Wednesday, July 30, 2014

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

TDM/Toxicology/DAU

B-389

Point of Care PK Quantitation Device for Pharmacokinetic Guided Dosing of Paclitaxel as a Companion Diagnostic Device

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Background: Paclitaxel chemotherapy is the cornerstone of most anti-cancer regimens due to its potent cytoxic activity against tumor cells. The variability of paclitaxel dosing can be as high as 10X across patients, therefore, dosing at a fixed dose even when adjusted for body weight will leave a significant portion of the patients underdosed - getting no benefit from the treatment- and another portion overdosed- getting undue toxicity. Therefore, paclitaxel therapy would benefit from TDM guided dosing. A full pharmacokinetic (PK) study is required to determine whether patients are getting the appropriate and efficient dosage. However, at the current state, a full pharmacokinetic requires not only multiple high-volume blood draws and extended stays in the hospital but also testing method by LC/MS/MS, which are both time-consuming and expensive. Here we describe the development of Point of Care TDM (Therapeutic Drug Monitoring) device for PK guided dosing of paclitaxel as a companion diagnostic device.

Methods: The 8A10 and 3C6 mAbs (mAbs against paclitaxel) were purified from the antibody-rich harvested medium using MabSelect (GE Healthcare, Pittsburgh, PA). Commercial antibodies (29B7B3C and 69E4A8E; Santa Cruz Biotechnology Inc.) were also tested. To synthesize BSA-paclitaxel, we used the method of J-G Leu et al. as described in Cancer Res. (1993) 53:1388-1391. BSA-paclitaxel and mAbs were labeled with colloidal gold using the gold labeling kit from BioAssay Works, LLC, Ijamsville, MD.

Results: Rapid test for paclitaxel based on the lateral flow system was developed. Of the mAbs tested, only 8A10 and 3C6 were useful. The assay requires the unique configuration of immobilizing the mAb against paclitaxel onto the membrane followed by flowing the BSA-paclitaxel-colloidal gold through in presence of test analyte. This configuration seems more effective than the traditional configuration where BSA-paclitaxel is immobilized on the membrane and colloidal-gold labeled anti-paclitaxel mAb as flow through. This resulted in a competitive assay for paclitaxel where the signal decreased as the concentration of paclitaxel analyte in blood increased. Coupled with the current lateral reader technology - especially the one developed by Qiagen- a rapid quantitative assay for paclitaxel is possible. The assay demonstrated good linearity and range suitable for paclitaxel TDM. The application of this assay in a preclinical pharmacokinetic study yield reasonably comparable result to LC/MS method.

Conclusions: A quantitative lateral flow platform coupled to a reader was developed to easily detect the paclitaxel concentrations in small amount of blood samples. Individual pharmacokinetic profiles can be obtained and used to determine the suitable treatments. In addition, the lateral flow PK quantitative assay can be deployed at point-of care (in home, doctor's office or central lab).

B-390

Comparison of Roche Methadone Screening Assay with DRI Methadone Metabolite (EDDP) Screening Assay

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Geisinger Medical Laboratories offer immunoassay based screening. Methadone, one of the drug classes we test, is a synthetic opioid used for narcotic addiction and pain management. Urine drug testing is used to monitor medication compliance for these patients. We observed significant number of false positive methadone screens (Roche). In a five-month period, 88 urine samples were screened positive for methadone, but 27% were negative by GC-MS. Because of the high rate of false positive results, we evaluated an alternative screening assay "DRI Methadone Metabolite (EDDP)". The following results were obtained:

EDDP Screening (DRI)	Methadone Screening (Roche)	GC-MS Confirmation
POS (n=29)	POS (n=24)	POS (n=24)
		NEG (n=0)
	NEG (n=5)	POS (n=4, false NEG methadone)
		NEG (n=1, false POS EDDP)
NEG (n=25)	POS (n=10)	POS (n=0)
		NEG (n=10, false POS methadone)
	NEG (n=15)	POS (n=0)
		NEG (n=15)

The EDDP screening assay (DRI) was more accurate with no false negative and only 1 false positive out of 54 urine samples tested. We also noted that many false positive methadone screens were from patients who were taking tramadol and suspected that is the interfering substance causing the false positive results. We therefore replaced the Roche methadone screening assay with the DRI methadone metabolite (EDDP) screening assay.

B-391

Light-Sensitivity of Chlordiazepoxide

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Objective Due to conflicting information in the literature, this study was conducted to determine if Chlordiazepoxide (CDP) is light sensitive at a variety of sample storage conditions.

Relevance If CDP patient specimens are not protected from light for greater than 24 hours, clinical results could be inaccurate leading to erroneous clinical results. Furthermore, the light-sensitivity of CDP is not sufficiently addressed in the current literature.

Methodology This study utilized High Performance Liquid Chromatography with Ultra-Violet Visible Spectroscopy (HPLC/UV-Vis) to compare three identical serum samples at various concentrations throughout the analytical measurement range (0.5 µg/mL - 12.0 µg/mL). Briefly, the three samples were split into 4 aliquots and stored protected and unprotected from light at both ambient and refrigerated temperatures for analysis on days 1, 3, 7, 16, 21, and 28.

Validation After 28 days, the three aliquots stored protected from light yielded an average degradation of 20.7% at ambient storage and essentially negligible degradation at refrigerated storage while the three aliquots not protected from light yielded an average degradation of 93.5% at ambient storage and 22.8% at refrigerated storage.

Conclusions Some degradation of CDP found at ambient temperatures was determined to be due to the storage temperature; however, by comparing the light-protected samples vs. the non-light protected samples at the ambient storage or refrigerated storage temperatures, the data clearly shows significant degradation of CDP due to the exposure to direct light which could significantly affect patient results.

B-392

Modified HPLC-UV Method without Evaporation Step for the Determination of Serum Ribavarin Level in Patients with Hepatitis C

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Bcakground: The measurement of serum concentration of anti-viral drug ribavarin is indicated for some patients with hepatitis C, for therapeutic drug monitoring. A simple and fast high performance liquid chromatographic method for the determination of ribavarin in serum sample is developed and validated according to FDA method validation guidelines without the evaporation step. Method: Serum sample (500 μL), internal standard (50 μL), and 20 mM ammonium acetate buffer of pH =8.5 (500 μL) were mixed and allowed to centrifuge for 5 minutes. After centrifugation, the supernatant was transferred into phenyl boronic acid cartridges (previously conditioned with 1mL of 3% formic acid and then with 1mL of 20mM ammonium acetate buffer pH 8.5) for solid phase extraction followed by washing step with 20 mM ammonium acetate buffer (1 mL) under vacuum not exceeding 10 psi and discarded. ribavarin and internal standard were eluted with 3% formic acid (300 μL) and was injected (90µL) into HPLC system. Serum samples from 37 patients were run simitnutenosly on both methods and compared. Results: A linearity between 0.1 - 6.25 mg/L with 0.06mg/l as limit of detection was obtained. The correlation coefficient was 0.950 with p value of 0.92 showing a good reproducibility of results. The mean

accuracy was checked at three different concentrations and found to be 108% for each level. The mean recoveries (extraction efficiency) of ribavarin and internal standard from serum were found to be 65.5 % and 71.2% respectively at concentrations ranging from 0.1 to 6.22 mg/L for ribavarin and 50mg/L for internal standard. The intra assay precision were determined at 0.5, 1.2, & 2.2 mg/L and % CV were found to be 7.1%, 5.1% % 7.4% respectively whereas injection reproducibility were calculated 5.6%, 0.7% and 0.6% at three levels. Maximum 1hour and 30 minutes was consumed to complete this assay whereas it takes about 3 hours and 30 minutes if the step of evaporation and gravity elution was used in the older method. Conclusion: The newly developed HPLC method was faster, accurate and sensitive. It may be applied for the estimation of ribavarin level in serum samples.

B-393

A HPLC-High Resolution Accurate Mass Spectrometric Method for the Quantification of Doxorubicin and Doxorubicinol

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Background: While chemotherapeutic agents have been commonly used in the treatment in a majority of cancers, these drugs are typically associated with significant adverse effects. Although chemotherapeutic regimens reduce mortality, morbidity may be high due to significant drug-induced toxicities. The anthracycline anti-neoplastic agent doxorubicin is a common treatment modality for hematologic malignancies, as well as a variety of solid tumors. Doxorubicin, and its active metabolite, doxorubicinol, is associated with severe cardiotoxicity; therefore, it is important to generate tools to measure doxorubicin concentrations following intravenous administration to minimize drug-mediated toxicity. Both invasive (blood) and non-invasive (saliva) collection schemes have been pursued for other drugs, and this is largely due to ease of collection, particularly in pediatric populations. Here, we present a liquid chromatographic-high resolution mass spectrometric (LC-HRMS) method for the dual quantification of doxorubicin and its metabolite in both serum and saliva.

Methods: Standard solutions of doxorubicin and its active metabolite, doxorubicinol, as well isotopically-labeled internal standards for each, were prepared in water and spiked into drug-free human serum and saliva. Following protein precipitation, a 10 µl aliquot was injected onto our LC-HRMS system. Chromatographic separation was achieved using a Thermo Scientific PFP HPLC column (50 × 2.1 mm, 5 µm particle size) and eluted under a gradient elution containing acetonitrile with 0.1% formic acid. Both doxorubicin and doxorubicinol were detected over 5 minutes using a Q-Exactive hybrid quadrupole-orbitrap mass analyzer (Thermo Scientific). A HESI (heated electrospray ionization) source was used and the instrument was operated in full scan (*m/z* 250-750) mode with a resolution of 70,000 at m/z 200.

Results: The high resolution Q-Exactive mass analyzer was able to detect both doxorubicin (m/z 544.1813) and doxorubicinol (m/z 546.1970) in serum and saliva with a mass tolerance of 5 ppm from theoretical mass. Assay development and subsequent validation was performed following the recommendations of the FDA for bioanalytical method validation. The analytical measuring range of the assay for both doxorubicin and doxorubicinol was 5 to 1000 ng/ml. Calibrator solutions were evaluated at drug concentrations of 5 ng/ml, 15 ng/ml, 50 ng/ml, 75 ng/ml, 125 ng/ml, 250 ng/ml, 500 ng/ml and 1000 ng/ml. Linearity was assessed as the average slope of $1/x^2$ weighted linear regression analysis. Across five independent injections from serum-extracted specimens, the average slope for doxorubicin and doxorubicinol were 0.953 and 0.951, respectively. Quality control solutions were prepared in serum with theoretical concentrations of 30 ng/ml and 400 ng/ml for both drugs. Intra-assay precision across five injections were determined to be 2.9% and 2.5% for the parent and metabolite drug, respectively.

Conclusion: A LC-HRMS assay has been developed and validated for the simultaneous quantification of doxorubicin and its active metabolite in both serum and saliva specimen sources.

