a controlled clinical trial for patients "exposed" to environmental nicotine.

Conclusion: This model of nicotine historical distribution is consistent with nicotine administered via patch or directly from smoking. It is conceivable that any "active" form of nicotine administration (e.g., gum, patch, etc.) will be consistent with this model. Combined with patient interviews and prescription history, this model can provide information about patients' potential adherence to nicotine replacement therapy.

B-370

Quantitative Analysis of Ethyl β-D-glucuronide (EtG) in Human Umbilical Cord Tissue by Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)

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Background: Prenatal alcohol exposure can significantly affect neonatal development and may result in adverse outcomes known as fetal alcohol spectrum disorders (FASD) which encompass a range of physical, behavioral and cognitive impairments. Since gestational alcohol consumption is often underreported, a biomarker for identification of *in utero* alcohol exposure is needed. The aim of this study was to develop a method for determination of Ethyl β-D-glucuronide (EtG), a direct ethanol metabolite, in umbilical cord tissue as a biomarker for *in utero* alcohol exposure.

Methods: Residual umbilical cord tissue specimens submitted to ARUP Laboratories for drug testing were de-identified and qualitatively screened for the presence of EtG using the LC-MS/MS method described here. Specimens with undetectable EtG were used to prepare six calibrators and control samples. Both spiked and authentic positive specimens were sliced and weighed (1.0g ±0.025). 3.0 ml acetonitrile, deuterated internal standard (IS) EtG-d5 and stainless steel UFO beads were added for homogenization on the Bead Ruptor, followed by centrifugation at 0°C. The supernatants were loaded on UCT Clean-Up® solid phase extraction (SPE) columns preconditioned with methanol and water. The columns were washed with water and methanol before elution with 2% formic acid in methanol. The eluate was dried under nitrogen (40°C) using a TurboVap® (Biotage) and reconstituted in the aqueous mobile phase. Chromatographic separation of EtG and the IS was achieved on a Phenomenex Hydro-RP C18 column (50mm x 2.1mm i.d., 2.5um) with a binary mobile phase of 0.1% formic acid in water and 0.1% formic acid in acetonitrile with gradient elution at a flow rate of 0.350 mL/min, with a total run time of 5 minutes. Quantification of the analytes was performed by AB Sciex Triple Quad 5500 mass spectrometer using positive turbo-ion-spray ionization in multiple-reaction-monitoring (MRM) mode with two mass transitions m/z 220.9 → 84.9 (quantifier) and 220.9 → 74.7 (qualifier) for EtG, and m/z 226.1 → 84.8 (quantifier) and 226.1 → 74.8 (qualifier) for the IS. A calibration range of 5.00-220 ng/g was established in umbilical cord tissue by a weighted ($1/x^2$) least square method. Accuracy, imprecision (% coefficient of variation), matrix effect, interference, carryover and stability were evaluated to determine the overall method performance.

Results: Good linearity was obtained (r = 0.9996). The limit of detection (LOD) was 1ng/g and the limit of quantitation (LOQ) was 5 ng/g with inaccuracy of <± 20% and CV of <20%. The within-run and between-run bias and imprecision of the method were <±15% and <15%, respectively. The relative matrix effect was <5% and the absolute extraction efficiencies for EtG ranged from 72%-89.2%. The selectivity of the assay was acceptable and no carryover was detected. Extracted samples were stable for 1 week at room temperature, 3 weeks at 4 °C and to freeze-thaw conditions. The method has been successfully used to quantify EtG in authentic umbilical cord tissue samples.

Conclusion: SPE-based sample preparation and an LC-MS/MS method for quantification of EtG in umbilical cord tissue homogenates have been developed and described here. This assay could be utilized for routine detection of *in utero* alcohol exposure.

B-371

Novel 5-plex Panel for the Detection of Acute Kidney Injury with Improved Analytical Performance

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Background: Acute kidney injury (AKI) is currently classified by the KDIGO guidelines based on serum creatinine and urine output. However, since creatinine is a lagging index of impending AKI, a novel set of more sensitive AKI biomarkers has been qualified in rodents. Studies are now underway to qualify a similar set of human biomarkers, but no high-throughput multiplex for these biomarkers is currently available to support an earlier and more robust classification of AKI. This work describes the development and optimization of a novel multiplex immunoassay panel comparing its performance with predicate ELISA methods for KIM-1, NGAL, cystatin C, clusterin, and osteopontin.

Methods: Randox Biochip technology was used to develop a multiplex immunoassay panel for the urinary biomarkers mentioned above using proprietary and commercially available antibodies. The analytical performance of this method was compared against singlicate ELISA methods using about 30 normal subjects, and data extracted from testing urine of each analyte by ELISA from >1000 subjects with normal kidney function or potential AKI.

Results: The functional sensitivities of assays, as revealed by the precision (%CV) of the lowest non-zero standard (n=6 each) were 24.4pg/mL (%CV=14.0), 0.89ng/mL (%CV=3.3), 1.22ng/mL (%CV=8.8), 7.02ng/mL (%CV=13.6), and 50.9ng/mL (%CV=8.5) for KIM-1, NGAL, cystatin C, clusterin, and osteopontin, respectively. These sensitivities were compared with the ideal lower limits of the dynamic ranges determined by ELISA from >1000 patient samples (40pg/mL, 1.0ng/mL, 1.5ng/mL, 10ng/mL, and 80ng/mL, respectively). The ideal upper limits of the dynamic range were 4000pg/mL, 100ng/mL, 150ng/mL, 1000ng/mL, and 8000ng/mL, for KIM-1, NGAL, cystatin C, clusterin and osteopontin, respectively. The effective upper limits of measurement and precision (%CV) were 4067.4pg/mL (3.8%), 97.8ng/mL (3.9%), 184.7ng/mL (8.0%), 1021.0ng/mL (2.9%), and 8028.7ng/mL (9.9%), respectively. Cross reactivity was tested for each analyte at the midpoint of the standard curve when spiked with x10 concentration of the highest standard of the other panel antigens, producing no significant cross-reactivity. Cross reactivity from various related, but non-panel proteins that may be present in urine were also tested for cystatin C, clusterin and KIM-1, again producing no significant cross-reactivity. Cross-reactivity of non-panel proteins for NGAL and osteopontin are pending. Correlation (r^2) of 29 urine samples for the KIM-1 multiplex assay vs ELISA (range 46 to 1447pg/mL) was 0.994; correlation of NGAL multiplex vs ELISA (n=28; range 1.1 to 197ng/mL) was 0.962; cystatin C multiplex vs ELISA (n=28; range 2.6 to 80.3ng/mL) was 0.872; clusterin multiplex vs ELISA (n=27; range 24 to 710ng/mL) was 0.812; and osteopontin multiplex vs ELISA (n=29; range 58 to 4517ng/mL) was 0.899. Slopes were 0.994, 0.544, 0.636, 1.67, and 0.685, respectively.

Conclusion: The development and optimization of the Randox Biochip 5-plex panel suggests that KIM-1, NGAL, cystatin C, clusterin and osteopontin will be suitable to replace the slower, more laborious, and presumably less sensitive ELISA methods for clinical trial and diagnostic use when the ongoing full analytical validation and clinical confirmations are completed.

B-372

Influence of function and co-administered calcineurin inhibitors on monitoring the trough mycophenolic acid concentration using particle enhanced turbidimetric inhibition immunoassay

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Background: Mycophenolate mofetil (MMF) is an immunosuppressive drug widely used in the patients after solid organ transplantation. Mycophenolic acid (MPA), major pharmacologically active metabolite of MMF, has been monitored by high performance liquid chromatography (HPLC). Recently, immunological methods such as the enzyme-multiplied immunoassay technique and homogeneous particle enhanced turbidimetric inhibition immunoassay (PETINA) has been widely used due to their simplicity. However, there is a concern about cross-reactions with MPA-glucuronide (MPAG) as well as other minor metabolites in these immunoassays. The aim of this study was to evaluate the influence of the renal function and the co-administration of calcineurin inhibitors (CNI) on the MPA and MPAG concentrations and then develop a formula that took into account these clinical factors.

Methods: Forty-two solid organ transplant patients receiving MMF in combination with tacrolimus (TAC) or cyclosporine A (CsA) were enrolled in this study. Blood samples were collected from the recipients prior to receiving any MMF doses. HPLC separations of MPA and MPAG were performed independently. MPA or MPAG concentrations measured by HPLC using an ERC ODS-1161 column and Shimadzu LC-10ADLP system (Shimadzu, Kyoto, Japan). In addition to the HPLC assay, the MPA concentrations in the same samples were also measured by PETINA, using the Flex reagent cartridge MPAT and the Dimension Xpand Plus system (Siemens Healthcare Diagnostics, NY, US). All patient laboratory data were obtained from the medical records.

Results: There were no significant differences between MPA plasma concentrations and MMF dosage per body weight. In contrast, MPAG plasma concentrations were positively correlated with the MMF dosage per body weight (r=0.79, p<0.001). Moreover, while the MPAG concentration was negatively correlated with the

estimated glomerular filtration rate (eGFR) (r=-0.56, p<0.001), there was no correlation between the MPA concentrations and the eGFR. There was no significant difference in the MPA and MPAG concentration per MMF dose between the patients who were co-administered TAC versus CsA. While there was a trend for the patients co-administered CsA to have higher MPAG concentrations than those who were co-administered TAC, this difference did not reach significance. After taking the renal function and co-administration of CNI into account, we developed a formula to correct the PETINA values to more precisely reflect the HPLC values for MPA. The multiple regression equations for the model is as follows: Estimated MPA concentration = -0.010 + 0.8882×MPA (PETINA) + 0.001×eGFR -0.013×CNI (TAC=0 or CsA=1).

Conclusion: Although MPAG accumulates in conjunction with the worsening of the renal function, the MPA concentration is not affected by the renal function. Thus, it is difficult to predict the MPA concentration based on the MMF dosage or renal function. By being able to successfully develop a formula that excluded the influences of cross-reactions with the accumulated MPA metabolites, this made it possible to correct the PETINA data to values that more precisely reflected the HPLC values for MPA.

B-373

Determination of Benzoylecgonine in urine with dilute-and-shoot and LCMS/MS analysis.

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Cocaine (COC) is one of the alkaloids present in leaves of two species of the genus Erytroxylum, commonly known as coca, with contents between 0.17 and 0.76% of COC. It is a potent local anesthetic and powerful sympathomimetic agent with stimulating effects on the central nervous system and has being considered the most potent stimulant of central nervous system of natural occurrence, which is why it is used as an abuse drug. The main biotransformation product of COC is benzoylecgonine (BE), which is excreted in urine and used as a biomarker for exposure to the drug. For many years, GC-MS has been considered the gold standard method for the analysis confirmatory of drugs of abuse. However, the LC-MS/MS have gained space in this field, mainly after the evolution of the instrumentation. The objective of this work was to develop a simple and fast method for the determination of BE in urine by LCMS/MS. Chromatographic separation were performed on Poroshell 120 EC-C18 column (50 mm x 4.6 mm x 2.7 µm) using a guard column Poroshell 120 EC-C18 (5 mm x 4.6 mm). Isocratic separation was obtained with mobile phase constituted by 50% of methanol and 0.1% of formic acid at a flow rate of 0.500 mL min-1.The chromatographic run time was 2.0 min. All experiments were performed on an Agilent 6460C (Santa Clara, CA) triple quadrupole LC-MS/MS system, with an Agilent 1290 Infinity LC system. The source was operated in a positive mode. The sample preparation was performed by centrifuge of 100 µL of sample, calibrator or internal quality control, at 14.000 rpm for 10 minutes. 25 µL of supernatant were transferred to a 2.0 mL glass vial and diluted with 960 µL of aqueous solution containing 5.0% of methanol and 0.1% of formic acid. 15 µL of deuterated internal standard (BE-D3) solution was added. The mixture was shaken and 1.0 µL was injected into the chromatographic system. The method was validated achieving a LoQ of 12.0 ng mL1, linearity of 12.0 to 1,000.0 ng mL-1 and imprecision within-run and total were less than 5.3%. The method was compared to other GC-MS method by analysis of 20 fortified urine samples and achieve 0.99 of coefficient correlation. The accuracy was observed by two samples of CAP UDC proficiency test, and was achieved a standard deviation index (SDI) of 0.1 and 0.6. In conclusion, a simple method based of dilute-and-shoot extraction has been developed and validated successfully, with a good precision and a good correlation with the gold standard GC-MS method.

B-374

Development and validation of nicotine and cotinine in human serum by liquid chromatography electrospray ionization tandem mass spectrometry.

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The use of tobacco is one of the main preventable risk factors leading to major diseases, like cancers and death worldwide. Concentrations of nicotine and its metabolites in blood are indicative of tobacco exposure. A simple, rapid and sensitive Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) was developed and validated for quantification of nicotine and its major metabolite cotinine in serum. The sample extraction is a simple protein precipitation with trichloroacetic acid 10% using only 100 µL of sample and 25 µL of internal standard (2-Phenylimidazole). Detection was performed on a 6460 MS system (Agilent Technologies) operate in a positive mode. Chromatographic separation was obtained on a Zorbax Eclipse Plus C18 RRHD column (2,1 X 50 mm 1,8 um) - Agilent with an isocratic mobile phase containing methanol, water, acetonitrile, ammonium formate and formic acid at a flow rate of 600 µL.min^{1}. The method had a chromatographic run time of approximately 2.5 min. The linear range obtained for cotinine was 4.0-500.0 ng.mL^{-1} and for nicotine was 3.0-40.0 ng.mL^{-1}. The limit of detection (LOD) were 2.0 ng.mL^{-1} for cotinine and 1.5 ng.mL^{-1} for Nicotine. Within-day and between-day imprecision was less than 8% for cotinine and less than 11% for Nicotine. The accuracy was between 87-104% for cotinine and 88-101% for Nicotine. The analytes were determinate with satisfactory sensitivity, accuracy, repeatability and linearity. In conclusion, the LC-MS/MS method was developed and validated for the quantitative analysis of Nicotine and Cotinine in serum and has been applied successfully evaluation of the tobacco exposure.

B-375

Performance Evaluation of Dimension TAC Assay and Comparison with Other Commercial Tacrolimus Assay

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Background: Therapeutic monitoring of tacrolimus (TAC) is essential for reducing the organ rejection and *adverse* effects. The measurements of TAC in whole blood is performed by immunoassays or liquid chromatography-tandem mass spectrometry (LC-MS/MS) and many automated platforms have been developed. The aim of the present study was to evaluate the analytical performance of Dimension TAC assay (Siemens Healthineers, USA) which was upgraded reagent from the previous Dimension TACR assay. **Methods:** The evaluation was performed based on the Clinical and Laboratory Standards Institute (CLSI) guidelines. The evaluation consisted of determination of the precision, linearity, limit of blank (LoB), limit of detection (LoD), limit of quantitation (LoQ), and reagent lot-to-lot using three lot number. A correlation study was conducted using Dimension TACR assay, Architect Immunoassay (Abbott Diagnostics), Elecsys (Roche Diagnostics), MassTrak LC-MS/MS (Waters Corporation). We collected each sample more than 40 from kidney, liver and heart transplant recipients. **Results:** The total CV for the low, middle and high level quality control materials were 7.3%, 5.1% and 5.7%, respectively. The linear range where the coefficient of determination was >0.99 of the Dimension TAC assay was 1.61-31.72 ng/mL. The LoB, LoD, and LoQ was 0.29 ng/mL, 0.47 ng/mL, and 1.02 ng/mL, respectively. Correlation analysis indicated that results of the Dimension TAC assay was comparable to Dimension TACR assay, Architect Immunoassay and Elecsys in liver and heart transplants [correlation coefficients (r)=0.856~0.982]. In kidney transplants, Dimension TAC assay showed the less correlation with Architect Immunoassay and Elecsys [r= 0.558 and 0.775]. The results of these assay were slightly higher than those of MassTrak (mean bias 1.563-2.619 ng/mL) in all transplant groups. And we found few lot-to-lot reagent variation in the reagents which were evaluated [r >0.993]. **Conclusion:** The overall analytical performance of Dimension TAC assay is acceptable for therapeutic monitoring in clinical practice. This assay showed the higher concentrations than mass spectrometry which was consistent with results in previous study.

B-376

Performance Characteristics of Capillary Blood Methotrexate Polyglutamates by Volumetric Absorptive Microsampling Collection Method Coupled with LC-MS/MS

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Background: Red blood cell (RBC) Methotrexate triglutamate ($MTXPG_3$) levels have utility in the dosing optimization of methotrexate therapy. We sought to 1) develop an LC-MS/MS method for quantifying RBC $MTXPG_3$ levels from capillary blood collected on volumetric absorptive microsampler (VAMS), and 2) compare the performance characteristics of this microsampling method to the reference method (venous blood collected by venipuncture).

Methods: Rheumatoid arthritis (RA) subjects (n=101) under methotrexate therapy consented to donate capillary (10 µl per specimen on VAMS) and venous blood (10 ml per specimen). RBCs from venous blood were isolated and stored at -80°C before analysis. Dried capillary blood was eluted with water containing deuterated

(d3) $MTXPG_3$ as the internal standard. Following deproteinization (70% perchloric acid) and centrifugation, a 20 µL aliquot of the supernatant extract was injected onto a pentafluorophenyl column (2.1x50 mm, 2.6 µm) connected to a TSQ Quantiva mass spectrometer with heated electrospray operating in positive mode. Transitions consisted of a 713->308 m/z transition, and 716->311 transition for $MTXPG_3$ and $MTXPG_3$-d3, respectively. RBC $MTXPG_3$ levels from venous blood were also measured by LC-MS/MS.

Results: The LC-MS/MS method presented with intra-day and inter-day coefficient of variation below 15%. In 72 consecutive specimens, RBC $MTXPG_3$ levels from venous blood (average [SEM]: 36.8±2.8 nmol/L packed RBCs) were proportionally 2.2 fold higher than those recovered from capillary blood (17.6±1.4 nmol/L) (r^2=0.87) and this conversion factor (2.2) was applied to convert capillary blood levels to their RBC equivalent. The method was prospectively validated in 29 RA subjects. Capillary $MTXPG_3$ blood levels were 17.2±1.1 nmol/L, 37.9±2.3 nmol/L RBC equivalent and similar to RBCs $MTXPG_3$ levels from venous blood (35.5±2.2 nmol/L RBC; r^2=0.9, Slope=1.1). Figure 1 illustrates the correlation between the two methods for the 101 subjects enrolled.

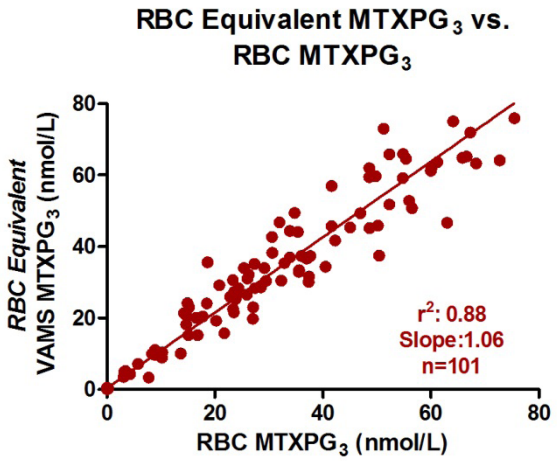

Figure 1.

Conclusion: We have validated a method for quantifying $MTXPG_3$ in capillary blood collected on VAMS.

B-377

Evaluation of Preanalytical Stability of Thiopurine metabolites in RBC and whole blood samples

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Background: Measurement of thiopurine metabolite is helpful to monitor adverse effects and to asses compliance in patients with thiopurine treatment. Maintaining stability of the metabolites in is necessary to get reliable test results. We evaluated the preanalytical stability of thiopurine metabolites in RBC and whole blood samples under various storage conditions to investigate the effects of preanalytical process

Methods: 6-thioguanine nucleotides (6-TG) and 6-methylmercaptopurine (6-MMP) were measured by the liquid chromatography-tandem mass spectrometry. Analyses were performed on an API 4000 tandem mass spectrometer (Applied Biosystems, Foster City, CA, USA) equipped with an Agilent Technologies Series 1200 HPLC system (Agilent, USA). Quantitative analysis was performed in multiple reaction-monitoring mode (m/z 168.0>150.9 for 6-TG, m/z 158.0>110.0 for 6-MMP, m/z 171.0 >154.0 for 6-TG-$^{13}C_2{}^{15}N$, m/z 161.1 > 110.1 for 6-MMP-d_3) with a total running time of 3 minutes for each sample. Intra- and inter-day imprecisions were lower than CV 10%. To obtain pure RBC , we centrifuged the EDTA whole blood sample at 4000rpm for 10min followed by removing remnant plasma. After washing twice with 0.9% saline, isolated RBCs was stored in each condition until analysis. 6-TG and 6-MMP in RBC samples were measured in four aliquots at 2 concentrations to evaluate their stability under various conditions. Freeze-thaw stability (3 cycles at -70 °C), short-term stability (at room temperature and -4°C, 0 min, 30 min, 1, 2 and 4 hrs), and long-term stability (at -20°C and -70°C, 0, 1, 3, 7, 14, 21, 90 and 180 days) of the metabolites in preprocessed RBC specimens were evaluated. In addition, stability of the metabolites in whole blood samples without preprocessing was tested at room temperature and -4°C up to 7 days (0, 4, 8 hrs, 1, 2, 4 and 7 days). **Results:** The concentrations of 6-TG and 6-MMP in RBC samples showed no significant changes at room temperature and 4 ° C until 4 hours. In frozen preprocessed RBC samples, thiopurine metabolites were stable at -70° C for up to 6 months, but 6-TG concentrations were decreased to 70% of the initial values at -20 C. The concentrations of 2 metabolites (6-TG/6-MMP) were significantly decreased in comparison with the initial concentration.in whole blood samples; 74%/ 85% for 4 days and 35%/ 42% ° for 7 days at room temperature, 93%/ 95% for 4 days and 74/ 88% for 7 days at 4 ° C. The effect of 3 freeze- thaw cycles on the sample stability was negligible.

Conclusion: Sample storage and handling process are critical for accurate measurement of thiopurine metabolites. We recommended that the patient's whole blood sample should be processed as soon as possible and stored at -70 ° C until analysis.

B-378

Omecamtiv Mecarbil (OM) in Patients with Heart Failure: Development of an Immunoassay to Guide Omecamtiv Mecarbil Dose Adjustment

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Background: Heart failure affects approximately 23 million people worldwide, including more than 5 million in the United States. Omecamtiv mecarbil (OM), a novel selective cardiac myosin activator, is being studied as a potential treatment for heart failure with reduced ejection fraction. The Phase 2 COSMIC-HF study indicated that a pharmacokinetic (PK)-based dose titration strategy was useful to adjust the treatment dose of OM for heart failure patients. GALACTIC-HF is a global Phase 3 double-blind, randomized, placebo-controlled study designed to enroll approximately 8000 heart failure patients that also implements a PK-based dose adjustment strategy. The QMS OM Immunoassay has been developed for the rapid (time to first result is approximately 10 minutes), quantitative determination of OM concentration in K_3-EDTA plasma. The assay is being investigated in central laboratories in the US, EU and Asia to support GALACTIC-HF.

Methods: The QMS OM assay is a homogeneous particle-enhanced turbidimetric inhibition immunoassay. It is based on competition for anti-OM antibody binding sites between OM in the sample and OM coated onto microparticles. A concentration-dependent classic agglutination inhibition curve can be obtained to determine the OM concentration in the sample. The system has two reagent components, six calibrators that span 0 to 1200 ng/mL, as confirmed by an LC-MS/MS reference method, and multi-level controls.

Results: The following representative performance characteristics were obtained at the manufacturer laboratory. The studies were performed following CLSI guidelines, where applicable. The assay demonstrated a lower limit of quantitation of 85 ng/mL. Assay precision was determined by measuring controls, patient plasma pools and spiked plasma samples over the course of 20 days. Precision ranged from 1.0% to 3.1% CV (within) and 2.2% to 3.3% CV (total). The linear range of the assay was determined to be from 85 to 1200 ng/mL. The assay accurately recovered spiked OM samples at concentrations spanning this linear range. Specificity testing results suggested minimal to no cross-reactivity or interference with OM metabolites, medications potentially co-administered with OM in heart failure patients or endogenous substances commonly existing in human whole blood. One hundred forty-six (146) OM plasma samples measured by an LC-MS/MS reference method were tested by the QMS OM Immunoassay. The Passing-Bablok regression analysis yielded an equation of y = 1.04x - 6.4 and an R value of 0.99, indicating a strong correlation with the platform used in PK analyses (LC-MS/MS). The shelf life stabilities of reagents, calibrators and controls are greater than 12 months when stored as indicated. On-board reagent stability is 30 days. No sample carry-over was detected. To demonstrate Phase 3 clinical study readiness, the manufacturer's laboratory and two central laboratories in the US and EU demonstrated over a twenty (20)-day period lab-to-lab reproducibility less than 3% CV and bias versus LC-MS/MS less than 7%, among other performance characteristics.

Conclusion: QMS OM Immunoassay turn-around-time, precision, accuracy and other performance characteristics support its use in the GALACTIC-HF Phase 3 clinical study.

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Comparison of drug testing rates and profiles in an urban vs a rural area in the province of Quebec, Canada

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Background: Drug addiction is often associated to a population living in underprivileged areas. Drug profiles may vary according to demographics, level of urbanization, crime rate and educational level. Few data exist on the patterns of drug abuse in urban and more rural communities in Quebec. In addition, many drug users and addicts consume more than one drug simultaneously. The objective of this project is to compare drug testing profiles of an underprivileged urban area to those of a semi-urban/rural area.

Methods: We compared drug testing in a major city's downtown area (Montreal, pop. 2 million) to the one of a small city in a remote area (Rimouski, pop. 50 thousand). Drug testing data for the year 2016 were extracted from Montreal's Laboratory Information System (LIS) and from Rimouski's laboratory. Incomplete results and screening in children less than 12 years old were excluded. Drugs of abuse screening panels (THC, opiates, cocaine, amphetamines, and benzodiazepines) were analyzed on Beckman-Coulter DxC 800 and 600. Reagents used (Beckman-Coulter) were the same except for the opiate analysis of the Montreal laboratory (CEDIA, Thermo Fisher). Data were analyzed according to sex and age, positive results for drug testing and frequency, origin of the request (emergency, outpatient, inpatient) as well as consumption profile.

Results: 68.5% (1980/2890) of drug screenings performed in downtown Montreal were positive compared to only 42% (640/1525) in Rimouski. In both cities, screening was more frequent in men (72% and 68%) than in women (28% and 32%), with similar rates of positivity for both sexes. The majority of positive results were found in individuals aged between 31 and 40 years old. Multiple drug abuse appeared to be more frequent in Montreal where 40.7% (806/1980) of screenings were positive for 2 to 3 drugs compared to 33.2% (212/640) in Rimouski. A similar pattern was observed for 4 drugs or more (5.2%, 49/1980 vs 1%, 5/640). In addition, Montreal's drug types differed from Rimouski's, with opiates and cocaine accounting respectively for 24% and 17.4% of all positive results (749 and 544/3126) in the former, compared to only 5.2% and 6.7% (47 and 61/911) in the latter, suggesting a different pattern of addiction between both populations. Rimouski had a slightly higher rate of positivity for THC (43.1% vs 33.6%) and benzodiazepines (26.9% vs 15.3%).

Conclusion: Clients screened for drug abuse were comparable between the two cities in terms of gender and age distribution. However, the rates of positivity and multiple drug abuse found in Montreal were much higher overall, with hard drugs such as opiates and cocaine being the main drugs of abuse. These results could indicate a higher drug addiction rate in Montreal's underprivileged neighbourhoods compared to Rimouski, a city known for it's relatively high quality of life. A deeper analysis of the results would be needed to confirm such a hypothesis.

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Validation of a LC-MS/MS method for measuring Voriconazole with ABSciex 4500 and comparison to a GC-MS method

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Background: Voriconazole is an antifungal drug indicated for patients with active invasive infections and for prophylaxis to prevent new fungal infections in high-risk immunocompromised patients. Voriconazole pharmacokinetics are nonlinear and inter-individual variability is high. Therefore, there is great difficulty for clinicians to effectively keep the patient's serum concentrations in the therapeutic range. The need for therapeutic drug monitoring of voriconazole is great and turnaround time of results is crucial for effective dosing. As no FDA approved methods are available, we developed a method using GC-MS. However, as our laboratory acquired new LC-MS/MS equipment the decision was made to validate voriconazole in this platform, to improve method performance and turnaround time.

Objective: Our goal was to determine the performance specifications of a LC-MS/MS method for measuring Voriconazole, and to compare them to our previous GC-MS method.

Methodology: The following parameters were evaluated to validate the LC-MS/MS method: Linearity, LLMI, matrix effect, carryover, accuracy, dilution, precision, interferences and sample stability. Moreover, these parameter results were compared to our previous GC-MS method validation data, as well as proficiency testing results, QC statistics and sample run time.