B-394

Rapid Enzyme Hydrolysis by a Novel Recombinant Beta-Glucuronidase in Benzodiazepine Urinalysis

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Background: Benzodiazepines are widely prescribed drugs that are readily abused for their sedative effects and as such are very frequently targeted in therapeutic drug monitoring. Only trace amounts of parent drug are present in urine due to extensive metabolism and conjugation of benzodiazepines and their glucuronides are major urinary species. Hydrolysis to cleave the glucuronides prior to analysis is necessary for improved detection. For benzodiazepine analysis, hydrolysis by enzyme is preferred over acid because the latter produces benzophenones, which convolute result interpretation. Enzyme hydrolysis can be costly and time-consuming, with reported incubation times ranging from 0.5 to 20 hours. The assessment and application of a novel recombinant beta-glucuronidase for rapid hydrolysis in benzodiazepine urinalysis is presented.

Methods:IMCSzyme™ recombinant beta-glucuronidase was buffered to recommended optimum pH and evaluated. Aliquots of drug-free urine fortified separately with glucuronides of oxazepam, lorazepam, temazepam at 2500 ng/mL were hydrolyzed with the enzyme in triplicate. The hydrolysis efficiency was assessed at 55◦C at incubation times of 0, 15, 30 and 60 mins and at room temperature at incubation times of 0, 5 and 10 mins. The optimized enzyme hydrolysis was applied to 20 randomly selected positive authentic urine samples for each analyte and compared to hydrolysis by commonly used beta-glucuronidase from abalone under its validated optimized conditions. Hydrolysis efficiency for alpha-hydroxyalprazolam glucuronide was evaluated solely with patient samples positive for that compound. Hydrolyzed urine samples were analyzed on a TLX-4 Multiplexed HPLC with Agilent 1200 Series Binary Pumps coupled to a Thermo Scientific™ TSQ Quantum Ultra™ Triple-Stage Quadrupole Mass Spectrometer using a previously validated method. Analytical column performance was monitored.

Results: The validated benzodiazepines liquid chromatography tandem mass spectrometry (LC/MS/MS) method had a linear range of 20-5000 ng/mL for oxazepam, lorazepam, temazepam and alpha-hydroxyalprazolam. At 0 mins (immediately run after addition), mean analyte recovery >92% from all hydrolyzed glucuronide controls was observed at both 55◦C and room temperature. Complete hydrolysis of the glucuronide controls (mean analyte recovery ≥ 100%) was observed at 15 mins (shortest incubation time tested) at 55◦C and at 10 mins at room temperature. This was considerably faster than the optimized incubation time of 30 mins for the abalone beta-glucuronidase. Hydrolysis at room temperature was also more convenient, eliminating heat activation. Mean analyte recovery changed by no more than -2% at longer incubation times at both temperatures. In patient samples, total oxazepam, lorazepam and temazepam compared well between the two enzyme sources. Recovery of alpha-hydroxyalprazolam was >30% higher using the recombinant beta-glucuronidase versus that from abalone, highlighting the differential hydrolysis of alphahydroxyalprazolam by the abalone enzyme under conditions optimal for the other analytes. The IMCSzyme™ recombinant beta-glucuronidase appeared to be a cleaner extract and the maximum number of analytical column injections seen with abalone-treated samples was exceeded with this recombinant source.

Conclusion: The superiority of the IMCSzyme™ recombinant beta-glucuronidase was demonstrated with fast benzodiazepine hydrolysis at room temperature. The use of this enzyme decreases processing time due to reduced incubation time, requires no heat activation and affords improved column life.

B-395

Comparison of paired immunosuppressant levels in venous and dried blood spot levels in post-transplant patients

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Background: Transplant patients are routinely and chronically monitored with laboratory testing to assess risk of rejection, infection, and to optimize therapeutic drug doses. Therapeutic drug monitoring of tacrolimus and sirolimus plays a significant role in the clinical follow-up of transplant patients receiving immunosuppressant (IMS) therapy. Success of transplant and favorable patient outcome relies on maintaining adequate therapeutic drug levels. Drug dosing must be individualized due to the narrow therapeutic range of these drugs, significant inter-individual variability, and intra-individual variability in absorption and metabolism. We developed, validated, and implemented a clinical liquid chromatography-mass spectrometry (LC-MS/MS) assay for simultaneous quantitation of tacrolimus and sirolimus in dried blood spots (DBS) collected remotely by patients and mailed into the laboratory. The purpose of this research is to assess the clinical utility of remote collection of dried blood spots for immunosuppressant monitoring, and compare the IMS level in paired collections of venous whole blood and dried blood spots.

Methods: The validation of the LC-MS/MS assay in dried blood spots was described previously, and the assay correlated well with our routine whole blood assay. To clinically correlate sirolimus and tacrolimus levels in capillary blood collected from a finger poke with venous whole blood, pediatric, post- transplant patients were asked to provide up to three paired collections of DBS and venous whole blood (total of 25 collections per drug). To be eligible, Seattle Children's patients needed to be

> 1 yr old, status post a heart, liver, or kidney transplant, and currently monitored for sirolimus or tacrolimus levels. Thirty-one patients consented and completed at least one paired collection. The phlebotomist only ordered the clinical whole blood immunosuppressant level, but collected both the venous and the blood spot. The participant took the dried blood spot card home with them with a pre-addressed, postage-paid envelope and mailed it back to the lab. The concentration and the turnaround times of the dried blood spot were compared with the whole blood sample. The recorded data include the unique study ID number, age of the patient, organ transplanted, immunosuppressant therapy, date and time of collection, whole blood level, date and time DBS was received, date and time DBS was analyzed, and DBS level.

Results: Tacrolimus in DBS correlated well with venous levels ($y = 1.03x + 0.84$, $R^2 = 0.93$, n=25). Overall, a small, but statistically significant negative bias was observed (-0.6 ng/mL, $p = 0.0013$). A chart review was performed to assess if clinical management would have changed, and none of the cases revealed a clinically significant change. Sirolimus in DBS also correlated with venous levels ($y = 0.83x + 1.17$, $R^2 = 0.84$, n=23). Overall, a small, negative bias was observed which was not statistically significant (-0.5 ng/mL, $p = 0.09$). Turnaround time from collection to receipt of DBS cards was on average 5.8 days, and ranged from 3 to 18 days.

Conclusions: In summary, analysis of IMS levels in DBS is possible, and the difference noted between capillary and venous blood is within the clinically acceptable limits.

B-396

A fast and sensitive high performance liquid chromatographic method for measuring 6 tricyclic antidepressants in human plasma

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Background: Monitoring certain tricyclic antidepressants (TCA) in blood is needed due to the large inter- and intra-individual variations. The objective of this work was to develop a high performance liquid chromatography (HPLC) method to measure 6 commonly prescribed TCA drugs, namely amitriptyline, imipramine, doxepin, nortriptyline, desipramine, and nordoxepin in plasma. **Methods:** Five hundreds µL of plasma samples and 50 µL internal standard solution (5 µg/mL of trimipramine and protriptyline in 0.1N HCl) were vortex mixed, followed by solid phase extraction using C18 cartridges. Chromatographic separation was achieved on a cyanopropyl bonded phase column (4.6x150 mm, 5 µm) with an isocratic elution of 22:18:60 mix of 10mM phosphate buffer (pH 7.0):methanol: acetonitrile at 1.8 mL/min. Detection was by UV at 214nm and quantification was based on peak height ratios using 5 level calibration. This method was compared with a separate HPLC-UV method using left-over patient specimens and spiked samples. **Results:** Baseline separation of all 8 compounds was achieved within 8.0 min. The obtained linearity ranges, mostly 20-500 ng/mL, fully encompassed the therapeutical ranges (within 50-300 ng/mL) of all the analytes. Precision was assessed at three different levels, and intra-assay and total CVs were <7.8%. No interference was found from lipemic, hemolytic, icteric and uremic plasma samples. Agreeable results were obtained in the method comparison study (Table). **Conclusion:** This newly developed HPLC-UV method offers fast and sensitive quantification of 6 common TCA drugs in human plasma.

Table 1. Method Comparison.

	n	Slope (95% CI)*	Intercept (ng/mL)*	Correlation Coefficient	Standard Error of Estimate (ng/mL)	Bias (%)
Amitriptyline	35	1.006 (0.968-1.044)	-5.3 (-13.1-2.4)	0.9943	14.1	5.5
Imipramine	32	0.939 (0.885-0.993)	2.1 (-7.9-12.1)	0.9880	15.7	4.9
Doxepin	22	1.046 (0.995-1.097)	-5.7 (-153.7-4.3)	0.9945	11.7	-0.4
Nortriptyline	33	1.032 (0.973-1.091)	-7.8 (-19.5-3.8)	0.9878	20.2	3.2
Desipramine	32	0.963 (0.930-0.989)	1.1 (-4.0-6.2)	0.9967	8.2	3.3
Nordoxepin	23	0.967 (0.943-0.990)	0.9 (-3.6-5.5)	0.9986	5.7	1.5

*Numbers in parenthesis indicate 95% confidence intervals.

B-397

Quantitation of (-)-Δ9-Tetrahydrocannabinol(THC) in Dried Blood Spots Using LC-MS/MS

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Background: Tetrahydrocannabinol (THC) is the major psychoactive ingredient in the cannabis plant. It has been shown that THC has complex effects on the central nervous system, correlated with the development of anxiety and psychotic disorders. After smoke inhaling of cannabis, THC is absorbed and incorporated into the bloodstream. THC is detectable for many hours after consumption and is a good indicator of recent cannabis consumption. LC-MS/MS is considered a useful tool for assessing the THC level in blood. The aim of the present study was to develop and validate a much simplifier and efficient high-throughput LC-MS/MS approach for the rapid quantification of THC in dry blood spot (DBS) samples.

Methods: The DBS was created by spotting 25uL spiked whole blood onto blank Whatman 903 paper and allowing a full 24 hours to dry at room temperature. A 3 mm punch was placed into a 1.5 mL vial with 100 uL exaction solution (acetonitrile with 10 mM ammonium acetate). The vial was vortexed for 10 minutes and soaked for 60 minutes. Then centrifuged and the supernatant transferred to an LC for injection. No SPE or evaporation step was required. A Shimadzu UFLC XR system was used. Sample was loaded onto Chromolith-RP18E column (100X3 mm, 3 um) held at 40^{0}C. A gradient LC method was created at a flow rate of 600 ul/min and a total LC run time of 4 minutes. Mobile phase A is 0.1% formic acid in 100% H2O and B is 0.1% formic acid in 100% ACN. An IONICS 3Q 220 mass spectrometer system was used which is equipped with heated coaxial flow ion source and a "Hot Source-Induced Desolvation" (HSID™) interface was used.

Results: The Calibration curve of the neat THC solution showed a good linearity over a range of 0.05-100 ng/mL with correlation value of R^2=0.994. At LLOQ of 0.05 ng/mL, the accuracy is 96% and CV is less than 10%. For DBS sample, no matrix interference was observed. Six calibration curves were generated with single injection over the range of 1-100 ng/mL. Good linearity with 1/x weighting was obtained for each curve with correlation value R^2 >0.99. The average accuracies for the six sets of THC spiked DBS samples at 1 ng/mL, 10 ng/mL, and 100 ng/mL are 100.9%, 99%, and 99.4% respectively. And the CVs at 1 ng/mL, 10 ng/mL, and 100 ng/mL are 4.2%, 4.7% and 4%, respectively.

Conclusion: A fast, accurate, and precise LC-MS/MS method with IONICS 3Q 220 mass spectrometer was developed for direct measurement of THC in DBS. Significant time can be saved in the absence of SPE or LLE sample preparation. In the neat standard THC solution, an LLOQ of 0.05 ng/mL was achieved with accuracy of 96% and CV of 3.6%. Six sets of single injection calibration curves for DBS extraction showed good linearity over a range of 1 to 100 ng/mL. Averaged accuracy was between 99 and 101%, and the CVs were < 5%. This LC-MS/MS method confirms its clinical applicability for THC level monitoring in DBS.

B-398

Establishment of Expected Ranges for the Random Serum Concentration of Lacosamide and Desmethyl Lacosamide

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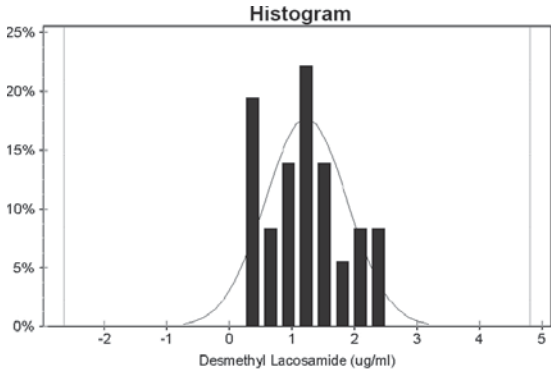
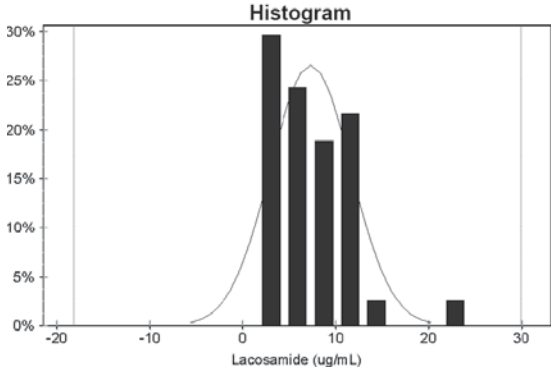
Background: Lacosamide (LCM) is an antiepileptic drug approved by the Food and Drug Administration for adjunctive treatment of partial onset seizures. LCM has a novel mode of action by selectively enhancing the slow deactivation of the sodium gated channels. LCM reaches peak concentration within 1-4 hours when taken orally and has a half-life of 13 hours. In humans the major metabolite is O-desmethy lacosamide (ODL). Theraputic drug monitoring (TDM) of LCM and ODL is used to help optimize therapeutic dosing, while limiting adverse effects. TDM is also used for establishing an individualized therapeutic range and to assess compliance to therapy. **Method:** Random serum samples (n=45) were obtained from adult subjects taking LCM adjunctive therapy for partial onset seizers as part of a clinical trial. The subjects consisted of 7 males and 16 females. These subjects were on either a 200mg/day or 400mg/day dose of Lacosamide. The random serum samples were analyzed on a previously validated LC-MS/MS method for LCM and ODL. **Results:** A statistical analysis was performed, in Excel, using the central 95% criteria to establish an expected concentration range. For subjects taking 200mg/day of LCM the random serum concentration of LCM and ODL was found to be 2.2 to 9.8 µg/mL and up to 1.6 µg/mL respectively. For subjects taking 400mg/day LCM the random serum concentration of LCM and ODL was found to 3.1 to 19.8 µg/mL and 0.5 to 2.5 µg/mL respectively. Histograms, see figure, were constructed using EP Evaluator to show distribution. **Conclusion:** The expected random concentration of LCM and ODL for patients taking 200-400mg/day of LCM is 2.2 to 19.8 µg/mL and up to 2.5 µg/mL respectively.





B-400

13-Panel toxicological drug screen of umbilical cord tissues with enzyme-linked immunosorbent assays (ELISAs)

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Relevance: Current best practice for detecting *in utero* drug exposure is to test newborn's meconium samples. However, a meconium sample is not available in 8-20% of births in the United States. Umbilical cord is readily available for collection in every birth. Previous studies have shown that an umbilical cord drug test performs equivalently well as a meconium drug test. The detection of drugs requires high sensitivity and fast turn-around for further interventions. Our **objective** is to develop and validate a multiplex 13-panel ELISA drug screen that provides equivalent sensitivities as the chromatography-mass spectrometry (GLC-MS) based confirmatory methods.

Method: An aliquot of approximately 0.5g of umbilical cord was homogenized in 3.0mL of acetone followed by centrifugation. The supernatant was filtered, 0.2% succinic acid in acetone was added, the sample taken to dryness, and finally reconstituted with 700 micro-liter buffer. Laboratory determined volumes of the extracts were aliquoted to each of thirteen ELISA plates using automated liquid handling devices for development according to drug assay manufacturer package inserts. The assay principle is homogeneous-competitive immunoassay, where the intensity of the developed color is inversely proportional to the sample's drug concentration. The absorbance of each sample well was normalized to that of the negative controls (B/B_0) of the same ELISA plate. Validation was performed according to Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines. We set the cut-off levels (decision points) listed below: 0.1ng/g for Tetrahydrocannabinoids; 0.5ng/g for Cocaine, Opiates, Oxycodone, and Buprenorphine; 1.0ng/g for Barbiturates; 2.0ng/g for Phencyclidine, Benzodiazepines, Methadone, and Meperidine; 4.0ng/g for Propoxyphene and Tramadol; and 5.0ng/g for Methamphetamine.

Results: Five controls with concentrations ranging between 50-150% of cut-off levels were prepared for analysis of precision and linearity. Coefficients of variation (CV%) were 0.9-12.4% within-run (n = 4) and 2.9-19.4% between-run (5 runs, n=20). The mean ± 2 standard deviations (SDs) for levels at ±50% of cut-off did not overlap with the decision points. Correlation coefficients (R^2) of B/B_0 versus concentrations (expressed in logarithm) were 0.9567-0.9977, demonstrating acceptable linearity. The mean $B_0-3.3$SDs determined lower limits of detection ranging 3.0-41% of cut-off levels. The immunoassays did not present hook effect and carry-over at least at 100 times of cut-off levels. The assays did not show interference from common over-the-counter or prescription drugs at 1000 ng/g, except dihydrocodeine for Opiates and Oxycodone assays. ELISA screened positive samples were subsequently quantitated using previously validated confirmatory methods to detect determinant drugs and metabolites of each class at the same cut-off. We found that 83.6-98.0% of the ELISA screened positive samples were eventually confirmed positive.

Conclusion: Fetus exposure to drugs results in adverse effects on newborn health, and early intervention is crucial. Although GLC-MS is a preferred methodology to detect minute amounts of harmful substances deposited into the umbilical cord, solely depending on this technology is operationally difficult to support such critical care. We validated and determined the 13-panel multiplex ELISA screen method to be appropriate for implementation and equivalently sensitive as the more time-consuming drug-class specific GLC-MS methods.

B-401

Evaluation of a liquid chromatography tandem mass spectrometry analytical method for the quantification of a panel of antiepileptic drugs and their metabolites in human plasma

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Background: the evaluation of an analytical method for the quantification of 17 different antiepileptic drugs and metabolites in human plasma is reported. The panel includes levetiracetam, theophylline, felbamate, lacosamide, rufinamide, carbamazepine, oxcarbazepine, carbamazepine diol, carbamazepine epoxide, 10-hydroxycarbamazepine, PEMA, primidone, phenytoin, stiripentol, zonisamide, phenobarbital and valproic acid. The method is based on liquid chromatography tandem mass spectrometry performed on a Transcend™ LC system combined with a TSQ Quantum™ Access MAX triple stage quadrupole mass spectrometer, both from Thermo Scientific™. The MassTox® TDM Series A kit for antiepileptics from Chromsystems™ was used for the scope; the kit included mobile phases, an analytical column, calibrators, controls and extraction, precipitation and dilution buffers. The calibration range covered the therapeutic window of concentrations in plasma for each analyte.

Methods: following the instructions provided by the kit supplier, lyophilized calibrators (on three levels) and controls (on two levels) were resuspended with distilled water, protein precipitated using the precipitation buffer (containing 12 different internal standards), vortex-mixed, centrifuged and the supernatant diluted with the dilution buffer prior to injection. Blank plasma samples from different donors were also added to the batch. Each calibrator and control sample was prepared in duplicate. The analytes of interest were divided into three groups and analyzed using three different gradient elutions, with runtimes of 5.0, 3.8 and 2.2 minutes. A heated electrospray source was used for sample ionization in both positive and negative mode and detection was performed by Single Reaction Monitoring (SRM). Data acquisition and processing was performed using Xcalibur™ software. Specificity, linearity and accuracy of the method were evaluated for each analyte. Specificity was tested by checking the absence of interfering peaks in plasma samples from different donors not taking any antiepileptic drug. Linearity in the calibration range set by the kit supplier was evaluated using a linear interpolation with 1/x weighting for all the analytes. The percentage bias between nominal and experimental concentration for both calibrators and control samples was used to assess the accuracy of the method; the adopted acceptance criterion for accuracy was: bias within ±15% for calibrators and within ±20% for control samples.

Results: the analytical method proved to be specific and accurate, with no interfering peaks and a maximum percentage bias within the acceptance criteria for both calibrators and control samples. Linear calibration curves were obtained for all the analytes of interest, with a correlation coefficient (R^2) always above 0.99.

Conclusion: the reported method can be successfully applied to the quantification of a large panel of antiepileptic drugs and metabolites in human plasma by liquid chromatography tandem mass spectrometry using a TSQ Quantum Access MAX.

B-402

Quantitation of Pentobarbital in Serum Using Liquid Chromatography-Tandem Mass Spectrometry (LC/MS-MS)

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Background. Pentobarbital is a central nervous system depressant with sedative and hypnotic properties. It is crucial to monitor levels with an acceptable turn-around time. Gas Chromatography Flame Ionization Detection (GC/FID) is a commonly used

method for the analysis of barbiturates, but can be labor intensive. Our aim was to validate a quantitative pentobarbital analytical method by LC/MS-MS which will be used as a routine method in the clinical laboratory with a faster turn around time (TAT) and will be less labor intensive than GC/FID.