Results & Conclusions: In general, the performance characteristics of our new LC-MS/MS method surpassed those of our previous GC-MS method. For the LC-MS/MS method, linearity was achieved from 0.2-10 µg/mL, and with dilution, we could measure up to 30 µg/mL. No matrix effect, extraction recovery or ion suppression issues were detected. Carryover was tested to be less than 0.5% and CVs for between runs was determined to be < 8% for 3 different concentrations. Accuracy was assessed by method comparison, testing of PT samples and spike and recovery, all acceptable.

Comparing the LC-MS/MS method to the GC-MS method, we saw a significant reduction in sample prep time and run time leading to quicker turnaround times.

Furthermore, comparing proficiency testing events, the LC-MS/MS method was more accurate, with an average bias%= 31% for the GC-MS method to 5.8% for the LC-MS/MS method. Precision statistics from routine QC also showed a decrease in the CVs from the GC-MS method (CV= 11.9%) to the LC-MS/MS (CV= 3.3%) method. Finally, method time from sample prep to result reporting was reduced from about 7 hrs with GC-MS to 4 hrs with LC-MS/MS method.

B-381

Exploration of Ion Ratio Challenges with Routine THC GC-MS Confirmation Assays

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Background: Mass spectrometric identification of drugs confers a high specificity, in part due to the use of ion ratios. Failure of ion ratios can be due to co-migrating interferences, matrix effects, or less than optimal peak shape resulting in poor integration. Ion ratio failures can lead to further work-up of the sample which can be time-consuming and costly, to verify that the identified analyte is accurate. We present a case study in which we investigated the cause of ion ratio failures in four cases and provide a solution that uses a different THC solid phase extraction column.

Methods: Four human urine specimens with prior ion ratio failures were analyzed by gas chromatography-mass spectrometry (GC-MS). Base hydrolysis, solid phase extraction, and derivatization were performed on the urine samples prior to GC-MS. Two batches of the four specimens were analyzed to compare Agilent Bond-Elut CERTIFY and United Chemical Technologies (UCT) Clean Screen THC solid phase extraction columns. GC-MS was performed quantitatively and qualitatively on two Agilent 7890A and 7890B gas chromatographs coupled to 5975C and 5977A inert mass spectrometers.

Results: All four specimens extracted with the Bond-Elut column had ion ratios outside of the acceptance criteria varying from 128 to 164% of the predicted ion ratio. In-depth analysis of the TIC scans for the specimens reveal the monitored ions at the appropriate retention time, but also an interfering shoulder on the 473 m/z qualifier ion that contributes to the increased ion ratio, an additional non-analyte peak with unexpectedly high derivatization, and a co-eluting, unanticipated 399 m/z ion demonstrating less predictable ionization patterns. Repeating the analysis with UCT-extracted specimens eliminated the ion ratio failures of all four specimens (92 – 114% of calibrators).

Conclusions: While urine drug confirmation with mass spectrometry is widely conducted, challenges with ion ratio failures are often mitigated by specimen dilution, sending out to other facilities, or overlooked altogether. Directly exploring these ion ratio difficulties reveal interesting correlations between specimen, solid phase extraction column, and ion ratio success.

B-382

A retrospective analysis of oxycodone metabolism in patients with different CYP2D6 genotypes

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Objective: Oxycodone is one of the opioids being mostly abused and misused in pain management. In *vivo*, oxycodone is metabolized to oxymorphone and noroxycodone by cytochrome p450 enzymes, CYP2D6 and CYP3A4, respectively. The CYP2D6 gene is highly polymorphic, with more than 100 alleles having been determined, as a result affect its metabolic activity. Analysis of the correlation between CYP2D6 genotypes and urine concentrations of oxycodone and its metabolites will help us to understand the metabolism of the drug in human bodies.

Methods: CYP2D6 genotyping was performed using the QuantStudio 12K Flex Real-Time PCR system with buccal swab DNA. Urine concentrations of oxycodone,

oxymorphone and noroxycodone were tested with LC-MS/MS methods developed and validated in our laboratory.

Results: CYP2D6 genotyping has been done for total of 330 patients. 15 allelic variants (*1, *2, *3, *4, *5, *6, *9, *10, *13, *17,*29, *35, *41) were tested. The alleles with higher frequency are *1 (38%), *2 (16.5%), *4 (17.5), *5 (4.4%), *35 (5.0%), *41 (7.4%). The top four genotypes are: *1/*4 (10.9%), *1/*2 (10%), *2/*4 (6.4%), and *1/*41 (5.2%). CYP2D6 phenotypes were predicted based on the tested genotypes. Among 330 patients, 276 (83.6%) are extensive metabolizer/normal metabolizer (EM), 26 (7.9%) are poor metabolizer (PM), 18 (5.5%) are intermediate metabolizer (IM); and 10 (3.0%) are ultra-rapid metabolizer (UM).

Among the 330 patients, 116 were prescribed oxycodone and generated total of 476 urine samples in our database. 356 of the 476 urine samples (74.8 %) are from 95 extensive metabolizers; 68 urine samples (14.2%) are from 10 poor metabolizers, 26 urine samples (5.5%) are from 6 intermediate metabolizers, and 26 urine samples (5.5%) are from 5 ultra-rapid metabolizers. Ratios of oxymorphone and noroxycodone to the sum of parent drug and metabolites (oxycodone+oxymorphone+noroxyco done) were normalized by applying logarithmic transformation, respectively. An unpaired *t* test was used to analyze the normalized data. Our results indicated that urine concentrations of oxymorphone, a metabolite of oxycodone through the CYP2D6 enzyme pathway, are lower in CYP2D6 PM than those in EM, IM and UM significantly ($p = 0.0001$ for PM *vs* EM; PM *vs* IM; and

PM *vs* UM). However, there is no significant difference observed among EM, IM and UM (EM *vs* UM, $p=0.603$; EM *vs* IM, $p=0.237$; IM *vs* UM, $p=0.284$). Our data also indicated that there is no significant difference for urine concentrations of noroxycodone, another metabolite of oxycodone through CYP3A4 pathway, between CYP2D6 poor metabolizers and other metabolizers ($p>0.2$).

Conclusions: The metabolism of oxycodone to oxymorphone may be reduced in patients who are CYP2D6 poor metabolizer.

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Ranitidine Interference in Roche Amphetamine Urine Drug Screen

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Background: A urine specimen sent to our laboratory tested positive for amphetamines with the Roche Integra 400 amphetamines II immunoassay (500 ng/mL cutoff), negative for amphetamines with the Siemens Dimension EXL immunoassay (1000 ng/mL cutoff), and negative for amphetamines by LC-MS/MS performed at Mayo Medical Laboratories. The patient was taking 300 mg ranitidine daily, and no other prescription or over the counter drugs were declared. As of February 2017, Roche did not list ranitidine as a known interference in the Roche Integra 400 amphetamines II package insert. We investigated how much ranitidine was required to cause a false positive result using the Roche Integra 400 amphetamines II immunoassay with a 500 ng/mL cutoff.

Methods: Blank urine was spiked with ranitidine at concentrations ranging from 50-2500 ug/mL, and tested for amphetamines with the Roche Integra 400 amphetamines II immunoassay. The spiked samples were also tested with the Abbott Architect ci8200 amphetamine immunoassay (1000 ng/mL cutoff). In addition, a volunteer from the lab who was taking 150 mg ranitidine daily provided urine samples at 4 and 8 hours post-dose, and amphetamines were tested with the Roche Integra 400 amphetamines II immunoassay. Another volunteer from the lab took a single dose of 300 mg ranitidine, and urine and blood were collected at 4 and 8 hours post-dose. The urine was tested on the Roche Integra 400 amphetamines II immunoassay, and serum was sent to NMS Laboratories for ranitidine quantitation by HPLC.

Results: All ranitidine spiked samples, with the exception of the 50 ug/mL spiked sample, tested positive for amphetamines with the Roche Integra 400 amphetamines II immunoassay. The samples spiked with 2500 ug/mL and 1250 ug/mL tested positive on the Abbott Architect ci8200 amphetamines immunoassay, while the remainder of the samples, spiked with 625 ug/mL or less of ranitidine, were negative, which is consistent with the package insert. The lab volunteer who was taking 150 mg ranitidine daily tested negative for amphetamines at both 4 and 8 hours post-dose, while the lab volunteer who took 300 mg ranitidine, tested positive for amphetamines at both 4 and 8 hours post-dose, with the serum ranitidine concentration peaking at 390 ng/mL.

Conclusion: Standard doses of ranitidine, a frequently used prescription and over the counter H2-receptor antagonist, interfere with the Roche Integra 400 amphetamines II immunoassay, while much higher ranitidine doses are required to interfere with the Abbott Architect ci8200 amphetamines immunoassay. Laboratorians and clinicians need to be aware that ranitidine, a commonly used drug, interferes with amphetamine urine drug screens at varying concentrations depending on the manufacturer.

B-384

Improving the measurement of L-asparaginase: a standard-of-care drug used in pediatric oncology

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Background: L-asparaginase (ASNase) is used as treatment for acute lymphoblastic leukemia (ALL) in children. ASNase catalyzes the hydrolysis of L-asparagine, depriving leukemic cells of this essential amino acid needed for growth. Despite improving survival outcomes, clinical hypersensitivity and silent inactivation remains a commonly reported adverse event (~30%). Therapeutic drug monitoring (TDM) of ASNase is essential to monitor treatment efficacy and identify patients with clinical hypersensitivity and silent inactivation. Currently, no commercial reagents are available, resulting in a lack of standardization of methods. Furthermore, there is little data on enzyme stability, interferences and dilution effects. This study shows the development and investigation of a microplate-indooxine method for measuring ASNase activity. Importantly, we investigated the use of inactivated vs neat serum for blanking and sample diluent (affects LoQ and patient samples); stability studies; and the potential interference due to endogenous ASNase activity, which is a recent discovery and never before reported in the literature. **Methods:** Two main preparations of ASNase are approved for ALL treatment: pegylated *Escherichia coli* ASNase (PEG-ASNase) and *Erwinia chrysanthemi* derived ASNase (ErASNase). Both differ in their pharmacokinetic and biochemical properties, requiring unique calibration curves. ASNase stock solutions were prepared by dissolving lyophilizates in 0.9% physiological saline solution, then mixed 1:1 with pooled blank human serum. Calibration standards and QC materials were diluted with pooled blank human serum to final working concentrations. Within-run and between-day imprecision, functional sensitivity (LoQ) and linearity were evaluated. Patient samples were split to perform a method comparison. Sample, QC and calibrator stability, the use of different matrices for blanking and sample dilution, and the frequency of endogenous ASNase activity, were investigated. **Results:** For PEG-ASNase, within-run and between-day imprecision ranged between 4.3-15.3% and 7.5-10.8%, respectively. For ErASNase, within-run and between-day imprecision ranged between 5.4-7.5% and 4.1-10.9%, respectively. Linearity was verified up to 300 IU/L for PEG-ASNase, and 250 IU/L for ErASNase. The LoQ is 15 IU/L for both. How results are interpreted clinically was assessed to determine what proportion of patients would be classified differently between methods; a few patients were classified differently, indicating comparability between methods. Stability of ASNase over several hours at room temperature, at 4°C, and over several freeze-thaw cycles were evaluated. Interestingly, samples were stable at room temperature over several hours as well as up to 3 freeze-thaw cycles. Blank serum with endogenous ASNase activity was investigated. Differences were observed between neat vs. inactivated serum when used for blanking or for dilutions, indicating that patient results can be greatly affected. Although rare, some patient's samples contain very low levels of endogenous ASNase activity. **Conclusions:** This in-house microplate-indooxine based ASNase activity assay for PEG-ASNase and ErASNase has acceptable precision, linearity, and functional sensitivity, and compares well to other methods. Our examination of the effects of sample stability, the use of inactivated serum for blanking and dilutions, the addition of glycerol to provide added sample stability to QC/calibrators, and endogenous ASNase activity demonstrate better patient testing and monitoring performance of the method over time.

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Method validations for identification and quantification of fentanyl analogs

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Background: Fentanyl analogs are an emerging issue in clinical and forensic toxicology. While fentanyl is known to be 100 times as potent as morphine, the risk of lethal overdose appears to be exacerbated for fentanyl analogs, such as carfentanil, which has been postulated to be 10,000 times more potent than morphine. A targeted screening assay was developed to detect fentanyl analogs at low levels in both urine and post-mortem blood. Additionally, fentanyl immunoassay kits were studied for screening for fentanyl analogs. Finally, an LC-MS/MS assay was developed for quantitation of fentanyl analogs. This assay was used to determine sequential carfentanil levels in an overdosed patient enabling estimation of half-life of this drug.

Methods and results: Screening methods were evaluated for detection of carfentanil, furanyl fentanyl, U47700, AH-7921, MT-45, W-15, W-18, norcarfentanil and furanyl norfentanyl in urine and whole blood. Only carfentanil and furanyl fentanyl were

identified using Immunalysis and Thermo Fisher Scientific immunoassays, with limits of detection > 1 ng/mL. The LC-MS/MS screening method encompassed minimal sample preparation, reverse phase chromatography and MRM. Presence of a drug was determined by MRM ratios and retention time. The limit of detection for carfentanil and furanyl fentanyl were similar for urine and blood (0.2 ng/mL), while it was > 1 ng/mL for all other fentanyl analogs tested. The LC-MS/MS quantitative method was for analysis of carfentanil, furanyl fentanyl, MT-45, AH-7921 and U47700 in whole blood. It encompassed liquid-liquid extraction and lipid-removal procedures, reverse phase chromatography and MRM. The calibration range was 0.05 to 40 ng/mL. The assay was validated in keeping with SWGTOX guidelines. With the exception of MT-45, accuracy of analysis was within 10% of spiked values and %CV was < 10% at 0.4 and 5 ng/mL. For carfentanil half-life determination, heparinized whole blood from a carfentanil-overdosed patient was collected on admission, day 1 and 2 post-admission and analyzed for carfentanil. Medical treatment involved the administration of 2 mg of i.v. naloxone by first responders at the scene, however the patient remained unresponsive. The patient received supportive breathing by bag and was intubated at hospital. Upon admission, carfentanil concentration in blood was 22.4 ng/mL. The patient awoke 34 h after admission with an estimated corresponding carfentanil level of 0.45 ng/mL.

Conclusions: The LC-MS/MS screening method achieved a limit of detection of 0.2 ng/mL for carfentanil and furanyl fentanyl and had a lower limit of detection than either immunoassay evaluated. Neither immunoassay had reactivity with fentanyl analogs U47700, MT-45 or AH-7921 in addition to metabolites norfentanyl and furanyl norfentanyl. For these fentanyl analogs, the LC-MS/MS screening method had a limit of detection > 1 ng/mL. Using a more involved sample preparation, an LC-MS/MS method was developed with limit of quantitation of 0.05 ng/mL for carfentanil and furanyl fentanyl, which has been a satisfactory limit of quantitation for all cases of toxic exposure examined to date. Based on our analysis of an overdosed patient, the half-life for carfentanil in humans is 5 – 6 hours.