Methods. Pentobarbital present in serum is extracted using a methanol/acetonitrile protein precipitation, followed by dilution and analysis by Shimadzu Prominence 20A Liquid Chromatograph, followed by the AB SCIEX QTRAP® 4500 Mass Spectrometry System (LC/MS-MS). A 20 uL specimen is mixed with a methanolic, deuterated internal standard (pentobarbital-D5), which initiates protein precipitation. The proteins are then further precipitated with the addition of acetonitrile. A portion of the supernatant is transferred and diluted with methanol:water (50:50) for injection into the LC/MS-MS. Qualitative identification is based on the presence of the specific MRM transitions for pentobarbital at the correct retention time. Quantitative measurement is accomplished by normalization of the peak area with the area of the internal standard for each specimen, including matrix specific calibrators, and quality control (QC) materials. Each sample is separately processed by the instrument software. The program automatically constructs a calibration curve, using the peak abundance data from the calibrator samples. QC are extracted and analyzed with patient samples. All data is subjected to analyst and technical QC review, prior to acceptance for reporting of results.

Results. The relative intra-laboratory reproducibility standard deviations were, in general, better than 5% at concentrations in the therapeutic range. The estimated lowest limit of detection (LOD) was zero 0.09 ug/mL and lowest limit of quantitation (LOQ) was 0.29 ug/mL. LOQ for serum pentobarbital using CV<20% was <0.5 ug/mL and was used as clinical cut-off. The mean true recoveries for samples in the analytical measurement range 0.5-100 ug/mL were between 96-106%. Correlation with GC-FID was excellent (y=0.9x1.1, r=0.99, p<0.05, n=21). Sample recoveries (n=3) following five freeze/thaw cycles were 98-111%. TAT was approximately 2 hours from receiving of specimen to reporting result to physician.

Conclusion. The quantitative pentobarbital method by LC/MS-MS is an easy and user-friendly method with an excellent analytical sensitivity. It requires one-step for extraction of pentobarbital from serum, and then a direct injection into the LC/MS-MS system. We were able to achieve excellent TAT with our method, and were able to meet our institution's goal for TAT.

B-403

Drug monitoring and toxicology: simultaneous determination of total mycophenolic acid and its glucuronide by HPLC-UV

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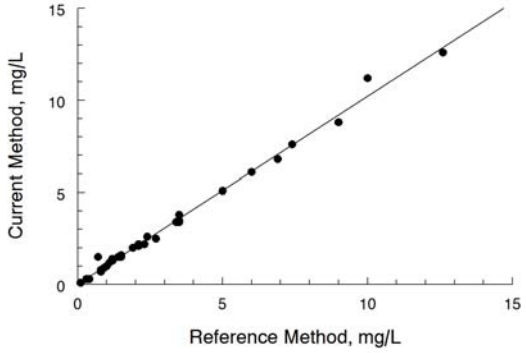
Background: Mycophenolic acid (MPA) is a potent immunosuppressant. MPA is primary metabolized to an inactive glucuronide MPAG, which is transported from liver into bile. Biliary MPAG then enters the GI tract, where it is converted back to MPA, which is then recycled into the bloodstream *via* the enterohepatic circulation pathway. Several studies have documented that variation in MPA plasma concentrations are unpredictable; variability in plasma concentration of MPA both within and between individuals is high. Therapeutic drug monitoring (TDM) of MPA plasma concentrations can help the clinician to develop personalized therapy strategies to avoid toxicity and maintain efficacy. To support the clinical studies of mycophenolate mofetil and to provide the clinical service for the TDM of MPA and MPAG ordered by the researchers and physicians, a simple and rapid HPLC method using UV detection has been developed.

Methods: The analytical procedures include single dilution step, protein precipitation, ultracentrifugation and gradient elution chromatography. Separation of MPA, MPAG and internal standard clonazepam was achieved by using a 5-μm Microsorb-MV C18 column (150 x 4.6 mm). These compounds were quantified by UV absorbance at 306 nm.

Results: Linearity was verified over ranges of 0.1-20 mg/L and 1-200 mg/L for MPA and MPAG, respectively. Recoveries of MPA and MPAG ranged between 93 and 105%. Both within-run (n = 6) and between-run (n = 30) precisions were lower than 10%. Figure 1 illustrates a comparison between the current method and reference method for MPA. The linear regression statistics indicated an r value of 0.993 (P < 0.0001). The linear regression equation for correlation was $y = 1.018\ x + 0.031$; where y, the current method and x, the reference method. No interferences with other common drugs were observed.

Conclusion: The analytical method is very suitable for both routine clinical use and pharmacokinetic studies.



B-404

Detection of 13 Opioids by LC-MS/MS from dried urine spots

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Introduction: Dried specimens offer a convenient and economical approach for collection, storage and transport of clinical specimens. Although, dried blood spots have been used as the specimen of choice in clinical laboratories (Biochemical Genetics) for years, dried urine spots (DUS) have not been established for routine use in clinical laboratories. Traditionally, drugs of abuse in clinical laboratories are detected by immunoassay (IA) and confirmed by GC-MS, which is time consuming and expensive. LC-MS/MS can offer a one-step detection and confirmation method for detection and measurement of drugs.

Objectives: To develop a LC-MS/MS method to detect multiple drugs in DUS in one-step.

Methods: 13 opioids (morphine, hydromorphone, oxymorphone, codeine, hydrocodone, oxycodone, heroin, 6- mono-acetyl morphine (6-MAM), fentanyl, norfentanyl, naloxone, tramadol, and meperidine) were analyzed in dried urine spot extraction. Briefly, 15 µL of de-identified urine samples containing the above drugs was spotted onto Whatman 903 Protein Saver Cards (GE Healthcare Biosciences). Drugs were extracted using a mixture of methanol, acetonitrile and water (4:4:1). Extracts, evaporated at RT, reconstituted in 5% acetonitrile before injecting into the LC-MS/MS. Urine samples were spiked by standards ranging from 100-2000 ng/mL. HPLC: Agilent 1290 (Palo Alto, CA) with a Restek Ultra Biphenyl column (100mm x 2.1 mm x 5µm). Mobile phase A was 1 mM ammonium formate and 0.1% formic acid in water; Mobile phase B was 0.1% formic acid in acetonitrile. Separation was achieved using a gradient elution program starting at 99% A for 1 minute, decreasing to 95% A for one minute, and then decreasing to 50% A until 13.6 minutes, holding at 50% A until 15.6 minutes, and returning to 99% an over 0.1 minutes. Flow rate was 0.5 mL/min and the injection volume was 10 µL.

Mass spectrometric detection was performed using an Agilent 6460 triple quadrupole mass spectrometer. Source parameters were optimized as follows: Electrospray voltage +2500V, Sheath gas temperature 380 °C, sheath gas flow 11 L/min, Nebulizer gas was 30 psi, Source gas temperature was 300 °C and the gas flow was 9 L/min. MRM transitions for all analytes were selected and optimized using the Agilent Optimizer software using 1000 ng/mL of each analyte in methanol. Data analysis was performed using Agilent MassHunter Quantitative analysis software

Results:

Our preliminary recovery results from DUS by LC/MSMS were: Morphine, 97%; Oxymorphone, 97%, hydromorphone, 100%; naloxone, 100%; codeine, 100%; 6-MAM, 99%; hydrocodone, 99%; oxycodone, 100%; heroin, 98%; fentanyl, 97%; norfentanyl, 97%; tramadol, 100%; and meperidine, 97%. These results matched those generated from identical liquid urine same by traditional immunoassay followed by GC/MS.

Conclusions: We modified a LC-MS/MS method to detect 13 opioids from DUS, simultaneously. DUS offers a convenient and economical approach for specimen collection, transportation and storage. This one-step LC-MS/MS detection confirmation method eliminates the need for the traditional multi-step approach of immunoassay and GC-MS and can greatly reduce the operational cost. More drugs of abuse are being tested.

B-405

Effectiveness of Afternoon Dosing Policy on Digoxin Level Monitoring

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Background: Determining serum or plasma digoxin levels is helpful in titrating drug dosage as well as assessing compliance and toxicity. However, inappropriately timed sample collection in relation to dose may result in level misinterpretation with the risk of over or under dosing. To circumvent inappropriately timed collections, the policy of afternoon dosed digoxin (PM dosing) in all inpatient care facilities in Alberta Health Services Edmonton Zone was instituted on November 5, 2008. This policy was also adopted in other but not all Northern Alberta health care facilities. The purpose of this study was to evaluate the effect of the policy change on digoxin dosing time by comparing pre- and post-implementation periods.

Method: The evaluation was conducted by comparing the time periods of approximately four years before and four years after policy implementation (November 1, 2004 to October 31, 2012). All digoxin levels during this period were included. Those with available dosing information were grouped into AM (0001h to 1159h) and PM (1200h to 2400h) dosing groups as well as into inpatients and outpatients, which also included collection at emergency departments.

Results: A total of 58,236 digoxin levels were performed in Northern Alberta from November 1, 2004 to October 31, 2012. There was a steady decline in the number of tests done annually (45.8% decline over eight years), likely reflecting the decreased prescription of digoxin in recent practice. Of the 58,236 requests, 23,324 (40%) had dosing information provided on the requisition forms. Within this latter group, the proportion where dosing occurred PM was significantly higher after policy implementation (mean 47±2.1% vs 28±0.73%, $p < 0.05$). For Edmonton Zone inpatient facilities, the proportion of requests with PM dosing increased significantly in the four year period after policy implementation (mean 56±3.4% vs 27±1.5%, $p < 0.05$). The proportion of digoxin levels at increased risk of toxicity (level >2.0 nmol/L) was significantly higher pre-policy implementation (mean 13±2.5% vs 5.5±0.58%, $p < 0.05$); whereas, the proportion of samples below target digoxin level was higher post-policy (mean 32±6.3% vs 16±2.9%, $p < 0.05$). The proportion of samples within the target range (0.6 to 1.2 nmol/L for heart failure) was similar pre- and post-policy (mean 42±2.6% vs 46±4.8%, $p > 0.05$).

Conclusion: This study demonstrates the sustained effect of increased compliance to PM dosing since the institution of this policy in Alberta Health Services Edmonton Zone in November 2008. However, other intervention strategies are required to further improve policy adherence, hence minimizing the chance of inappropriately timed collections. The lack of dosing information on the requisition forms is another issue that precludes a complete assessment.

B-406

Development of a High-throughput LC-MS/MS Assay for Pain Management Panel from Urine

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Background: The widespread use and the potential abuse of opiates, sedatives, and stimulants drugs have increased the need and in some cases the requirement to screen patients on a routine basis. Pain panels continue to grow in complexity as more prescription and non-prescription compounds are added. This has made the job of toxicological analysis even more challenging. To fulfill these requirements, a fast, reliable, and accurate LC-MS/MS method has been created for the analysis of a pain panel comprised of 30 drugs on an IONICS 3Q 120 triple quadrupole mass spectrometer.

Methods: First, the mixed drug standard solution was spiked into the urine matrix, then diluted with the mobile phase A (100% H2O, 0.1% formic acid) to make a series of concentrations ranging from 0.016 to 16 ng/mL. The internal standard concentration used was 10 ng/mL. The calibrator solutions will be directly injected without further treatment. IONICS 3Q 120 mass spectrometer equipped with a heated coaxial flow ion source and "Hot Source-Induced Desolvation" interface was used. The time-managed MRM in Molana™ software was used to optimize the dwell time for each MRM transition based on the retention times and the number of MRM transitions within given experiments. The separation was performed on a Shimadzu Prominence LC system. A 10 uL sample was loaded onto a Restek Ultra II Biphenyl column (50 x 2.1 mm, 5um) kept at 40 °C. A gradient method was created with a flow rate of 600 uL/min and a total LC cycle time of 7.5 minutes. Solvent B was composed of 0.1% formic acid in 100% methanol.

Results: A total of 57 MRM transitions were used to monitor 30 drugs including internal standards. No matrix interferences were observed. LC system carryover was checked to ensure the validity of the data. An overlay of the extracted chromatograms of 30 drugs in a 7.5 minute LC run showed that all of the anlaytes were clearly separated. The calibration curves showed good linearity for all the analytes across the whole concentration range with a coefficient $R^2>0.99$. All calibration curves used a linear weighting regression of 1/x. The LLOQs for the 30 drugs were in the range of 0.032 to 2 ng/mL. At LLOQs, the accuracy was between 84-114%, and CVs were < 10% for all analytes.

Conclusion: The results in this study show that in a 7.5-minute LC run, this LC-MS/MS method can effectively separate the 30 pain panel drugs. The quantitation results also indicate that this method is accurate, precise, and reproducible. The LLOQs for all the 30 drugs is in the range of 0.032 to 2 ng/mL, which is 2 to 3 orders lower than the typical screening cutoff concentration (300 ng/mL), and much lower than the typical confirmation cutoff concentration (50 ng/mL) for most of the drugs of abuse. Therefore, this LC-MS/MS method with IONICS 3Q 120 mass spectrometer is an effective combination for clinical pain management to monitor patient drug use and program adherence or for drugs of abuse or other workplace drug testing.

B-407

Development of Abbott Phenytoin Assay for the ARCHITECT *c*Systems Automated Clinical Chemistry Analyzers

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Introduction: The study objective is to develop a sensitive immunoassay intended for the quantitative measurement of phenytoin in human serum or plasma on the ARCHITECT *c*Systems. The measurements obtained are used in the diagnosis and treatment of phenytoin overdose and in monitoring levels of phenytoin to help ensure appropriate therapy.

Methods: The Abbott Phenytoin Assay is a liquid ready-to-use, homogeneous enzyme immunoassay. The two reagent kit uses specific antibodies to detect phenytoin in the sample, with minimal cross-reactivity to various over-the counter, structurally unrelated compounds. The method is based on the competition for a fixed amount of specific antibody binding sites between enzyme [glucose-6-phosphate dehydrogenase (G6PDH)]-labeled phenytoin, and phenytoin contained in the sample. In the absence of phenytoin from the sample, the specific antibody binds the G6PDH-labeled phenytoin and causes a decrease in enzyme activity. If phenytoin is present in the sample, it occupies the antibody binding sites, which allows the G6PDH-labeled phenytoin to interact with the substrate, resulting in enzyme activity. This phenomenon creates a direct relationship between the phenytoin concentration in sample and enzyme activity. By measuring the enzyme's ability to convert nicotinamide adenine dinucleotide (NAD) to NADH, its activity is determined spectrophotometrically at 340 nm.

Results: The performance of the Abbott Phenytoin Assay was evaluated on the Abbott ARCHITECT *c*8000 analyzer. Based on guidance from Clinical and Laboratory Standards Institute (CLSI) protocol EP17-A2, the assay demonstrates a Limit of Quantitation of $\leq$ 1.8 µg/mL using inter-assay precision $\leq$ 7% CV or $\leq$ 0.7 µg/mL SD and bias within 10% or 1.0 µg/mL over an extended period. The assay is linear from 1.8 to 40.0 µg/mL using guidance from CLSI protocol EP6-A. Assay precision was evaluated using CLSI guideline EP5-A2. A tri-level commercial control and six human serum samples containing phenytoin at concentrations ranging from 3.9 µg/mL to 36.1 µg/mL were tested. Each sample was assayed in duplicate twice a day for 20 days with at least two hours between runs. The precision ranged from 1.6 %CV to 3.3 %CV for Within-Run and 2.0 %CV to 4.1 %CV for Total-Run. The assay accurately recovered spiked phenytoin at levels representing sub-therapeutic, therapeutic, and toxic samples. No significant interference was observed with various endogenous substances or compounds whose chemical structure or concurrent therapeutic use would suggest possible cross-reactivity. The assay did not exhibit obvious cross reactivity with phenytoin derivatives and metabolites at the concentrations tested with the exception of fosphenytoin. Abbott ARCHITECT Phenytoin patient correlation studies: new vs. current on-market assay yielded a regression equation of $y=1.11x + 0.12$ and a correlation coefficient of 0.99. The new reagent has an onboard stability of 40 days and calibration curve stability of 7 days.

Conclusion: The Abbott Phenytoin Assay enables measurement of phenytoin in human serum or plasma with high precision across the linear range. The ability to monitor levels of phenytoin with high accuracy can help ensure appropriate therapy. The assay has applications on the ARCHITECT *c*16000, *c*8000, and *c*4000.

B-408

Urine drug screening: using GC-MS/MS to augment LC-MS/MS screens

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Background & Objective Clinical toxicology services, including the detection of pharmaceuticals and drugs of abuse in biological samples, are routinely offered by many hospital laboratories and can play an important role in patient management. In addition to immunoassay screening for routine drugs of abuse, our laboratory offers a liquid chromatography tandem mass spectrometry (LC-MS/MS) method to screen patient urine samples for a large number of clinically relevant compounds. Immunoassays are not available for many of the compounds in our method, which makes it a challenge to verify results by two methods. Here we report the development and validation of a gas chromatography tandem mass spectrometry (GC-MS/MS) method for comprehensive urine drug screening with method performance evaluated in part by concordance with our LC-MS/MS method.

Methods GC-MS/MS was performed on a Bruker ScionTQ mass spectrometer with an EI source and a Bruker 436 GC with a 30m BR-5ms column. Chomatographic runs began at 80°C and ramped to 295°C over 20 minutes. Retention times (RT) were established and two multiple reaction monitoring (MRM) transitions were developed for each of the approximately 150 compounds in the method. Compounds were identified by RT and the presence/ratio of the two MRM transitions. For method validation analytes were spiked into 0.5 mL urine, isolated using solid phase extraction (SPE), eluted in either the acid/neutral or basic fractions, and evaporated to dryness at 40° under N_2 flow. Extracts were reconstituted in 50:50 methanol:acetonitrile and 1 µL was injected. Our validation included determining the lower limit of detection (LLOD), matrix effects, and SPE recovery for each compound. At least 30 patient samples were analyzed by both GC-MS/MS and LC-MS/MS.

LC-MS/MS was performed on an Agilent HPLC with an ABSciex 3200 QTRAP mass spectrometer in positive-ESI mode. Compounds were identified by a combination of RT, one MRM transition and a match between the collected product ion spectrum and our in-house-built spectral library.

Results Overall the GC-MS/MS method performed quite well. LLODs ranged from 5 ng/mL to 250 ng/mL. A small fraction of compounds were not observed in any validation samples, likely due to extremely low volatility. Method validation included determining matrix effects using two donor urine matrices and evaluating the recovery of our solid phase extraction method for acid/neutral and basic drugs. Comparison of patient samples between LC-MS/MS and GC-MS/MS showed good concordance. As expected, many of the compounds that were missed by GC-MS/MS were large and/or quite polar, for example glucuronide conjugates.

Conclusions GC-MS/MS has many advantages for identification of drugs in biological matrices. GC-MS/MS instruments are generally less expensive than LC-MS/MS instruments and can analyze some compounds that LC cannot and GC-MS/MS can offer lower limits of detection than GC-MS systems. We have recently had cases involving pentobarbital and 1,4-butanediol, neither were detected using our LC-MS/MS but were easily observed with our GC-MS/MS. Use of both technologies in our laboratory allows us to screen patient urines for a wider variety of compounds with a greater assurance of accuracy.

B-409

Ultrafast Quantitative Analysis of Illicit Drugs and Benzodiazepines in Urine Using High-throughput SPE/MS/MS

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Introduction Pathologists, employers and law enforcement officials use drug testing extensively today. Drug screening typically involved immunoassay analysis followed by a confirmatory test by GC/MS or LC/MS detection. Steady increases in the need for greater analytical capacity and throughput have placed demands on traditional technologies. The RapidFire (RF) platform provides an automated solid-phase extraction (SPE) system that gives a throughput of approximately 12 seconds per sample to the mass spectrometer.

In the present study, we evaluated the ability of the RF/MS/MS system to quantitatively measure a panel of illicit drugs or benzodiazepines in urine. Imprecision, accuracy and linearity results achieved with this ultrafast RF/MS/MS system were comparable to LC/MS/MS.

Methods Blank matrix containing internal standards was spiked with the analytes of interest in a range of concentration to prepare the calibration curve. The illicit drug panel consisted of 6-monoacetylmorphine, benzoylecgonine and phencyclidine. This panel was subjected to dilution (1/10) with an aqueous solution containing the internal standards prior to online SPE. The benzodiazepine panel consisted of temazepam, 7-amino clonazepam, nordiazepam, alpha hydroxy alprazolam, lorazepam, alprazolam and oxazepam. Benzodiazepine samples were subjected to enzymatic hydrolysis followed by centrifugation and then diluted (1/50) prior to online SPE. Online SPE methods were optimized for each panel. Analysis of all samples was performed at a rate of <12 seconds per sample using a RapidFire high-throughput system coupled to a triple quadrupole mass spectrometer.

Results The analytes in the illicit panel had good linearity with R2 values >0.995 within the measurement range of 2.5-500 ng/mL for 6-monoacetylmorphine, 5-1000 ng/mL for phencyclidine and 25-5000 ng/mL for benzoylecgonine. The average intra- and interday accuracy at LLOQ was 99% for 6-monoacetylmorphine, 105% for phencyclidine and 102% for benzoylecgonine. The average intra- and interday imprecision at LLOQ was 17% for 6-monoacethylmorphine, 7% for phencyclidine and 2% for benzoylecgonine. The standard curve of benzodiazepine analytes had excellent linearity within the measurement range (50-5000 ng/mL) with R2 values >0.999. The average intra- and interday accuracy and imprecision at LLOQ for all compounds were 103% and 8% respectively. No significant carry-over was detected for any illicit and benzodiazepine analytes. The linearity, accuracy and imprecision results from SPE/MS/MS analysis were comparable to those from traditional LC/MS/MS. The SPE/MS/MS analysis was more than 20 times faster than LC/MS/MS.

Conclusions Illicit drugs and benzodiazepines can accurately, precisely and rapidly be quantified using an ultrafast SPE/MS/MS system. This system is an alternative to traditional LC/MS/MS, combining efficiency and speed.