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Falsely elevated total phenytoin by EIA in a uremic patient treated with fosphenytoin

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Background: Phenytoin is a commonly prescribed anticonvulsant drug for partial and generalized tonic-clonic seizure. Fosphenytoin, a phosphate ester prodrug of phenytoin, is readily water-soluble and can be administered via intravenous or intramuscular injections resulting in less severe side effects than phenytoin. Fosphenytoin is pharmacologically inactive and metabolized to the active phenytoin *in vivo* by phosphatases in liver, erythrocytes, and other tissues. Fosphenytoin can also be dephosphorylated *in vitro* by serum alkaline phosphatase. Here we report a case of a uremic patient, who was treated with fosphenytoin, showing falsely elevated total phenytoin results by both Abbott ARCHITECT EIA and Roche Modular EIA methods compared to an HPLC method. We also report the results of cross-reactivity studies for fosphenytoin by ARCHITECT EIA and HPLC phenytoin methods. **Methods:** For the investigation of the index patient, we measured total phenytoin by HPLC, Abbott ARCHITECT EIA, and Roche Modular EIA, along with free phenytoin by HPLC. For the cross-reactivity study, stock fosphenytoin solution was diluted in 0.9% physiological saline and spiked into pooled human serum or saline at a range of concentrations. Samples were then incubated at room temperature for 0, 2, and 17 hours and phenytoin concentrations measured by ARCHITECT EIA and HPLC. To further elucidate whether high urea in patient specimens affect phenytoin immunoassays *in vitro*, fosphenytoin was also spiked into pooled serum containing normal or high urea levels. Samples were then incubated at room temperature for 0, 2, and 6 hours. Urea concentrations were measured by Ortho Vitros colorimetric method and phenytoin concentrations measured by ARCHITECT EIA and HPLC methods. Percent cross-reactivity were calculated. **Results:** In the index patient, total phenytoin differs between ARCHITECT EIA and HPLC by 120 to 215% in different specimens. Investigation of discordant result by Roche Modular EIA also show a discrepancy with HPLC values. Cross-reactivity studies showed that fosphenytoin cross reacts with the phenytoin immunoassay depending on the presence of serum alkaline phosphatase, and does not depend on *in vitro* urea concentrations. Comparisons between spiked pooled serum and spiked saline showed a 10% cross reactivity of fosphenytoin by the ARCHITECT phenytoin EIA assay. This was not reported in the manufacturer package insert. **Conclusions:** Fosphenytoin may crossreact with phenytoin immunoassays. This results in unpredictable discordant phenytoin results with specimens containing fosphenytoin. Therefore, it is recommended that for patients treated with fosphenytoin, therapeutic drug monitoring of phenytoin should be measured 2-4 hr post-dose to ensure all fosphenytoin have been metabolized into phenytoin. Additionally, the index patient is uremic and may contain other metabolites that are immunoreactive to phenytoin immunoassay antibodies. This is consistent with a previously reported observation that falsely elevated phenytoin results may be due to the oxymethylglucuronide metabolite that is accumulated in patients with renal insufficiency. Therefore, free fraction of phenytoin by HPLC is recommended for therapeutic drug monitoring for uremic patients, patients with chronic liver disease, and patients with hypercholesterolemia to prevent misinterpretation.

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Development and Validation of an LC-MS/MS Method for the Quantification of Capillary Blood Hydroxychloroquine Levels Collected on Volumetric Absorptive Microsampler

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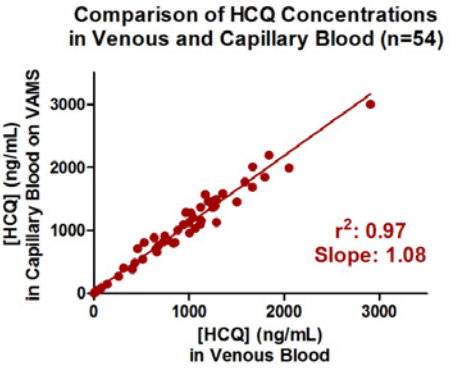
Background: Hydroxychloroquine (HCQ) blood levels have established utility in patients with chronic autoimmune rheumatic diseases. We sought to develop and validate an LC-MS/MS method for capillary blood hydroxychloroquine (HCQ) levels collected on volumetric absorptive microsampler (VAMS).

Methods: Capillary blood on VAMS (10 µL per specimen) and venous blood in EDTA (10 ml per specimen) were collected in 54 subjects receiving HCQ (200-400 mg/day). VAMS were eluted into water containing deuterated (d4) HCQ internal standard (IS). Following deproteinization with perchloric acid, supernatants were injected onto a Kinetex C8 column coupled to a TSQ Quantiva mass spectrometer with a heated electrospray ionization source operating in positive mode. Mobile phase was 0.1% formic acid and 0.01% triethylamine in water with an acetonitrile gradient. Transitions consisted of m/z 336 → 247 and m/z 340 → 251 for HCQ and IS, respectively. Venous blood HCQ levels were also measured using LC-MS/MS.

Results: Lower limit of quantification for capillary blood HCQ levels on VAMS was 10 ng/mL and was linear from 10 to 2000 ng/mL. Accuracy was within 15% of target value and intra/inter-day coefficients of variation were below 15%. No carry over, matrix effect, or interference from endogenous or exogenous substances were observed. HCQ concentrations recovered from capillary blood (day 3 elution, average 970±90 ng/ml) were similar to those observed in venous whole blood (average 873±83 ng/ml) ($r^2 = 0.97$; Deming slope = 1.08). HCQ collected on VAMS was stable for 10 days at ambient temperature and at least for 24 hours at 50°C**.**

Conclusion: HCQ can be accurately measured in capillary blood collected on VAMS. Our method offers a valuable alternative to venous blood for therapeutic drug monitoring of HCQ.



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Quantitation of Gabapentin, Pregabalin, Lamotrigine, Topiramate and Clobazam in Serum by LC- MSMS Using Low Sensitivity Ions to Expand the Linear Range

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Background: Blood drug testing is an effective tool for compliance monitoring, dose correlation and clinical tolerance. The aim of this study was the development of a fast and cost-efficient LC-MSMS method for simultaneously detecting and quantifying five anticonvulsant drugs in serum covering a projected quantitation range of 2 to

50,000 ng/mL (4+ orders of magnitude). Calibration curves constructed using primary product ions for gabapentin, pregabalin, lamotrigine and topiramate demonstrated signal saturation and deviation from linearity. To solve this problem, a strategy for expanding the linear range using secondary 'low sensitivity' product ions was developed. Deuterated analogs of pregabalin and topiramate were used as internal standards.

Method: Ten microliters of a 50 µg/mL internal standard mixture, containing deuterated standards in methanol, was added to fifty microliters of patient serum followed by 400 µL of acetonitrile. The solution was mixed for 15 seconds and centrifuged at 13,000 rpm for 5 min. at room temperature. The supernatant was transferred to a glass tube and evaporated to dryness at 37° C for 10 min using a gentle stream of air. The extracted sample was reconstituted with 200 µL of mobile phase A, mixed, transferred to an autosampler vial and injected onto a RESTEK Ultra bi-phenyl analytical column (5 µm, 50x2.1 mm) maintained at 40° C. Mobile phase A was 0.1% formic acid in water. Mobile phase B was 0.1% formic acid in acetonitrile. The acquisition method utilized 10 µl injection volume, 0.6 mL/min flow rate, and a gradient program of 98% A, increased to 80% B over 4 min. Run time was 5 min (injection to injection). The HPLC system consisted of Shimadzu pumps and autosampler. MSMS was performed on a Sciex 4500 triple quadruple mass spectrometer with TurboIonSpray© source monitored in positive and negative modes using primary (high sensitivity) and secondary (low sensitivity) product ions.

Results: Specificity was assessed by retention times and unique quantifier/qualifier transition peak area ratios. Intra-assay imprecision at two concentrations averaged 7.8% CV. A primary product ion was used to quantitate clobazam in the low (nanogram) range; linearity ranged from 2 to 1,000 ng/mL. Secondary product ions were used to quantitate gabapentin, pregabalin, lamotrigine and topiramate in the microgram range; linearities ranged from 100 to 50,000 ng/mL. Extraction efficiencies were greater than 90%. Matrix effects of native analytes were similar to deuterated analogs and did not affect quantitation. No carryover, endogenous or exogenous interferences were observed, with analyte stability at room temperature for 24 hrs. Qualitative correlations between our procedure and commercial LC-MSMS methods showed 100% agreement at cutoffs. Quantitative correlations showed less than 10% differences.

Conclusion: We present the development and validation of a LC-MSMS procedure for the quantitative determination of five anticonvulsants in serum at therapeutic levels employing small amount of a single specimen, deuterated internal standards, and a single extraction - without derivatization and additional chromatographic resolution. By monitoring both primary and secondary product ions, the linear measurement range is expanded, and the need for re-assay with dilution or pre-dilution is eliminated. This novel method is suitable for routine clinical use.

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Development of a Plazomicin Immunoassay as a Potential Aid for Therapeutic Drug Management (TDM) in the Potential Treatment of Serious Bacterial Infections Due to Multi-drug Resistant (MDR) Enterobacteriaceae, including Carbapenem-Resistant Enterobacteriaceae (CRE)

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Background: Rapid spread of multi-drug resistant (MDR) bacteria is rendering current antibiotic therapies less effective. Plazomicin is a next generation aminoglycoside being developed for the treatment of serious bacterial infections due to MDR Enterobacteriaceae, including carbapenem-resistant Enterobacteriaceae (CRE). Plazomicin is synthetically derived from sisomicin and contains structural modifications allowing it to maintain activity in the presence of common aminoglycoside-modifying enzymes that inactivate existing aminoglycosides. The QMS® Plazomicin Immunoassay is a homogeneous particle-enhanced turbidimetric inhibition immunoassay under development for the quantification of plazomicin in human plasma and serum using automated clinical analyzers to help monitor plazomicin concentrations in patients receiving plazomicin.

Methods: The QMS Plazomicin Immunoassay consists of two reagent components, namely a monoclonal anti-plazomicin antibody and plazomicin-coated microparticles, six calibrators spanning from 0 to 40 µg/mL and tri-level controls. The immunoassay is based on competition between free plazomicin present in a sample and plazomicin derivative coated onto microparticles for antibody binding sites. The plazomicin-coated microparticle reagent is rapidly agglutinated in the presence of the anti-plazomicin antibody reagent and the rate of absorbance change is measured photometrically. When a sample containing plazomicin is added, the agglutination reaction is partially inhibited, slowing down the rate of absorbance change. A concentration-dependent agglutination inhibition curve is obtained with maximum rate of agglutination at the lowest plazomicin concentration and lowest agglutination rate at the highest plazomicin concentration. The QMS Plazomicin Immunoassay performance characteristics were evaluated on the Beckman Olympus AU680 analyzer in both plasma and serum through a set of analytical studies following CLSI guidelines.

Results: The assay range was determined to be 0.4-40 µg/mL. The lower limit of quantitation (LLOQ), was 0.4 µg/mL. The assay was linear with less than 6.3% error for plazomicin concentrations between 0.8-40 µg/mL. Assay precision and accuracy in plasma and serum spiked with 2, 4, 15, and 30 µg/mL of plazomicin were determined by testing twice per run, two runs per day for 5 days. This yielded within-run precision between 1.8 and 3.2 %CV and total-run precision between 3.1 and 6.7 %CV, with accuracy greater than or equal to 95.6%. No significant interference was observed with various endogenous substances, aminoglycosides, or common concomitant medications. No known plazomicin metabolites have been identified to test for potential cross reactivity with the assay. Sample carry-over was 0.158 µg/mL or below. Method correlation studies comparing the QMS Plazomicin Immunoassay and a LC-MS/MS reference method using clinical plazomicin plasma samples yielded a Passing-Bablok's regression equation of y = 1.03x - 0.122 and a correlation coefficient of 0.9770 (n=82). Onboard reagents were stable for a minimum of 30 days and the calibration curve was stable for 7 days.

Conclusion: The QMS Plazomicin Immunoassay enables measurement of plazomicin with acceptable precision and accuracy between 0.4 - 40 µg/mL. The assay's ability to accurately measure plazomicin has the potential to enable individualized dosing for patients receiving plazomicin, a novel antibiotic also under development. This project has been partially funded under BARDA Contract No. HHSO100201000046C.

B-390

CEDIA® Heroin Metabolite (6-AM) Application for the Ortho Clinical Diagnostics VITROS® 4600 Chemistry System and VITROS 5600 Integrated System

T. Prestigiacomo, T. Huynh, M. Aquino, K. Cheung. *Thermo Fisher Scientific, Fremont, CA*

Introduction: Heroin (3, 6-diacetylmorphine) is a Schedule 1 substance and commonly abused opioid within the United States and in the rest of the world. It is generally synthesized through chemical modification of morphine, a naturally occurring alkaloid. Heroin can be administered by intravenous and subcutaneous injection or by nasal insufflation. It is rapidly metabolized (half life of 9 minutes) to Monoacetylmorphine (6-AM) by various esterases in the blood; then it is converted to morphine within the liver through hydrolysis. 6-AM is a distinctive metabolic marker for heroin use because it cannot be formed by acetylation of morphine within the body. Use and possession of heroin is illegal and is associated with a number of adverse effects including lung complications, kidney disease, and bacterial infection of blood vessels. The ability to detect heroin within overdose patients and drug offenders proves vital for health practitioners and the members of the criminal justice system. **Method:** The CEDIA 6-AM Assay utilizes the enzyme β-galactosidase, which has been genetically engineered into two inactive fragments (EA and ED). The heroin metabolite present within human urine samples and the 6-AM conjugated to an inactive enzyme fragment (ED-LC) compete for antibody binding sites. Because 6-AM inhibits the binding of ED-LC to the antibody, its presence allows the two inactive enzyme fragments to better re-associate into an active enzyme. The concentration of 6-AM within the sample will affect the complementation of the enzymes fragments. Enzyme activity results in an absorbance change that is directly proportional to the concentration of 6-AM in the sample; this change can be measured spectrophotometrically. The VITROS 4600 Chemistry and 5600 Integrated Systems are new applications for the CEDIA 6-AM Assay for the qualitative detection of heroin metabolite in human urine with a cutoff of 10 ng/mL. Analyzer performance was determined for precision, limit of blank, and accuracy. Correlation studies using the two instruments were conducted in comparison to liquid chromatography-mass spectrometry (LC-MS) values.