B-410

Compliance Rates In Chronic Cancer Pain Patients.

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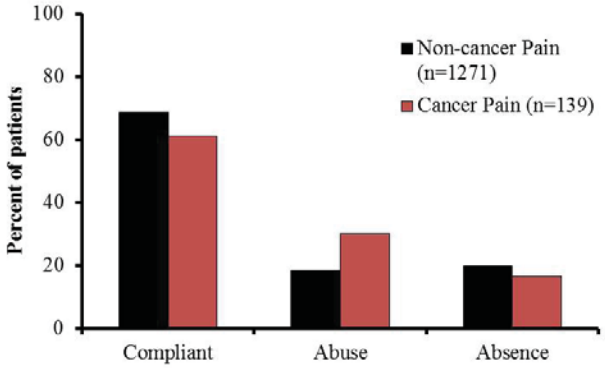
Background: A distinction is often made between patients on prescription opioid therapy for chronic cancer and chronic non-cancer pain. This distinction can have wide reaching implications, such as impacting which patients are and are not subject to new legislation. No evidence exists to suggest that these two patient populations differ in terms of the prevalence of opioid misuse.

Methods: This retrospective cohort study included 1,271 non-cancer and 139 cancer patients on prescription opioids who submitted urine samples to our laboratories between May of 2012 and April of 2013. All patients were subject to chart review as part of normal clinical operations by a single reviewer. Chronic cancer pain was based on cancer or cancer therapy related pain being the primary diagnosis in the chart. Urine samples were analyzed for the presence or absence of prescription opioids (oxycodone, hydrocodone, methadone, morphine, hydromorphone, fentanyl, buprenorphine, meperidine, propoxyphene) and the heroin-specific metabolite 6-monoacetylmorphine using a clinically validated, laboratory-developed liquid chromatography tandem mass spectrometry (LC-MS/MS) assay. Cocaine abuse was determined by an immunoassay (Syvia EMIT II) and methamphetamine was determined with a separate validated LC-MS/MS assay. Benzodiazepines and other analgesics (tramadol,carisoprodol, etc) not detected in our assays were not considered.

Results: Cancer patients showed a compliance rate statistically indistinguishable from that of non-cancer patients (61% and 68%, respectively). The patterns of abused drugs were not substantially different between these two populations. Both populations were three times more likely to abuse prescription opioids than illicits (heroin, cocaine and methamphetamine).

Conclusion: Chronic cancer pain patients are equally likely to misuse prescription opioids, and in particular to use non-prescribed prescription opioids. This misuse puts them at risk for adverse outcomes and clinicians should be aware of these dangers when managing chronic cancer pain.



B-412

Rapid measurement of tacrolimus, cyclosporin A and sirolimus in blood by paper spray-tandem mass spectrometry (PS-MS/MS)

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Background: therapeutic drug monitoring (TDM) of immunosuppressant(s) is critical in preventing organ rejection after transplant. Automated immunoassays provide quick turnaround time but are costly and lacking standardization. Conventional tandem mass spectrometry (MS/MS) requires a liquid chromatography (LC) system and assay requires pre-analytical manipulation which is not amenable to random access testing. Paper spray (PS) ionization is a technique that generates gas phase analyte ions directly from dried blood spots for quantitative MS/MS analysis, without complex sample preparation, nor a LC system. We planned to evaluate PS-MS/MS for simultaneous tacrolimus, cyclosporin A and sirolimus TDM in a clinical diagnostic laboratory, by examining assay precision, accuracy, and analytical measurement range (AMR), as well as assay specificity and possible analyte ion suppression phenomenon.

Methods: 200 µL of whole blood, calibrators, or quality control material were mixed with 50 µL of stable isotope labeled internal standard mixture ([^{13}C, $^{2}H_2$]-FK506, [$^{2}H_4$]-cyclosporin A, and [$^{2}H_3$]-rapamycin). The blood mixture (10 µL) was spotted onto triangular shaped card paper contained in disposable cartridges and dried at 40°C. Cartridges were analyzed using an automated paper spray ion source coupled to a triple quadrupole MS/MS (TSQ Vantage™; Thermo Scientific). Small amount (up to 120 µL) of methanol and chloroform mixture with added sodium acetate was delivered by the ion source to blood containing paper in cartridge before a voltage of 3.5 kV was applied to generate ion spray. Sodium adduct ions of each immunosuppressant, along with corresponding internal standard ions were detected in the selected reaction monitoring (SRM) mode and quantitated by the area under the curve of collected 50 scan within 1.0 min, with an average analysis time of 3 min per sample. Each analyte result was confirmed by using a second SRM.

Results: the PS-MS/MS method has acceptable AMR and precision, and results correlate well with those of a FDA approved immunoassay currently used in clinical labs. Patient samples may be analyzed in batches by PS-MS/MS, or analyzed by adding on to batch throughout the day, much like in a random access fashion.

Conclusions: PS-MS/MS is much simpler in comparison to a conventional LC-MS/MS system. It simultaneously provides accurate results for tacrolimus, cyclosporin A and sirolimus, with fast turnaround time amenable to random access testing protocols.

B-413

Development of Abbott Theophylline Assay for the ARCHITECT *c*Systems Automated Clinical Chemistry Analyzers

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Introduction: The study objective is to develop a sensitive immunoassay intended for the quantitative measurement of theophylline in human serum or plasma on the ARCHITECT *c*Systems. The measurements obtained are used in the diagnosis and treatment of theophylline overdose and in monitoring levels of theophylline to help ensure appropriate therapy.

Methods: The Abbott Theophylline Assay is a liquid ready-to-use, homogeneous enzyme immunoassay. The two reagent kit uses specific antibodies to detect theophylline in the sample, with minimal cross-reactivity to various over-the counter, structurally unrelated compounds. The method is based on the competition for a fixed amount of specific antibody binding sites between enzyme [glucose-6-phosphate dehydrogenase (G6PDH)]-labeled theophylline, and theophylline contained in the sample. In the absence of theophylline from the sample, the specific antibody binds the G6PDH-labeled theophylline and causes a decrease in enzyme activity. If theophylline is present in the sample, it occupies the antibody binding sites, which allows the G6PDH-labeled theophylline to interact with the substrate, resulting in enzyme activity. This phenomenon creates a direct relationship between the theophylline concentration in sample and enzyme activity. By measuring the enzyme's ability to convert nicotinamide adenine dinucleotide (NAD) to NADH, its activity is determined spectrophotometrically at 340 nm.

Results: The performance of the Abbott Theophylline Assay was evaluated on the Abbott ARCHITECT *c*8000 analyzer. Based on guidance from Clinical and Laboratory Standards Institute (CLSI) protocol EP17-A2, the assay demonstrates a Limit of Quantitation of ≤ 2.0 µg/mL using inter-assay precision ≤ 7% CV or ≤ 0.7 µg/mL SD and bias within 10% or 1.0 µg/mL over an extended period. The assay is linear from 2.0 to 40.0 µg/mL using guidance from CLSI protocol EP6-A. Assay precision was evaluated using CLSI guideline EP5-A2. A tri-level commercial control and six human serum samples containing theophylline at concentrations ranging from 3.5 µg/mL to 37.0 µg/mL were tested. Each sample was assayed in duplicate twice a day for 20 days with at least two hours between runs. The precision ranged from 1.4 %CV to 2.3 %CV for Within-Run and 1.9 %CV to 3.3 %CV for Total-Run. The assay accurately recovered spiked theophylline at levels representing sub-therapeutic, therapeutic, and toxic samples. No significant interference was observed with various endogenous substances or compounds whose chemical structure or concurrent therapeutic use would suggest possible cross-reactivity. The assay did not exhibit obvious cross reactivity with theophylline derivatives and metabolites at the concentrations tested with the exception of caffeine. Abbott ARCHITECT Theophylline patient correlation studies: new vs. current on-market assay yielded a regression equation of y=1.07x - 0.07 and a correlation coefficient of 1.0. The new reagent has an onboard stability of 40 days and calibration curve stability of 7 days.

Conclusion: The Abbott Theophylline Assay enables measurement of theophylline in human serum or plasma with high precision across the linear range. The ability to monitor levels of theophylline with high accuracy can help ensure appropriate therapy. The assay has applications on the ARCHITECT *c*16000, *c*8000, and *c*4000.

B-414

Isotope Dilution Gas Chromatography-Mass Spectrometry (GC/MS) Method for the Analysis of Hydroxyurea

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Background: Hydroxyurea (HU) is used in the treatment of various malignancies such as chronic myelogenous leukemia, squamous cell carcinomas, polycythemia vera and essential thrombocytosis. It is also used in the treatment of sickle cell disease where it is shown to decrease painful crisis and reduce number of transfusions. There are limited studies on the pharmacokinetics of hydroxyurea. An accurate, precise and sensitive method is needed to support such studies and also the monitoring of therapeutic adherence. Current methods for measurement of HU include colorimetry, high performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS). While available GC-MS methods are sensitive and specific, they involve use of internal standards that are structurally different from HU and prone to error due to poor (e.g., < 3%) extraction efficiency. Here we describe a novel GC-MS method for the determination of HU that involves stable labeled HU as an internal standard.

Materials and Methods: HU was purchased from Sigma-Aldrich and the internal standard, HU [13C] 15N2 was custom synthesized from Toronto Research, Canada. The calibrators and controls were prepared in drug-free plasma. To 0.5 mL plasma, 1 mL of phosphate buffer (pH 6.0) and 0.1 mL of internal standard were added. HU was extracted with ethylacetate. The extract was dried and trimethylsilyl derivatives of HU were prepared. 1 µL of the derivatization mixture was injected into the GC-MS. Selected ion monitoring was used for identification and quantification of HU. The monitored m/z ions were: HU (quantification ion 277, qualifier ions - 292 and 249), HU [13C] 15N2 (quantification ion 280, qualifier ion - 295).

Results and Conclusion: The method was evaluated for reportable range, accuracy, within and between-run imprecision, and limit of quantification. Reportable range (linearity) of the method was from 0.1 to 100 µg/mL. All results were accurate within allowable error of <10% or 0.03 µg/mL. Within-run and between-run imprecision

were <5 and <10% respectively. Lower limit of quantification, at inaccuracy of <10% or <0.03 µg/mL, was 0.1 µg/mL. To check specificity and selectivity of the method, 20 negative samples were analyzed. All the drug free samples quantified less than the lower limit of quantification and failed qualifier ion ratios. Samples were stable for at least 4 h, 2 months and 6 months at room temperature, -20°C and -70°C respectively. Samples were also stable after 3 freeze/thaw cycles. Extraction efficiency for 1, 5, 10 and 50 µg/mL samples averaged 2.2%, 1.8%, 1.6% and 1.4% respectively. These data demonstrate that our new, isotope dilution GC/MS method for analysis of HU is accurate, sensitive, precise and robust.