Results: All studies were evaluated using CLSI guidelines. Three levels of 6-AM controls were used in the studies. The within-run precision ranged from 0.2 to 0.5% CV and the total precision, 1.5 to 2.6%CV. The limit of blank on the VITROS 4600 Chemistry and 5600 Integrated Systems resulted in 0.43 ng/mL and 0.35 ng/mL, respectively. Accuracy was measured using patient correlation against LC-MS values. The VITROS 4600 Chemistry System yielded 92.7% Positive Agreement, 97.8% Negative Agreement, and 95.0% Total Agreement ($n_{negative}$=45, $n_{positive}$=55, n_{total}=100). The VITROS 5600 Integrated System yielded 92.7% Positive Agreement, 97.8% Negative Agreement, and 95.0% Total Agreement ($n_{negative}$=45, $n_{positive}$=55, n_{total}=100).
Conclusions: All measured studies demonstrated acceptable performance, validating

use of the CEDIA Heroin Metabolite Assay on the Ortho Clinical Diagnostics VITROS 4600 Chemistry and 5600 Integrated Systems. The assay will provide an effective detection system to screen individuals who use heroin in its various forms.

B-391

DRI® Hydrocodone Application on the Ortho Clinical Diagnostics VITROS® 4600 Chemistry System and VITROS 5600 Integrated System

T. Prestigiacomo, M. Aquino, T. Huynh, K. Cheung. *Thermo Fisher Scientific, Fremont, CA*

Introduction: Hydrocodone is a commonly used semi-synthetic opioid derivative. It is synthesized from codeine, a naturally occurring alkaloid. Due to its ability to relieve moderate and severe pain, hydrocodone has grown in usage, particularly within the United States. The analgesic effect of the drug begins within 20-30 minutes of taking it and can last up to 8 hours. Furthermore, an increase in the abuse of hydrocodone in its various forms has been observed in recent years. Thus, an effective monitoring system is necessary for clinicians and law enforcement to determine drug levels within addicts and criminals. **Method:** The DRI Hydrocodone Assay utilizes a drug-labeled variant of glucose-6-phosphate dehydrogenase (G6PDH) and the effects of competitive inhibition. When in the presence of select antibodies, G6PDH competes with free drug present within a sample for antibody binding sites. When the drug-labeled enzyme binds to the antibodies, enzyme activity is decreased as a result. Thus, drug concentration and enzyme activity are directly proportional. This relationship can be determined by monitoring the conversion of NAD to NADH, which is measured spectrophotometrically at 340 nm. The VITROS 4600 Chemistry System and VITROS 5600 Integrated System are new applications for the DRI Hydrocodone Assay for the qualitative and semiquantitative determination of hydrocodone in human urine at a cutoff of 300 ng/mL. The analyzers were subjected to precision, limit of blank (calculated as three times the standard deviation of the negative calibrator), and linearity studies. **Results:** All studies were evaluated in adherence to CLSI guidelines. Total precision was conducted over the span of 20 days. In this timeframe, the low and high control values were compared to the cutoff calibrator in both qualitative and semiquantitative methods. Within-run precision results ranged from 0.1% to 0.3% CV qualitatively and 1.1% to 1.9% CV semiquantitatively between the two instruments. Total precision results ranged from 0.2% to 0.5% CV qualitatively and 1.9% to 3.2% CV semiquantitatively between the two instruments. The limit of blank was determined to be 3.01 ng/mL on the VITROS 4600 Chemistry System and 3.17 ng/mL on the VITROS 5600 Integrated System. Additionally, linearity was evaluated by comparing calibrator blends to their nominal values. The VITROS 4600 Chemistry System yielded 95.2% to 102.0% recovery, and the VITROS 5600 Integrated System yielded 94.5% to 101.7% recovery. **Conclusions:** All studies aforementioned demonstrate acceptable performance of the DRI Hydrocodone Assay on the Ortho Clinical Diagnostics VITROS 4600 Chemistry System and 5600 Integrated System. Further studies characterizing positive and negative agreement between methods will be conducted.

B-392

DRI® Methadone Metabolite (EDDP) Application on the Ortho Clinical Diagnostics VITROS® 4600 Chemistry System and VITROS 5600 Integrated System

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Introduction: Methadone is a synthetic opioid that has been used for decades by clinics and other addiction-treatment facilities to manage opioid dependency. After administration, methadone is metabolized to normethadone by N-demethylation, which is converted by dehydration to the primary metabolite, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP)[1]. Effective monitoring of methadone and its metabolite is essential for ensuring compliance. **Method:** The DRI Methadone Metabolite Assay is a competitive assay between drug labeled with glucose-6-phosphate dehydrogenase (G6PDH) and free drug in the urine sample for a fixed number of antibody binding sites. Antibodies detect EDDP without crossreactivity to the parent drug, methadone. Drug concentration and enzyme activity are directly proportional to the conversion of NAD to NADH, which is measured spectrophotometrically at 340 nm. The VITROS 4600 Chemistry and 5600 Integrated Systems are new applications for the DRI Methadone Metabolite Assay for the qualitative and semiquantitative determination of methadone metabolite in human urine at a cutoff of 1000 ng/mL. **Results:** All studies were evaluated using CLSI guidelines. Total precision was evaluated for 20 days in which low and high control levels were compared to the cutoff calibrator level in both qualitative and semiquantitative modes. For the VITROS 4600 Chemistry System, qualitative %CV results ranged from 0.3-0.4% and semiquantitative %CV results ranged from 1.4-1.7%. For the VITROS 5600 Integrated System, qualitative %CV results ranged from 0.6-0.9% and semiquantitative %CV results ranged from 1.3-1.7%. The Limit of Blank (i.e., 3 times the SD of the negative calibrator) was determined to be 29.4 ng/mL on the VITROS 4600 Chemistry System and 21.6 ng/mL on the VITROS 5600 Integrated System. One hundred and ten (110) patient samples were analyzed for positive and negative agreement compared to liquid chromatography-mass spectrometry (LC-MS). Qualitative positive and negative agreement was 100% and 90%, respectively, on both instruments. Semiquantitative positive agreement was 98% on the VITROS 4600 Chemistry System and 100% on the VITROS 5600 Integrated System. Semiquantitative negative agreement was 91.7% on both instruments. **Conclusions:** All studies demonstrated acceptable performance, validating the use of the DRI Methadone Metabolite Assay on the Ortho Clinical Diagnostics VITROS 4600 Chemistry System and VITROS 5600 Integrated System.

B-393

ARK Fentanyl Assay for the Beckman Coulter AU680 Automated Clinical Chemistry Analyzer

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Background: Fentanyl is a highly addictive potent synthetic opioid that is widely used for chronic pain management and surgical anesthesia. The drug is a controlled Schedule II substance and was introduced in 1960 as a replacement for other opioids in cardiac surgery. Currently, the drug is available as injectable solution for surgical anesthesia and transdermal patches at concentrations of 25, 50, 75, 100 mg/h for chronic pain management. Other than medical applications, fentanyl has also been sold to drug users, primarily heroin abusers and resulted in hundreds of overdoses. The severity of the situation became apparent when the above average numbers of overdoses were observed in many regions in United States. Since fentanyl is 50-100 times more potent than heroin and present in biological samples at very low concentrations, administration and monitoring of fentanyl present a great challenge in clinical and forensic laboratories. There is an increasing need for a high throughput screening method for the detection of fentanyl in human urine.

Methods: The ARK Fentanyl Assay is a liquid stable, homogeneous enzyme immunoassay, intended for the qualitative and/or semi-quantitative determination of fentanyl in human urine at a cutoff concentration of 1.0 ng/mL on automated clinical chemistry analyzers. Two reagents, calibrators (0.0, 1.0, 2.0, 4.0, and 10.0 ng/mL) and controls (0.5 and 1.5 ng/mL) compose the test system. The 1.0 ng/mL Calibrator is the Cutoff for distinguishing "positive" from "negative" samples. Precision over 20 days, histogram overlap analysis of Control and Cutoff concentrations, recovery and specificity were evaluated on the Beckman Coulter AU680.

Results: Semi-quantitative precision was determined for 0.5 (11.3%CV), 1.0 (5.5%CV) and 1.5 (6.0%CV) ng/mL. Qualitative determination of fentanyl in Low and High controls did not overlap with the Cutoff by histogram analysis. Recovery of fentanyl ranged from 91.6% (0.75 ng/mL) to 104.7% (6.0 ng/mL). Norfentanyl metabolite tested positive at 300.0 ng/mL. Fentanyl analogues despropionylfentanyl, hydroxyfentanyl, acetylfentanyl, butyrylfentanyl, carfentanil, and sufentanil tested positive at 75.0, 1.0, 1.0, 2.0, 500.0 and 600.0 ng/mL respectively. Other opiates were not crossreactive. The sensitivity (true positive, 100 samples) and specificity (true negative, 50 samples) was 96.2% and 98.0%, respectively, versus LC-MS/MS (fentanyl cutoff 0.2 ng/mL).

Conclusion: ARK Fentanyl Assay determines fentanyl in human urine accurately and sensitively in either semi-quantitative or qualitative modes with fast turn-around times. Detection of fentanyl use in pain management, compliance or misuse/abuse with a superior cutoff concentration for a screening assay is an important new addition to clinical chemistry.

B-394

Two efficient sample preparations for opiates testing in urine

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Background:

In 2014, nearly two million Americans either abused or were dependent on prescription opioid pain relievers. 91 Americans die every day from an overdose involving either prescription opioid or heroin. Today´s abuse profile combine both clinical and street drugs. Combining efficient hydrolysis based on purified beta-glucuronidases with an automatable clean-up or extraction method enables provides robust and defendable results.

Methods:

Two different sample preparation methods were tested for recovery, linearity, precision and enzyme hydrolysis. Three analytes (6-MAM, codeine, and morphine) and their deuterated internal standards (IS) were spiked to blank urine at different concentrations for extraction method validation. Codeine-6-beta-D-glucuronide, morphine-3-beta-D-glucuronide and 6-MAM were spiked to blank urine at ULOQ level (equal to 2000 ng/ml free drug level) for enzyme hydrolysis recovery test.

For filtration method, 0.2 ml of master mix (including ammonium acetate buffer , 66 µL of EBG beta-glucuronidase and IS) and 0.1 ml of fortified urine was directly added to RubyPro protein crash plate (2cc/well 96-well plate). After incubation at 50°C for 15 minutes, 0.05 ml of methanol was added to each well. After 5 minutes, positive pressure was applied to elute the analytes, and eluate was injected to LC-MS/MS (Shimadzu 10AD-API 3000) directly.

For solid phase extraction (SPE) method, mixture of 0.25 ml of master mix(ammonium acetate buffer, 25 µl of BG100 beta-glucuronidase and IS) and 0.1 ml of fortified urine was incubated at 68°C for 30 minutes. Orpheus C18 SPE plate(50mg/well, 2cc/well plate) was pre-conditioned with 1 ml of methanol, then 1 ml of water. Hydrolyzed urine solution was loaded to C18 and then washed with 1 ml of each water and 5% methanol. Analytes were eluted with 0.5 ml of methanol, and directly injected to LC-MS/MS. LC-MS/MS was operated in positive ion mode. Reliasil C18 3 micron, 2.1x50 mm column was used for separation with acetonitrile and water gradient mobile phases. LC run time is 5 minutes.

Results:

For both methods, all analytes are linear in the range of 20-2,000 ng/ml, with precision within 15% and 6-MAM integrity is preserved with above 90% recovery. Recoveries of codeine and morphine are similar for both methods, above 85% for codeine and above 90% for morphine.

Conclusion: Robust and defendable results are achieved. Two accurate and automated sample prep methods for opiates drug testing are validated. Technically, the methods are a proof of concept solving three analytical challenges: hard-to-cleave analyte (codeine-6-glucuronide), protein-binding (morphine), labile analytes (6-MAM). Hydrolysis can be conducted directly on the Protein crash filter plate providing a faster, simpler to operate method, at a lower cost. It meets the typical screening requirement of PDM and pain-management. C18 SPE method removes more endogenous in the urine, and increases the lifetime of LC column and mass spectrometer. With 0.5 ml of elution, we eliminate evaporation time. This method may be preferred for opiates/opioids confirmation assays,e.g. in workplace drug-testing. Both method should now be challenged with the addition of further analytes to complete a broad pain-panel.

B-395

Predicting Drug Exposure in Breast-Feeding Infants: Using Physiologically-Based Pharmacokinetic Modeling of Escitalopram in Breast Milk to Simulate Infant Plasma Concentrations

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Background: Postpartum depression is a common disorder affecting between 10-15% of new mothers. Escitalopram (ESC) is considered to be the first line treatment for postpartum depression; however safety data about its use during breastfeeding is limited and the risk of drug exposure to the nursing infant remains unknown. To ensure new mothers are compliant to their medication regimen, along with safeguarding the nursing infant from potential drug exposure, it is essential to determine the safety profile of ESC during lactation and investigate the extent of drug exposure to the nursing infant. The objectives of this study are: i) develop an LC-MS/MS method to measure ESC in breast milk, ii) perform population pharmacokinetics (popPK) and physiologically-based pharmacokinetic modeling (PBPK) to simulate drug concentrations in milk and infant plasma so to estimate the risk of adverse events. **Methods**: Eighteen women receiving ESC therapy were recruited through the Drugs in Lactation Analysis Consortium (DLAC) framework at the Hospital for Sick Children. Breast milk was collected at 3-5 time points, post-dose, across the patient's dosing interval (24h). An LC-MS/MS method was developed for ESC and its metabolite, desmethylescitalopram (D-ESC), and validated in accordance with CLSI guidelines. To simulate the infant dose based on the concentration of drug in breast milk, popPK modeling was performed using the non-linear mixed effects program, NONMEM, using stochastic approximation expectation maximization and the ADVAN5 subroutine. After the infant dose was generated, PBPK modeling was then performed to simulate infant plasma drug concentrations using PK-SIM software. **Results**: Results from the development and validation of the ESC/D-ESC LC-MS/MS method will be presented. There was no significant difference seen between ESC concentrations in the foremilk and hindmilk. A one-compartment model (+ absorption) with a proportional residual error model was selected to best describe the time-concentration profile of ESC in milk. Based on this final popPK model, ESC concentrations in milk were simulated at steady-state in 1000 women and the median dosage an infant would be exposed to, via milk, was 9 µg/kg/day. The infant dose at the 99th percentile was 8.8% of the weight-adjusted maternal daily dose, which was 18 µg/kg/day. Using a PBPK approach, the median simulated infant $AUC_{0\text{-}inf}$ following ESC exposure through breast milk was 12 µg.h/L, which is 3.2% of the maternal $AUC_{0\text{-}inf}$. Both the dosage through breast milk and the median infant $AUC_{0\text{-}inf}$ fall well below the therapeutic range. **Conclusion**: The data from this study demonstrates that infant exposure to ESC through breast milk is less than 10% of the maternal weight-adjusted dose. These results indicate that the risk of an adverse events in nursing infants is low. Therapeutic drug monitoring using a robust analytical method, followed by pharmacokinetic modeling and simulation data, provide a comprehensive view of ESC excretion into milk that will help guide decision making for drug use during lactation. Filling in the gaps of knowledge that exist in this area is essential to understanding drug safety and reducing the risk of adverse events in infants exposed to drugs through breast milk.