B-415

Bupropion Exposure in an Infant: A Case Report

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Background: The last two decades has seen a substantial increase in the rates of women breastfeeding their infants. However, it has been reported that 66 - 80% of nursing women are on medication. While many drugs are safely taken by nursing mothers, there is accumulating evidence of toxicity in some breastfed infants. Information on drug excretion into milk is lacking for most drugs, and early phase drug studies exclude breastfeeding women. This uncertainty in the risk of drug exposure causes maternal non-adherence to therapy or avoidance of breastfeeding. This is a clinical problem in drug safety and is an important women's health issue, uniquely affecting both mother and infant. The objective of this study is to investigate the risk of drug exposure in nursing infants. We present a proof-of-principle case of infant bupropion exposure highlighing the importance and clinical applicability of this study.

Methods: To investigate infant drug exposure through breast milk, we established a drug safety monitoring program, Drugs in Lactation Analysis Consortium (DLAC), to measure several drugs commonly used by women breastfeeding. Breast milk is a complex lipid- and protein- rich matrix, with drugs partitioning to either the aqueous or lipid phase, thus requiring meticulous sample processing before analysis. We first investigated whether drugs partition to the aqueous or lipid phase of breast milk. We then worked to create a simplified drug extraction method using hexane, methanol and acetonitrile to facilitate efficient drug extraction from breast milk. Extraction efficiency and stability were determined. Methods were then developed to measure these drugs using LC-MS/MS. This process was followed in the case presented below.

Results/Case Report: A 6.5 month old previously healthy infant presented to SickKids Hospital with vomiting and seizures. She was exclusively breastfed; her mother was taking escitalopram daily for several months, with the recent addition of bupropion. The usual clinical workup was negative. As we suspected this might be a case of drug exposure, the infant's urine was tested and was positive for bupropion and escitalopram. Serial breast milk and serum samples were obtained and bupropion and its major metabolite, hydroxybuproion, were measured using HPLC-MS/MS. Using a pharmacokinetic model, we determined that the bupropion levels were significant around the time of the event. Average milk bupropion and hydroxybupropion levels were 20.3 ng/mL and 68 ng/mL, respectively. Predicted bupropion steady state in the infant around the time of the event was 0.12 ng/mL. The average observed infant serum hydroxybupropion level was 11.2 ng/mL. Discharge diagnosis was bupropion-induced seizures.

CONCLUSION: This case highlights the importance of the DLAC study. Given the increasing use of medication in nursing women, there is urgency to investigate the potential adverse events associated with infant drug exposure through breast milk. Future investigations will focus on population pharmacokinetic modeling to simulate and estimate infant drug exposure and assess potential risks associated with drugs and breastfeeding. The data generated from this study will help guide decisions for drug use in nursing mothers.

B-416

Rapid confirm and determination of d and l methamphetamine in human urine by liquid chromatography tandem mass spectrometry

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Background: Prescription methamphetamine (Desoxyn) is composed entirely of the d isomer. Over-The-Counter cold medications contain l isomer (Vicker's inhaler). Street-methamphetamine is either d-isomer or racemic mixture with d-isomer of 20-100%. In order to narrow down the potential sources of the methamphetamine, clinical toxicology laboratories need to accurately differentiate between d and the l isomer forms of methamphetamine. Because of the identical mass spectrum of d and l isomers, directly identifying specific isomer relies on the chromatographic separation of isomers in the racemic mixture. We therefore developed an analytical method to simultaneously confirm and determine d and l methamphetamine isomers in urine samples by LCMSMS.

Method: 100 ul urine from prescreened positive sample was mixed with 100 ul internal standard of isotope d and l methamphetamine isomers. Samples were concentrated by using one step liquid-liquid extraction with diethyl ether and ethyl acetate (1:1). At least two small amounts of concentrated standards were added to the sample. The overlay chromatograms of sample and added standards combining with the retention time of isomer of isotope internal standard were used to confirm the expected isomer. This separation of isomers was achieved with LC/MS/MS mass spectrometer with macrocyclic glycopeptide-based 10cm x2.1 mm chiral column and mobile phase of consisting 100% methanol with1% formic acid and 0.01% NaOH. The x-intercept of the standard addition calibration curve corresponds to the concentration of analyte. A query of instrument data processing software was written to perform the calculation for determination and conversion of d and l isomer concentrations to percentage of total racemic mixture of methamphetamine.

Results: The analytical curve for d and l methamphetamine was linear over the range of 40 ng/ml (40% of cutoff 100 ng/ml) to 800 ng/ml with correlation coefficient of 0.9988. The precision of method was evaluated based on three different concentrations and racemic mixture ratios of d and l methamphetamine over two weeks of intra runs. CVs (n=25) of 4.2 %, 3.0% and 2.0% were obtained. Limit of quantitation was determined as 40 ng/ml. Limit of detection was 10 ng/ml with retention time within ±2% of isotope internal standard. Recoveries ranged between 95% and 106% for spiked and pooled samples.

Conclusion : This analytical method was rapid, simple and specific. The accuracy, precision, repeatability and robustness were found to be within the acceptable limit.

B-417

Development of Abbott Carbamazepine Assay for the ARCHITECT *c*Systems Automated Clinical Chemistry Analyzers

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Introduction: The study objective is to develop a sensitive immunoassay intended for the quantitative measurement of carbamazepine in human serum or plasma on the ARCHITECT *c*Systems. The measurements obtained are used in monitoring carbamazepine levels to help ensure appropriate therapy. Since carbamazepine concentrations correlate better with pharmacologic activity than dosage, monitoring of blood levels can increase efficacy and safety.

Methods: The Abbott Carbamazepine Assay is a liquid stable, particle-enhanced turbidimetric inhibition immunoassay used for the analysis of carbamazepine in serum or plasma. The assay consists of two reagents and is based on competition between carbamazepine in the sample and carbamazepine coated onto a micro-particle for anti- carbamazepine antibody binding sites. In samples lacking carbamazepine, the carbamazepine -coated micro-particles rapidly agglutinate in the presence of anti-carbamazepine antibodies. The rate of absorbance change is measured photometrically, and is directly proportional to the rate of particle agglutination. In samples containing carbamazepine, the agglutination reaction is partially inhibited, slowing the rate of absorbance change. A concentration-dependent classic agglutination inhibition curve can be obtained, with maximum rate of agglutination at the lowest carbamazepine concentration and the lowest agglutination rate at the highest carbamazepine concentration.

Results: Performance of the Abbott Carbamazepine Assay was evaluated on the Abbott ARCHITECT *c*8000 analyzer. Based on guidance from Clinical and Laboratory Standards Institute (CLSI) protocol EP17-A2, the assay demonstrates a Limit of Quantitation of ≤ 1.9 µg/mL using inter-assay precision ≤ 7% CV or ≤ 0.3 µg/mL SD and bias within 10% or 0.4 µg/mL over an extended period. The assay is linear from 1.9 to 20.0 µg/mL using guidance from CLSI protocol EP6-A. Assay precision was evaluated using CLSI guideline EP5-A2. A tri-level commercial control and five human serum samples containing carbamazepine at concentrations ranging from 1.9 µg/mL to 18.1 µg/mL were tested. Each sample was assayed in duplicate twice a day for 20 days with at least two hours between runs. Precision ranged from 1.0 %CV to 3.0 %CV for Within-Run and 1.9 %CV to 6.3 %CV for Total-Run. The assay accurately recovered spiked carbamazepine at levels representing sub-therapeutic, therapeutic, and toxic samples. No significant interference was observed with various endogenous substances or compounds whose chemical structure or concurrent therapeutic use would suggest possible cross-reactivity. The assay did not exhibit obvious cross reactivity with carbamazepine derivatives and metabolites at

the concentrations tested with the exception of carbamazepine-10, 11-epoxide. Abbott ARCHITECT Carbamazepine patient correlation studies: new vs. current on-market assay yielded a regression equation of y=0.91x + 0.56 and a correlation coefficient of 0.97. New assay vs. HPLC yielded y=1.09x + 0.37 and a correlation coefficient of 0.96. The new reagent has a 45 day onboard stability and 7 day calibration curve stability.

Conclusion: The Abbott Carbamazepine Assay enables measurement of carbamazepine in human serum or plasma with high precision across the linear range. The ability to monitor levels of carbamazepine with high accuracy can help ensure appropriate therapy. The assay has applications on the ARCHITECT *c*16000, *c*8000, and *c*4000.

B-418

Chemotherapy with pharmacokinetic (PK) guided exposure optimization: US based experience with 5-fluorouracil (5-FU) in colorectal cancer (CRC) patients

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Background: 5-FU is the backbone of colorectal cancer chemotherapy. Numerous studies over the last 30 years have demonstrated wide pharmacokinetic variability of 5-FU, which can lead to undue toxicity and suboptimal treatment. Exposure optimization of 5-FU based on PK-guided dose adjustment has been tested in multiple studies, demonstrating improved response rates and reduced toxicity compared to body surface area (BSA) based dosing. Here we present the experience of a US based laboratory evaluating 5-FU plasma levels to optimize systemic exposure in CRC patients of US based oncologists. **Methods:** Between June 5, 2013 and January 17, 2014, 5-FU concentrations were determined in 631 patient samples at Saladax Biomedical Laboratories (a CLIA-certified lab) using a laboratory developed immunoassay (My5-FU). Systemic 5-FU exposure [the area under the concentration curve (AUC)] was calculated from the 5-FU level determined in a steady state sample collected 24±6Hr after the start of 5-FU continuous infusion from 240 CRC patients (n=250 therapy lines). The calculated AUC and dose adjustment recommendations to achieve the target range AUC (20 – 30 mg*hr/L) were provided in the laboratory report. Differences between cycles and the comparison between dose adjustment recommendations and actual dose changes were made between evaluable cycle pairs (defined as two consecutive cycles with AUC results). Actual vs. target AUC, recommended vs. actual dose adjustment, and ability to adjust exposure to target range were evaluated. **Results:** The majority of AUC results (62%) were outside the target range in the first sample from each therapy line where exposure was determined, irrespective of the 5-FU dosing or regimen used. For the 250 samples collected during the first cycle of therapy, 48% had AUCs below the target range, 38% were within the target range, and 14% were above the target range. In patients dosed at the standard of 2400 mg/m^2 (57% of doses), the majority of AUC results were still out of range. At this dose, 44% had exposures below the target range, 42% were within the target range, and 14% were above the target range (n=144). In 288 cycle pairs, 201 (69%) were outside the target range: doses were decreased in 48% of the 40 above the target range and doses were increased in 51% of the 161 below the target range. No dose change was made in 89% of the 87 within the target range. In 101 cycle pairs out of target range where a dose adjustment consistent with recommendation was made, 42% of these patients achieved exposure in the target range. **Conclusions:** 5-FU exposure optimization is feasible in the US clinical setting. Consistent with other reports, body surface area based 5-FU dosing resulted in frequent under dosing (48%). The majority (62%) of dose adjustment recommendations were followed in subsequent cycles. When AUCs were out of range and dose adjustments were made, 5FU exposure was optimized in almost half the patients with a single dose adjustment. PK-guided dose adjustment is a practical approach to personalize and optimize 5-FU exposure.