B-396

Increasing Sample Throughput for Quantitation of Anticonvulsant Drugs for Clinical Research Use

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Anticonvulsant drugs such as zonisamide, lacosamide, topiramate, levetiracetam, lamotrigine, clozapine, norclozapine, and oxcarbazepine metabolite, 10,11-Dihydro-10-Hydroxycarbamazepine have been measured in biological fluids in both clinical research and forensic analysis. Better techniques for monitoring concentrations of these drugs are needed to help optimize drug therapies in healthcare. In this study we evaluate an analytical method to measure multiple anticonvulsant drugs within a very narrow range of detection and sensitivity to meet clinical research laboratory requirements for analytical efficiency. Detection in biological fluids can be time consuming however, by utilizing online sample cleanup and multi-channel LC/MS detection, a fast cost effective analysis of anticonvulsant drugs can be performed. All standards were obtained from Cerilliant (Round Rock, TX) and analyzed as received. Serum curves were prepared in human serum, isotopic labeled standards were added, vortexed, and spun. Supernatant was put in autosampler vials and injected into the LC/MS. A volume of 5 uL was injected onto a Thermo Scientific™ TurboFlow™ Cyclone™ column, which was then focused onto an analytical column, Thermo Scientific™ Hypersil™ GOLD aQ. Compound separation was accomplished using a reverse-phase gradient with the Thermo Scientific™ Transcend™ II system with Thermo Scientific™ UltiMate™ 3000 RSLC pumps in a duration of seven minutes. The Thermo Scientific™ TSQ Endura™ was employed to detect the target analytes using electrospray ionization. Timed selected reaction monitoring (SRM) was utilized to maximize detection efficiency for the compounds analyzed. In order to increase throughput, multiplexing two LC channels was employed. In order to maximize efficiency, data windows are made as small as possible so the detector can always be acquiring data. With the analytical method developed here utilizing two-dimensional chromatography, 80-90% recovery off the TurboFlow column was obtained. Accuracy and precision data were collected in neat and serum matrices**.** The analytical performance gave RSD values that were less than 15.0% for all compounds tested. Additionally, accuracy was ±15% of the theoretical value for all the assays. The correlation coefficient values for all the compounds were below 0.995, showing linearity throughout all concentrations and analytes. Detection levels below

50 ng/mL can be obtained which meets clinical research laboratory requirements. In addition, multiple compounds can be analyzed in one run and can be separated chromatographically.

B-397

Development and Validation of a Liquid Chromatography-Tandem Mass Spectrometry Assay for the Simultaneous Quantitation of 5 Azole Antifungals and 1 Active Metabolite

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Background: Invasive fungal infections are deadly and prevalent in certain high-risk patient populations: patients with hematological malignancies, the immunosuppressed, and the critically ill. Successful azole antifungal medication therapy can be life-saving for these patients. To support azole therapeutic drug monitoring at our hospital, a liquid chromatography tandem mass spectrometry (LC-MS/MS) method was developed and validated for 6 analytes: fluconazole, voriconazole, posaconazole, isavuconazole, itraconazole, and its active metabolite hydroxyitraconazole. **Methods**: During preparation, 50 µL of sample was precipitated via mixing with 250 µL of an acidified acetonitrile solution [0.1% (v/v) 1M HCl] containing the internal standards for all 6 analytes (1 µg/mL). A 50 µL aliquot was then diluted with 200 µL 0.1% (v/v) formic acid in ultrapure water. Utilizing a Transcend LC system (Thermo Scientific), 20 µL of the sample was injected onto a reversed-phase column (Accucore RP-MS, 50 x 2.1 mm, 2.6 µm), coupled to a Thermo Scientific TSQ Vantage triple quadrupole mass spectrometer. To validate the method, the following experiments were performed: ion suppression, mixing study, interference, analytical measuring range (AMR), carryover, stability, precision, and method comparison. **Results**: *Ion suppression* Only fluconazole exhibited signal suppression versus a blank sample. *Mixing study* The mean percent difference between pooled-patient serum and charcoal-stripped serum (the calibrator matrix) ranged from -5.2% to 4.3%. A mixing study was also performed between pooled-patient serum and pooled-patient lithium heparin plasma, to expand the acceptable sample types. The mean percent difference ranged from -7.5% to 5.5%. *Interferences* The method was found to be free (percent difference <20%) from potential endogenous (hemolysis, icterus, lipemia, and uremia) and exogenous (>100 therapeutic drugs and common analytes) interferences. *AMR* The AMR for all analytes, except fluconazole, was established by triplicate analysis at 7 levels in spiked, pooled-patient serum: 0.2, 0.3, 0.6, 1.3, 2.5, 5.0, and 10.0 µg/mL. Fluconazole's AMR was evaluated in the same matrix, but at higher levels to be consistent with therapeutic target ranges: 0.5, 0.9, 1.9, 3.8, 7.5, 15.0, and 30.0 µg/mL. The analytical recovery ranged from 91.6% to 121.3%, with coefficients of variation less than 14.3%. *Carryover* No carryover was found (percent difference <1.8%) at twice the upper limits of the AMR. *Stability* The unextracted samples were found to be stable (percent difference <8%) for 15 days at room temperature and 4 °C. At -20 °C, all analytes were stable for 60 days (percent difference <8%). The extracted stability at 4 °C (percent difference <8%) was 7 days. *Precision* The intraday CVs ranged from 1.5% to 3.4%. The total CVs ranged from 1.8% to 3.6%. *Method comparison* Approximately 40 patient samples (spiked or endogenous) were compared to reference laboratories, for each analyte. The correlation coefficients ranged from 0.9658 to 0.9981. The range for the slopes and intercepts were 0.947 to 1.105 and -0.296 to 0.127, respectively. **Conclusion**: This LC-MS/MS method has been validated to support therapeutic drug monitoring of patients undergoing azole antifungal medication treatment. The method only requires simple sample preparation and a sub 2 minute analysis time.

B-398

Comparison of Centrifugal Filter Performance for Free Phenytoin Assays

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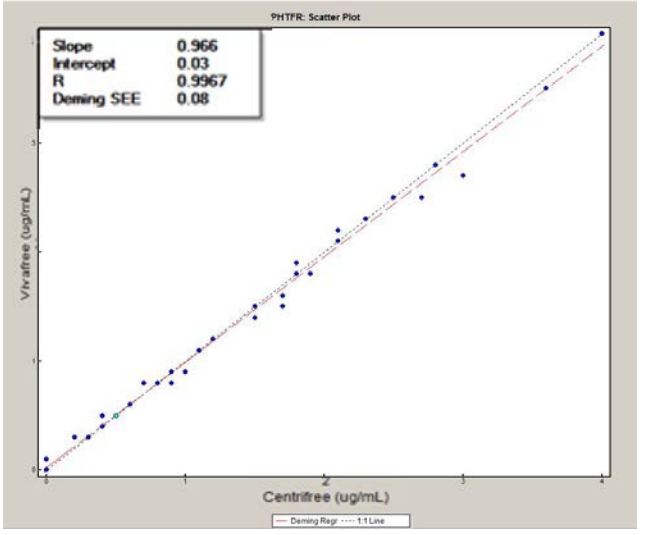
Background: Free phenytoin concentration samples require a preanalytic spin through a filtration system to separate free from bound drug. A good filtration system needs to produce an accurate result as well as a high yield. The objective of this study was to compare the performance of the Vivafree™ 2 centrifugal filters from VivaProducts versus the Centrifree® Ultrafiltration Device from Millipore for free phenytoin testing. Performance was evaluated by comparing the analytical results for the filtered samples and the yield of filtrate.

Methods: 40 samples were created using patient pools and the Roche COBAS FP Free Phenytoin Calibrators. Mixtures were created to span the AMR of 0.1-4.0 µg/mL. 500 µL of each mixture was then transferred to a filter from each manufacturer and spun for 20 minutes at 1810 g. The samples were transferred to cobas micro sample cups and processed on a Roche cobas Integra 800. Filtration yield was investigated by making a patient pool and spinning 3 of each manufacturers' filters with a range of volumes up to 1 mL. Also, 2 mL of sample in the Vivafree 2 filters was tested.

Results: Values of <0.1 were interpreted as 0.0 and values >4.0 were interpreted as 4.0. The correlation, slope and intercept are shown in the plot. Comparing the yields, the Centrifree filter produces a higher yield at lower sample volumes, producing 130 µL with 250 µL of sample vs. 50 µL yielded by the Vivafree. At 1000 µL of sample, the filters yield 180 vs. 187 µL respectively.

Conclusion: The Vivafree 2 and Centrifree filtration systems produce comparable analytical results. The Centrifree system has a higher yield at lower volumes, but the volumes are comparable at higher sample volumes. If larger volume yields are needed, the Vivafree filters could be utilized.



B-399

Validation of dilute and shoot and evaluation of SPE method for barbiturates and THC carboxylic acid panel in urine

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Background: Barbiturates and THCA panel is commonly tested in clinical labs by dilute and shoot approach which comes with high matrix interference at low concentration, and long term deterioration of the LC-MS system. The purpose of study reported here was to validate in lab developed dilute and shoot assay and evaluate solid phase extraction (SPE) approach as alternative. **Methods:** ExionLC - Triple Quad 4500 MS was operated in negative ion mode. Xenobiotics and buffer chemicals were purchased from Sigma Aldrich. *E. Coli* beta-glucoronidase (BG) was purchased from Campbell and Kura Biotec. HPLC-MS grade solvents were purchased from Pharmco-Aaper. Gazelle biphenyl column (1.8 µm, 50 × 2.1 mm) was used with a 30-95% methanol (0.1% FA) gradient mobile phase. Dilute and shoot procedure was as follows: 100 µL of urine was spiked with ISs, vortexed with pH 6.8 phosphoric buffer, mixed with 20 µL of BG solution and incubated (55 deg C, 30 min). 150 µL of ammonium acetate buffer in 40/60 water/methanol was added prior to vortexing and centrifuging. Assay was validated for accuracy, precision, linearity, carryover limit, matrix interference, xenobiotic interference and analyte stability across three validation batches on three different days. To develop an SPE method we screened Orpheus C18, and two types of polymeric DVB SPE plates (Celerity and Panthera Deluxe): each plate was conditioned with 1 ml of methanol and 1ml of water, loaded with 100 µL of fortified urine (spiked at cut-off and 30% of ULOQ concentration), while washing and elution procedures were varied. Elutes were evaporated, reconstituted (when needed) and analyzed. Recovery and reproducibility were tested along with different wash protocols using varying concentrations of methanol with or without 0.1-1% formic acid instead of water and varying elution solutions and volumes. At the end, we verified the final procedure by comparing one validation batch against the batch of the previously validated dilute and shoot method. **Results:** Dilute and shoot assay conformed to industry standards: accuracy and precision were within 20% of the target (25% at LLOQ) while linearity was assessed for calibrators across three validation batches and showed back-calculated concentrations of all calibrators (n=6) within 20% of target. AMR was determined to be 10 - 2500 ng/mL

for all barbiturates and 4 - 500 ng/mL for THCA. Optimized SPE method showed recoveries above 90% for barbiturates and 70% for THCA and improvement of matrix interference, ion suppression and enhancement. We could skip evaporation step easily by using 0.5 ml of methanol elution. Cross validation of two bathes of dilute and shoot assay against the optimized SPE procedure showed that SPE procedure gives comparable results with desired improvement pertaining to better sample clean up.

Conclusion: We developed a fast SPE method with about 10 minutes per plate processing time. This improved method reduced matrix interference more than double while it could easily be compared against the validated dilute and shoot method.

B-400

Automated blood sampling with paper spray ionization mass spectrometry: improving workflow and the safe handling of human blood for personalized medicine and clinical trials

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Traditional, manual methods of blood collection are imprecise with regard to the time of collection and volume of blood removed from the patient. Collection times are significant in pharmacokinetic studies and in personalized medicine where a patient's rate of absorption and clearance of a drug is evaluated. If each blood draw collects an excess of sample, then frequent sampling can induce anemia. In addition, with the use of sharps common to manual collection, there is a risk of exposure to blood-borne pathogens for the person collecting the sample. Likewise, there is a risk of nosocomial infection to immune-compromised patients through repeated access to indwelling catheters. Improvements are justified for both sample collection and analysis. Herein, a workflow is demonstrated that utilizes a device (Phlebot) which automatically and painlessly collects small (0.025 to 1.000 mL) programmable volumes of whole blood from an intravenous catheter at preset time points and deposits them in refrigerated, sealed vials pretreated with an anticoagulant. The procedure is painless and the device is as mobile as an infusion pump.

After centrifugation and collection of plasma, each sample was spotted onto paper and dried at room temperature to deactivate blood-borne pathogens and permit safe handling of samples. The paper was then analyzed by paper spray (PS) ionization tandem mass spectrometry (MS/MS). PS is an ambient ionization technique amenable to point of care analysis of biofluids. It quantitates small analytes in whole untreated biofluids in under one minute and has successfully been demonstrated with opioids, benzodiazepines, illicit drugs, immunosuppressants, and others. This technology allows for pharmacokinetic and pharmacodynamic measurements to be readily taken for personalized medicine in a painless manner for improved patient care.

For this proof of workflow study, acetaminophen was measured in human plasma. A healthy male subject (n=1) was dosed orally with 500 mg acetaminophen. Blood collections of 250 µL were programmed for 5 minute intervals over 2.5 hours into cold, presealed, heparinized vials held at 4°C. Plasma was recovered from the blood and transferred to vials with a random code which blinded their identity to the analyst. Samples were stored frozen. For analysis they were thawed, mixed with an internal standard solution, and then 10 µL of the sample was pipetted onto paper triangles. The sample was dried for 30 minutes at room temperature before being placed in front of the MS inlet where a solvent and high voltage were applied to the paper to ionize the analytes. The method has a limit of detection of 50 ng/mL, was linear (R^2 = 0.990), precise (CV < 9%), and accurate (QC error < 11%). A time vs. concentration plot of the data demonstrated an absorption and excretion profile consistent with the pharmacokinetics of acetaminophen in a healthy volunteer after a single oral dose. This proof of concept study demonstrates that this blood collection device and analysis technique allow for an improved workflow of blood collection and analysis for personalized medicine and clinical studies.

B-401

Introduction of Anti-Fungal Triazoles on an Agilent High-Performance Liquid Chromatography System in the Clinical Biochemistry Laboratory

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Background:

Triazole antifungals (Voriconazole, Posaconazole, Itraconazole) are synthetic antifungal drugs that are widely used for the management of fungal infections. As a result of their broad spectrum antifungal activity and fewer side effects, these benefits of Triazoles antifungals make them appropriate to the prevention and therapy of systemic fungal infections. However, Triazole antifungals demonstrate marked variability in patient plasma drug concentrations as a result of wide inter-individuality variability in absorption, metabolism, elimination, or interaction with concomitant medication. Therapeutic drug monitoring of these antifungals is therefore beneficial for optimizing clinical efficacy and reducing drug toxicity in patients receiving antifungal prophylaxis or therapy. A commercial assay kit for Itraconazole, Posaconazole and Voriconazole in Serum/Plasma by Chromsystems™ using HPLC methodology has been adapted for evaluation in our laboratory on the Agilent 1260 Infinity HPLC system.

Methods:

The Chromsystems Triazoles assay kit consisted of commercial mobile phases, precipitation reagents, internal standard, HPLC column, lyohilized calibrators and quality controls. The assay is able to quantitate Voriconazole, Posaconazole, Itraconazole and its Hydroxy-Itraconazole metabolite. Performance validation parameters of the assay included assay imprecision (within and total), linearity, recovery, limits of detection (LOD) and quantification (LOQ), and carry-over. Sample preparation was performed according to manufacturer's instructions using a 2-step protein precipitation method with kit precipitation reagents, vortex-mixed, followed by centrifugation and analysis of the supernatant on the Agilent 1260 Infinity HPLC system. Imprecision was assessed using manufacturer's quality control materials measured in 6 replicates over 5 days. Linearity, recovery and carry-over studies were performed using appropriately spiked Cerilliant Antifungal Certified Spiking Solutions® into drug-free patient serum matrix. Limits of detection and quantification were assessed by determining HPLC signals from drug-free patient serum matrix and serial dilutions of a low concentration Triazole sample respectively.

Results:

Within run and total imprecision for all 4 Triazole analytes were determined to be ≤6.0% on the Agilent HPLC. The assay demonstrated linearity across the analytical measurement range up to 20 mg/L for Voriconazole and up to 10 mg/L for Posaconazole, Itraconazole and Hydroxy-Itraconazole. Analytical recoveries obtained for all Triazoles ranged between 89-116%. The LOD was assessed to 0.2 mg/L for Voriconazole and ≤ 0.06 mg/L for Posaconazole, Itraconazole and Hydroxy-Itraconazole. LOQ was assessed to be ≤ 0.5 mg/L for all Triazoles. Results of carry-over studies were insignificant. Preliminary participation in a trial run in the United Kingdom National Quality External Quality Assessment program (UK NEQAS) for Anti-fungals showed good agreement with other HPLC peers.

Conclusions:

Overall, the Chromsystems Triazoles kit performed in agreement with the manufacturer's specifications except for LOQs which were determined at a higher concentration on our HPLC system. The kit method is easily adaptable to contemporary HPLC systems and benefits from the advantages of simple preparation procedures, simple isocratic elution, commercially prepared mobile phases and readily available calibrator standards, quality control materials and internal standard. The commercial kit enables workflow efficiency and ease of staff training in our laboratory and has been validated to be a suitable choice for high volume and busy clinical laboratories.

B-402

Targeting Drug Transport: Using Vitamins to Inhibit Bcrp-Mediated Transport of Methotrexate into Milk

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Background: : The health benefits of breastfeeding are well known for both infant and mother. During lactation, the Breast Cancer Resistance Protein (Bcrp) is highly induced in the mammary epithelium and is known to actively transport various toxins, drugs, and nutrients into milk. Use of medication in the postpartum period has been reported to be as high as 80%, causing concern for infant exposure of drugs through breast milk. The potential risk of infant drug exposure via breast milk causes women to discontinue breastfeeding prematurely or decide not to initiate breastfeeding altogether. Methotrexate (MTX) is an anchor drug used in various inflammatory disorders; however, it is considered to be contraindicated during lactation, largely due to the lack of safety information. The objective of this study was to develop a strategy to improve the safety profile of MTX during lactation by investigating if the administering of vitamins and isoflavonoids, which are known to interact with Bcrp, can competitively inhibit MTX efflux into milk, resulting in a lower level of drug in milk.

Methods: To determine the influence of co-administering a nutritional Bcrp substrate on MTX efflux into milk, FVB mice (lactation day 8-12) were administered either 20

mg/kg riboflavin (vitamin B_2), 100 mg/kg flavin mononucleotide (FMN), 20 mg/kg genistein (soy isoflavonoid) or 20 mg/kg of the synthetic Bcrp inhibitor, ko143, 30 minutes prior to administering 0.75 mg/kg MTX. Milk and plasma were collected 60 minutes post-MTX dose and analyzed by LC-MS/MS and the Abbott ARCHITECT, respectively.

Results: Genistein administration decreased milk MTX concentrations and increased MTX plasma levels, but not significantly. Administration of the synthetic Bcrp inhibitor, ko143, significantly decreased MTX concentrations in milk; however, it also significantly increased plasma MTX levels, indicating that ko143 alters drug elimination pathways non-selectively throughout the body. Conversely, a significant decrease in MTX milk concentrations was seen after injecting mice with riboflavin or FMN, without increasing plasma MTX concentrations; suggesting that mammary BCRP transporter were primarily inhibited while systemic BCRP transporters where not inhibited.

Conclusion: It is known that Bcrp is a major transport mechanism involved in the excretion of MTX into milk and in clearing the drug from the body through renal and hepatic mechanisms. Our data shows that MTX efflux into milk can be modulated using riboflavin or FMN, without increasing MTX plasma concentration. This is important as this data suggests co-administering high-dose vitamins can reduce milk drug levels without reducing systemic clearance so that the drug can be cleared from the body. This data may have clinical implications and could help reduce MTX exposure to the infant via breast milk. The data obtained from this study may help improve the safety profiles of drugs with Bcrp-mediated excretion into milk.

B-403

Performance of the Thermo Scientific QMS everolimus assay based on transplant type, metabolite differences and assay platform following everolimus immunosuppression.

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Background: Everolimus has a narrow therapeutic window and significant intra-individual variability, which requires a rapid turn-around-time and in-house measurement for dosing titration and adjustments. The Thermo Scientific Quantitiative Microsphere System (QMS®) everolimus immunoassay is the only FDA-approved immunoassay for everolimus measurement and is a homogeneous, particle-enhanced turbidimetric immunoassay. **Objective:** The analytical performance of the Thermo Scientific QMS® everolimus immunoassay on Indiko Plus was evaluated and compared to an LC-MS/MS method and QMS® immunoassay on AU680 based on transplant type and everolimus metabolites**.** We determined if significant metabolite cross-reactivity differences occur between LC-MS/MS and QMS® immunoassays. **Methods**: Whole blood EDTA-anticoagulated patient samples were pretreated with methanol and precipitation reagent (immunoassay) or zinc sulfate (LC-MS/MS) to remove proteins before analysis. An LC-MS/MS method for measuring everolimus and major metabolites (39-O-des-methyl, 16-O-des-methyl, 24-hydroxy, 25-hydroxy, 46-hydroxy and 11-hydroxy) was used and the transition *m/z* 980.9→389.1 was monitored for everolimus, with *m/z* 980.9→409.5 as qualifier. Total imprecision of the QMS® everolimus assay was determined by analyzing patient pools and commercial controls in duplicate with everolimus concentrations ranging from 4.6 - 16.9 ng/mL. Linearity was determined using diluted liver and kidney transplant patient pools of known everolimus concentrations (determined by LC-MS/MS). Functional sensitivity was determined by analyzing patient samples (duplicate analysis, 5 days) with everolimus concentrations ranging from 1.5-4.5 ng/mL. Thirty-four transplant samples (20 liver, 14 kidney) were used for the initial method comparison. Passing-Bablock regression, Spearman correlation coefficient, and Bland-Altman plot analysis were performed using EP Evaluator® software. **Validation and Results:** Total imprecision (%CV) for QMS® everolimus assay was 3.1% (mean=4.2 ng/mL), 3.3% (mean=9.3 ng/mL) and 2.8% (mean=14.3 ng/mL) for patient pools from liver samples. The kidney transplant samples showed a similar imprecision of 2.3% (mean=4.8 ng/mL), 3.2% (mean=8.5 ng/mL) and 5.5% (mean 14.7 ng/mL). Assay using patient pools from liver or kidney samples was linear from 2-15 ng/mL with equivalent slopes (1.000 and 1.001 respectively) and functional sensitivity was 1.5 ng/mL at <15%CV. Patient comparison studies revealed the following relationships: QMS® on the Indiko Plus=1.130(LC-MS/MS)-0.02 (r=0.91, average bias=0.51ng/mL); QMS® on the AU680=0.897(LC-MS/MS)-1.36 (r=0.90, average bias=(-)0.34 ng/mL. The methods were also compared in liver and kidney transplants with similar outcomes. Although the immunoassay methods compared favorably with LC-MS/MS, accuracy studies showed the QMS® immunoassay underestimated kidney transplant samples >10% and overestimated liver transplant samples >10% at low therapeutic values of 3.0 ng/mL. Evaluation of everolimus metabolites (39-O-des-methyl, 16-O-des-methyl, 24-hydroxy, 25-hydroxy, 46-hydroxy and 11-hydroxy) measured by LC-MS/MS found that the distribution of the everolimus metabolites was similar between liver and kidney transplant samples. However, the ratio of [pg] metabolite / [ng] everolimus was higher in kidney transplant patients and varied by metabolite (3-42%), especially for 24-hydroxy metabolite. **Conclusions:** Thermo Scientific QMS® everolimus immunoassay produces everolimus concentrations in patient samples that are comparable to LC-MS/MS values except at low therapeutic values. Accuracy studies revealed differences between liver and kidney transplant samples and may be due to differences in the concentrations of everolimus metabolites in liver versus kidney transplant samples.

B-404

miR-26a and miR-15b expression profiles as a potential early biomarker for clopidogrel-induced hepatotoxicity

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Background: Antiplatelet therapy, especially clopidogrel, is essential for prevention of thrombosis and atherosclerosis. Among clopidogrel adverse effects, the hepatotoxicity is a potential adverse effect related to liver damage. In a scenario of lack of early diagnostic for clopidogrel-induced hepatoxicity, new exosomes-derived miRNAs may be useful for improve the safety of this antiplatelet. Therefore, the miR-26a-5p, miR-145-5p, miR-15b-5p and miR-4701-3p in exosomes were evaluated *in vitro* aiming use then as potential biomarkers of clopidogrel-induced hepatotoxicity. **Methods:** HepG2 cells were cultured in RPMI containing 5% Exosome-Depleted Fetal Bovine Serum and supplemented with penicillin (10.000 UI/mL), streptomycin (10.000 UI/mL), and sodium bicarbonate (44 mmols/L) at 37°C in 5% CO_2 air. Clopidogrel treatments were performed during 24 and 48 h using the concentrations of 6.25, 12.5, 25, 50, and 100 µM. The cytotoxicity was evaluated by flow cytometry to analyze DNA fragmentation and cell cycle. Relative expression of exosomes-derived miRNAs was evaluated by RT-qPCR. **Results:** Our results revealed that in both periods of treatment the concentrations of 6.25 e 12.5 µM had similar profile that those observed in the control. In relation to the concentrations 25, 50, and 100 µM, it was observed a dose-dependent increase in DNA fragmentation. The miR-26a-5p was upregulated in concentration of 100 µM of clopidogrel and miR-15b-5p were downregulated in this toxic concentration. **Conclusion:** The increased DNA fragmentation in high clopidogrel concentration associated with miR-26a-5p upregulation and miR-15b downregulation are suggestive that these miRNAs profiles may be useful as an innovative diagnostic tool for early detection of clopidogrel-induced hepatotoxicity.

B-405

Pilot Study for determination of Infliximab Levels in Inflammatory Bowel Disease Patients

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Objectives: Therapeutics for Inflammatory bowel disease (IBD) have recently switched to the use of monoclonal antibodies against TNF-α (anti-TNF-α) to modulate inflammation and mucosal healing. One such drug, a chimeric IgG1 antibody (Infliximab) targets circulating and cell surface TNF-α. These agents have improved management of IBD patients especially those who did not respond to conventional IBD therapies. However, approximately a third of IBD patients lose response to anti-TNF-α drug therapy over time due to development of immunogenicity (produce anti-drug neutralizing antibodies), which accelerate systemic drug clearance leading to treatment failures. Generation of antibodies against anti-TNF-α agent is drug-specific. Measurements of anti-TNF-α drug trough and anti-drug antibodies levels have been utilized to evaluate clinical efficacy, safety and cost of anti-TNF-α therapeutics. Furthermore, there is a correlation between anti-TNF-α drug levels and their clinical efficacy. As these drugs have a narrow therapeutic window, knowledge of circulating trough levels can help clinicians in dose adjustments and/or switching to an alternative anti-TNF- agent in case of high clearance or lose of response. Thus, drug monitoring can help in treatment optimization which has been associated with improved clinical

outcome. In this pilot study, we evaluated a commercially available ELISA assay to measure therapeutic drug levels of Infliximab and anti-infliximab antibody levels and compared that with an established in-house assay in IBD patients undergoing treatment.

Design and Methods: Nine patients with IBD and on Infliximab were recruited for this study after obtaining ethics approval from the Hamilton Integrated Research Ethics Board (HIREB). Blood samples were collected in serum tubes just before infusion of their next Infliximab dose (trough levels) and 15 minutes after drug infusion (peak levels). Blood was left to clot at room temperature before centrifuging at 1500 x g. Serum was collected and stored at -20°C until use. Infliximab and anti-infliximab antibody levels were measured in duplicate using the Theradiag Duo Infliximab III ELISA kit.

Results: Five males and 4 females with age ranging from 21-56 years old were included in this study. The intra-run CV between duplicates ranged from 1.7-6.1% (maximum for assay = 8.8%) for both calibration standards and patient samples. The calibration curve was curvilinear as described by the manufacturer along the assay range of 0-16 µg/ml of Infliximab. Trough drug levels for patient samples ranged from 1.6-13.9 µg/ml while all peak samples were above the upper assay range of 16 µg/ml. We compared our patient sample data (trough levels only) with those of an in-house well established ELISA assay in Leuven, Belgium and found a good agreement between the two methods. The anti-infliximab antibody levels in patient samples were below the limit of detection of both assays.

Conclusions: This pilot study demonstrates that the Theradiag Duo Infliximab assay has a good precision and linearity within the assay measurement range. There was a good agreement between this method and an established in-house ELISA method in Leuven, Belgium. We are currently recruiting patients for a complete method validation study with a view to commence this test in clinical diagnostic laboratory.

B-406

Stimulant Drug Analysis by Chiral Liquid Chromatography Tandem Mass Spectrometry

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Background: Amphetamine-type stimulants have often been abused as recreational drugs or doping agents in sports. However, the less active enantiomers are often used in over-the-counter medication, such as nasal decongestants. Traditional screening methods such as immunoassays used in clinical diagnostics can exhibit interference from cross reactivity with drugs such phentermine and pseudoephedrine, resulting in false positives that can have legal and medical consequences for patients. This paper presents a chiral chromatography-tandem mass spectrometry method for quantification of methylphenidate, phentermine, phenylpropanolamine, and pseudoephedrine and chiral separation and quantification of D-amphetamine, L-amphetamine, D-methamphetamine, and L-methamphetamine in urine matrix. The method is used to both identify possible sources of unexpected positive screens due to over-the-counter medication and differentiate chiral enantiomers in order to eliminate potential errors during the analysis of patient samples.

Methods: Analytical Standards for methylphenidate, phentermine, phenylpropanolamine, pseudoephedrine, D-amphetamine, L-amphetamine, D-methamphetamine, and L-methamphetamine as well as deuterated internal standards were purchased from Cerriliant. Our method was automated using a Hamilton Microlab STAR liquid handling system. DPX CO-RE tips containing reverse phase (1 mg) and weak-anion exchange (2 mg) resins were used for sample preparation. Drug separation is performed using Shimadzu 20A Prominence LC. Phenomenex Lux AMP column (150mm x 3.0 mm, 3 µm) is used to achieve well-defined separation. Mobile phase A consist of 5mM ammonium bicarbonate, adjusted to pH 11 with ammonium hydroxide and mobile phase B consist of methanol. Sample are analyzed on ABSciex API 4000 and results are generated using Analyst 1.6.2 software.

Results: The precision for all stimulant drugs and enantiomers were less 15% CV. Accuracy for all drugs and enantiomers were less than 15% bias. The linear quantifiable range is 40 ng/mL to 2000 ng/mL with correlation coefficient of greater than 0.995.

Conclusion: The method was successful in quantification of individual peaks for the (D)- and (L)- enantiomers of amphetamines and methamphetamines, methylphenidate, phentermine, phenylpropanolamine, and pseudoephedrine with performance that was satisfactory in terms of linearity, precision, accuracy and limits of detection.

B-407

Evaluation of the Thermo Scientific CEDIA Cyclosporine PLUS assay on the Abbott Architect c8000 analyzer

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Background: Cyclosporine is an immunosuppressive drug commonly used post-transplantation. Both immunoassay and chromatographic methods are used for quantitation of cyclosporine. One advantage of immunoassays is the greater availability of assays on automated chemistry analyzers and the potential for 24/7 testing service. Another advantage compared to chromatographic analysis, typically requiring manual sample extraction, is the faster turnaround time. A third advantage is that testing can be set up and/or consolidated onto a main chemistry platforms rather than maintaining a separate instrument. The Thermo Scientific CEDIA cyclosporine PLUS assay is an enzyme immunoassay utilizing a mouse monoclonal antibody against cyclosporine. The objective of this study was to evaluate the Thermo Scientific CEDIA cyclosporine PLUS assay on the Abbott Architect c8000.

Methods: Studies included evaluating linearity, intra- and inter-assay imprecision, and accuracy with respect to a currently in-use method. The comparison method was a whole blood chemiluminescent immunoassay on the Siemens Dimension EXL analyzer.

Results: The assay was linear across the measurement range of 25-450 ng/mL (slope = 0.989, correlation coefficient (r) = 0.998). Within run imprecision was 5.4% at 48 ng/mL (n=10), 3.3% at 108 ng/mL (n=20), and 1.4% at 429 ng/mL (n=10). Day-to-day imprecision was 16.6% at 60 ng/mL, 3.6% at 187 ng/mL and 3.4% at 350 ng/mL (n=22 for each). Samples ranging from 43 to 420 ng/mL (n = 45) were analyzed over at least 5 days and indicated overall acceptable agreement between the Thermo Scientific CEDIA and Siemens Dimension EXL methods ($y = 1.05x – 8.99$, $r = 0.988$). The average bias between methods was -2.94%, but the method-to-method difference for individual samples was as high as 31%.

Conclusion: The Thermo Scientific CEDIA Cyclosporine PLUS assay on the Abbott Architect c8000 shows acceptable average bias compared to the Siemens Dimension EXL analyzer, but unacceptably high (>16%) day-to-day imprecision at a low concentration.

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B-408

Use of Urine Drug Screening Positivity Rates from Qualitative Liquid Chromatography Tandem Mass Spectrometry Definitive Testing to Identify Annual Trends in Drug Use

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Background: Comprehensive and specific information regarding trends in the prevalence of licit and illicit drug use within a specific region is often not widely available or current. Urine drug screening positivity rates for patients within a specific geographical area has recently been used to obtain this information. However, comprehensive studies of urine drug screening positivity rates for patients in Ontario, Canada have not yet been published. **Objective:** Identify multi-year trends in drug use by examining qualitative liquid chromatography tandem mass spectrometry (LC-MS/MS) based urine drug screening positivity rates. **Methods:** All LC-MS/MS urine drug screening results from 2014 (*N*=136,864), 2015 (*N*=153,329) and 2016 (*N*=106,687) were retrospectively reviewed. Following enzymatic hydrolysis and protein precipitation, all urine specimens received targeted LC-MS/MS screening which identified the presence of drugs within the following drug classes: anesthetic (ketamine); anticonvulsant (gabapentin); antidepressant (bupropion, trazodone); benzodiazepine (alprazolam, clonazepam, diazepam, flunitrazepam, flurazepem, lorazepam, nitrazepam, oxazepam, phenazepam, temazepam, triazolam); cannabinoid; opioid (buprenorphine, codeine, fentanyl, hydrocodone, hydromorphone, morphine, naltrexone, meperidine, methadone, oxycodone); stimulant (amphetamine, methylphenidate); and illicit (benzylpiperazine, cocaine, heroin, mephedrone, methamphetamine, MDPV, MDEA, MDMA). Relevant drug metabolites and related compounds (naloxone, levamisole) were also included in this test. A total of 63 different compounds were screened using their respective positive/negative cut-off concentrations. The positivity rates for all analytes were tabulated and partitioned by month of testing. **Results:** Over the examined three year testing period, urine drug screening positivity rates ranged from 76.6% (cotinine) to <0.01% (diazepam, flunitrazepam, 7-aminoflunitrazepam, flurazepam, desalkylflurazepam, phenazepam, JWH018, JWH200, MDEA, MDPV and mephedrone). From 2014 to 2016 significant

(p≤0.05) increases in urine drug screening positivity rates were observed for: gabapentin (5.4% to 6.8%); alprazolam (0.3% to 0.5%); α-hydroxyalprazolam (0.5% to 0.7%); buprenorphine (7.1% to 8.7%); norbuprenorphine (8.5% to 10.0%); 6-acetylmorphine (0.6% to 1.1%); naloxone (7.0% to 8.5%); naltrexone (0.1% to 0.3%); amphetamine (3.4 to 5.8%); methamphetamine (2.9% to 4.8%); cocaine (2.8% to 3.5%); and benzoylecgonine (7.6% to 11.5%). From 2014 to 2016 significant (p≤0.05) decreases in urine drug screening positivity rates were observed for: bupropion (2.6% to 2.4%); 7-aminonitrazepam (8.5% to 7.0%); oxazepam (6.6% to 5.8%); temazepam (5.0% to 4.6%); methadone (57.4% to 38.1%); EDDP (47.0% to 38.8%); and benzylpiperazine (0.05% to 0.02%). All other analytes included in the LC-MS/MS screening panel did not show significant annual differences within the tested patient population. THCA positivity rate was 29.6%, 28.9% and 29.5% in 2014, 2015 and 2016, respectively. **Conclusion**: From 2014 to 2016, significant annual trends of increasing (gabapentin; heroin; naltrexone; methamphetamine; cocaine; alprazolam; buprenorphine) and decreasing (methadone; temazepam; oxazepam; nitrazepam; and bupropion) drug use were identified through the review of urine drug screening positivity rates of community-based patients in Ontario, Canada. The relative changes in drug use within the examined cohort may be reflective of the relative decrease of methadone patient testing and related positivity rates. Tabulating and communicating urine drug screening positivity rates from qualitative definitive LC-MS/MS testing would serve to educate physicians about drug use within their communities.

B-409

Drug Confirmation -- Method Consolidation Reduces Cost-to-Payer, Instrument Run Time, Labor Costs, and Result Turnaround Times

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OBJECTIVE: Drug class-specific LC-MS/MS methods are routinely performed to confirm urine drug immunoassay (IA) results. However, more than 50% of specimens are positive for more than one drug class requiring the sample to be run by multiple methods. Hospital laboratories often have limited resources and are behooved to simplify test workflows to minimize cost and turnaround time while maximizing sensitivity and specificity. We tested 15,197 specimens by a single-run LC-MS/MS method to quantitate 49 analytes known to trigger positive results in our 9 drugs of abuse IA drug screen and evaluated this workflow against conventional class-specific methods to determine differences in cost-to-payer, within-lab turnaround time, instrument run time, and technologist hands-on time.

METHODS: The single injection, consolidated workflow is based on an enzyme-hydrolysis, isotope-dilution LC-MS/MS method that employs positive/negative switching to quantitate 49 analytes that are known to trigger positive results in our 9 drugs of abuse IA drug screens (amphetamines, barbiturates, benzodiazepines, buprenorphine, cocaine, opiates, oxycodone, methadone, and cannabinoids). We tested 15,197 specimens that were submitted to our laboratory from 22 hospital emergency departments that were positive for one or more of the IAs. The frequency of drug classes found positive per specimen, as well as the distribution of positive drug classes, were used to calculate costs, tech time, in-lab turnaround time, and instrument run time of the consolidated method. These same distributions were then used to determine estimated values for conventional workflows utilizing class-specific methods (i.e., amphetamines, barbiturates, benzodiazepines, buprenorphine, cocaine, opiates/oxycodone, methadone, and THC).

VALIDATION: The frequencies of positive IAs by drug class are opiates/oxycodone, 30%; benzodiazepines, 21%; amphetamines, 19%; cannabinoids, 16%; buprenorphine,7%; methadone, 3%; cocaine, 3%; and barbiturates, 2%. The frequencies of specimens that were positive for drug classes by IA are: one class, 49%; two classes, 30%; three classes, 14%; four classes 6%; and >4 classes, 1%. Based on these frequencies, known costs, and the requirements of each analytical workflow, we determined that a consolidated method reduces cost-to-payer by 1.8 fold. The consolidated method was largely favored over the class-specific methods for average sample prep time (5.9 versus 9.4 minutes/ sample), instrument run time (includes calibrators and quality controls) (13.5 versus 18.5 minutes/sample), and total within-lab turnaround time (33.3 versus 22.4 minutes/sample), respectively. It is projected that cumulative hands-on technologist time was reduced by 1400 hours over a period of a year by employing the consolidated testing method.

DISCUSSION: LC-MS/MS enables laboratories to quantitate many drugs/metabolites simultaneously in both positive and negative ion modes over short run times. As labor and instrumentation are two of the largest costs of sample testing, a consolidated testing approach has a marked impact on these variables. However, we observed some challenges with the consolidated workflow including additional complexity of preparing calibration materials, internal standards, and controls; added complexity of data analysis of many analytes; QC or calibration failures for single analytes in a panel; and the large amount of data to move to the LIS. Solutions were implementer to address these added complexities and the benefits outweigh the challenges.

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A Comparison of Methods for Measurement of Plasma Methotrexate in a Pediatric Population.

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Introduction Methotrexate (MTX) is an antineoplastic drug that prevents DNA synthesis via the competitive inhibition of the enzyme, dihydrofolate reductase. MTX is widely used to treat malignancies, such as osteosarcoma, acute lymphoblastic leukemia, and lymphoma. Following the administration of MTX, plasma levels are typically measured at 24, 48, and 72 hours post-dose, due to the inherent risk of nephrotoxicity. Traditional "random-access" methods for plasma MTX measurement include florescent polarization immunoassay (FPIA), and more recently, homogeneous immunoassay. Liquid-chromatography tandem mass spectrometry (LC-MS/MS) methods are also available, due to their utility for measurement of MTX in the context of carboxypeptidase treatment in cases of toxicity. In this study, we compared the performance of three methods for MTX: The Abbott TDx FPIA, the ARK™ homogeneous immunoassay, and an LC-MS/MS method. Performance characteristics were assessed in a subset of plasma specimens from a pediatric population. **Method** Assay imprecision on each platform was assessed by replicate measurement (n = 5) of MTX in patient plasma pools across the concentration range, 0 to 0.10 µmol/L. The lower limit of quantification (LLOQ) of each assay, determined by means of functional sensitivity, was assessed by replicate analysis (n = 5) of plasma pools created from pediatric patients (n = 5) across the concentration range 0.04 to 0.15 µmol/L. A direct comparison of the three methods was performed by measurement of patient specimens (n = 30) across the concentration range, 0.04 to 19.0 µmol/L. Comparison between patient samples using the three methods was assessed by Deming-regression analysis. **Results** Assay imprecision (%CV) assessed at MTX concentrations of 0.04, 0.08 and 0.10 µmol/L. The mean coefficient of variation (%CV) using the TDx and LC-MS/MS methods at 0.04, 0.08 and 0.11 µmol/L were 11.0%, 6.7% and 3.6% respectively. Similar data using the ARK™ assay produced %CV values of 13.6%, 4.4% and 3.1% at 0.06, 0.10 and 0.14 µmol/L respectively. Deming-regression across the concentration range, 0.0 to 1.0 µmol/L showed excellent correlation between the TDx and LC-MS/MS methods (r = 0.997). Values for slope ranged from 1.1844 to 1.2336. For ARK™ versus LC-MS/MS (r = 0.993), values were obtained for slope (range: 0.9923 - 1.009) and intercept (range: 0.022 - 0.039). The data suggested that the ARK™ method exhibits a slight positive bias compared with the other two methods. **Conclusions**: Our data suggests that there is a slight positive proportional bias with the ARK™ method relative to the other two methods. In conclusion, all three MTX methods are reproducible at concentrations used for medical decisions in plasma specimens obtained from pediatric patients.