B-419

Evaluation of Tacrolimus QMS assay by using Indiko and AU680 analyzers and comparison to Architect

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Background: Tacrolimus has been a widely used calcineurin inhibitor immunosuppressant for renal, liver and other transplants. Therapeutic ranges are: 10-15 ng/mL for post-transplant and 5 -10 ng/mL for maintenace, with more recent studies suggesting minimization range to be 3-7 ng/mL. According to CAP surveys, tacrolimus may be monitored by 4 immunoassays/analyzers, and by LC-MS-MS and Mass Spectrometry. More recently, the QMS turbidimetric immunoassay was developed for tacrolimus.

Objective: This study initially established the clinical efficacy of the QMS tacrolimus assays by using AU 680 and Indiko, followed by comparison to clinically used assay by using Architect.

Methods: QMS tacrolimus is a turbidimetric immunoassay. Sample preparation included mixing 200 microliter of samples - patient whole blood with 200 microliter of the extraction reagent. After vortexing and centrifugation, the supernatant was transferred to sample cups. Drug in the supernatant and drug coated on microparticle underwent competitive binding for a limited number of antibody binding sites. If tacrolimus was absent, tacrolimus-coated microparticle was agglutinated in the presence of antibody reagent. If tacrolimjus was present, agglutination was partially inhibited depending on tacrolimus concentration. Thus, agglutination rate was inversely proportional to everolimus concentrations, and was measured photometrically. Six calibrators ranged from 0 to 30 ng/mL.

Results: Precision studies of control samples showed the following mean concentrations and CVs: n= 10, AU 680, 4.14 ng/mL and CV = 2.6%, and Indigo, 3.96 ng/mL and 11.6%., and n =20, AU 680, 9.67 ng/mL and 2.4%., Indiko, 9.79 ng/mL and 2.3% respectively. Calibration stabilities for both AU680 and Indiko were shown to be 10 days. Comparison studies of the three analyzers for kidney transplant samples with concentration ranging from <2.0 to 25.8 ng/mL showed the following slopes, intercepts and correlation: Arch. vs AU 680 for n = 95, 1.224, 0.62 and 0.976., Arch. vs Indiko n = 95, 1.204, 0.59, and 0.965., and AU 680 vs Indiko for n = 97, 0.979, 0.03, and 0.982.

Conclusions: Tacrolimus may be monitored by the QMS assay using two autoanalyzers with adequate sensitivity and acceptable precision. While both QMS assays offered comparable tacrolimus determination suitable for monitoring renal transplant patient, the concentrations were about 20 to 22% higher than those obtained by the clinically used comparison method using Architect.

B-421

An Immunoassay for Methotrexate in Blood on ARCHITECT *i* System

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Background: Methotrexate (MTX) is a cancer therapeutic drug for leukemia, osteosarcoma, non-Hodgkin's lymphoma and others. MTX levels in blood are monitored in patients to ensure appropriate therapy and determine when to intervene with the counter-acting 'rescue' therapy. An assay for measurement of MTX in serum and plasma on the ARCHITECT *i* System (ARCHITECT Methotrexate) is presented.
Methods: ARCHITECT Methotrexate is a competitive immunoassay. First, the instrument mixes and incubates a sample (calibrators, controls, sera or plasma) with anti-MTX antibody-coated magnetic microparticles, biotinylated anti-MTX antibody and acridinium-conjugated MTX. Then, the instrument washes the microparticles and adds pre-trigger and trigger solutions to initiate the chemiluminescence reaction. Signals obtained as relative luminescent units are inversely proportional to the amount of MTX in the sample.
Results: The limit of quantitation was ≤ 0.040 µmol/L MTX. The direct measuring range was from 0.04 to 1.5 µmol/L and up to 12000 µmol/L with specimen dilution. The 20-day imprecision study showed a total CV ≤ 7.1% within the range of 0.056 to 1705.433 µmol/L MTX with 6 controls and 5 panels on 4 instruments using 3 lots of reagents (n = 80). Deviations from linearity were -4.1% to 7.2% within the range of 1.881 to 0.012 µmol/L MTX. Spike recovery ranged from 92% - 94% for samples at 0.045, 0.909 and 9.090 µmol/L of MTX. In the interference studies, the average levels of MTX in the individual interfering substance-spiked samples (10 endogenous substances including human anti-mouse antibody and rheumatoid factor, and 21 therapeutic drugs) were within 90% - 110% of that in the unspiked control samples. Method comparison of ARCHITECT Methotrexate to TDx/TDxFLx Methotrexate II (TDx) generated a Passing-Bablok correlation as [ARCHITECT] = 0.00 + 1.01 [TDx] for samples within the range of 0.040 to 1.415 µmol/L (n = 92) and [ARCHITECT] = 0.00 + 1.04 [TDx] for samples within the range of 0.040 to 1624.760 µmol/L MTX, n = 142). Specimen storage study denoted that specimens could be stored at room temperature for 24 hours or at 2-8°C for 48 hours since the MTX values of the stored specimens described above were within ± 10% deviation from that of the baseline control concentrations. MTX concentrations in specimens collected in K2-EDTA, sodium heparin, and lithium heparin tubes were within ± 10% deviation from that collected in serum tubes across the range of 0.040 to 10.00 µmol/L MTX. Reagent on-board stability showed that the ARCHITECT Methotrexate reagents could remain

on the analyzer for a minimum of 30 days with no more than 10% shift from baseline. **Conclusion:** The ARCHITECT Methotrexate assay under development was demonstrated to be an accurate, precise, sensitive and robust assay for the measurement of methotrexate in human serum and plasma.

B-422

Monitoring Rivaroxaban Anticoagulation Therapy

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Background Currently, laboratory monitoring of direct acting anticoagulants, dabigatran (direct thrombin inhibitor), rivaroxaban and apixaban (factor Xa inhibitors) is not being performed based on their predictable pharmacokinetic profile and a lack of FDA-approved clinical assays to measure drug levels.

Relevance Measurement of drug levels may be useful to ensure drug clearance (e.g., in patients with active bleeding or those undergoing invasive procedures with high bleeding risk), as well as to ensure compliance in patients who develop thrombosis. Furthermore, the lack of any antidote also supports monitoring of patients.

Objective The first objective was to develop and validate a liquid chromatography tandem mass spectrometry (LC-MS/MS) assay for measuring serum rivaroxaban concentration. The second objective was to monitor serum concentrations in patients receiving rivaroxaban.

Methods An LC-MS/MS assay for measuring serum rivaroxaban concentration was developed. Briefly, 200 µL of serum was added to 700 µL methanol containing 15 ng rivaroxaban-d4 (Toronto Research Chemicals, Toronto, Canada). The sample was vortexed for 30 seconds, centrifuged at 3,000 rpm for 10 minutes and the supernatant transferred to a test tube. The supernatant was dried under a stream of nitrogen at 50ºC for 45 minutes with the subsequent residue reconstituted in 100 µL acetonitrile: water (30:70). 30 µL of the reconstituted residue was then injected onto a C-18 50mm x 3mm column (Hypersil Gold, Thermo Scientific), and separated using an acetonitrile/0.01% formic acid and water/10mM ammonium formate/0.01% formic acid gradient over 8.0 minutes at 40ºC and analyzed on a mass spectrometer (Agilent 6460, Agilent Technologies, Santa Clara, CA) using multiple reaction monitoring mode (MRM). One and two MRM transitions were used as quantifier (m/z 436.1>144.9) and qualifiers (m/z 436.1>231.1 and 436>73.0) respectively. Rivaroxaban calibrators (2.5, 5, 10, 25, 50, 100, 200 and 500 ng/mL) and quality control samples were prepared in bovine serum. Thirteen serum samples from a total of twelve patients who were receiving rivaroxaban were also analyzed.

Results The LC-MS/MS assay was linear across an analytical measurement range of 2.5-500 ng/mL with a slope of 1.004 and a correlation coefficient (r^2) 0.998. The intra assay imprecision was 2.3 % at 2.9 ng/mL (n=10), 2.9 % at 25 ng/mL (n=20), 1.2% at 36 ng/mL (n=20), and 2.0% at 217 ng/mL (n=20). The inter assay imprecision ranged from 2.7-7.4%. The limit of detection was determined to be 0.5 ng/mL and the limit of quantitation was set at 2.5 ng/mL. The rivaroxaban concentration in the patient samples ranged between 3.0-220 ng/mL.

Conclusion We have developed an LC-MS/MS assay for measuring rivaroxaban concentration in serum. In our initial cross sectional study, rivaroxaban could be reliably quantitated in samples from patients receiving the drug. Measuring serum concentration of direct acting anticoagulants is of clinical value, particularly for managing active bleeding events and in patients undergoing elective procedures. In the future, developing LC-MS/MS based anticoagulant drug panels are an attractive option for clinical laboratories to facilitate an improved management of patients receiving anticoagulation therapy by novel anticoagulants.

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Development of Abbott Phenobarbital Assay for the ARCHITECT *c*Systems Automated Clinical Chemistry Analyzers

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Introduction: The objective of this study is to develop a sensitive immunoassay intended for the in vitro quantitative measurement of phenobarbital in human serum or plasma on the ARCHITECT *c*Systems. The measurements obtained are used in the diagnosis and treatment of phenobarbital overdose and in monitoring levels of phenobarbital to help ensure appropriate therapy. Monitoring serum phenobarbital concentrations has been shown to improve patient therapy by providing physicians with a tool for adjusting dosage.

Methods: The Abbott Phenobarbital Assay is a liquid stable, homogenous particle-enhanced turbidimetric inhibition immunoassay used for the analysis of phenobarbital in serum or plasma. The assay consists of two reagents and is based on competition between phenobarbital in the sample and phenobarbital coated onto a micro-particle for anti- phenobarbital antibody binding sites. In samples lacking phenobarbital, the phenobarbital -coated micro-particles rapidly agglutinate in the presence of anti-phenobarbital antibodies. The rate of absorbance change is measured photometrically, and is directly proportional to the rate of particle agglutination. In samples containing phenobarbital, the agglutination reaction is partially inhibited, slowing the rate of absorbance change. A concentration-dependent classic agglutination inhibition curve can be obtained, with maximum rate of agglutination at the lowest phenobarbital concentration and the lowest agglutination rate at the highest phenobarbital concentration.

Results: The performance of the Abbott Phenobarbital Assay was evaluated on the Abbott ARCHITECT *c*8000 analyzer. Based on guidance from Clinical and Laboratory Standards Institute (CLSI) protocol EP17-A2, the assay demonstrates a Limit of Quantitation of ≤ 2.0 µg/mL using inter-assay precision ≤ 7% CV or ≤ 0.7 µg/mL SD and bias within 10% or 1.0 µg/mL over an extended period. The assay is linear from 2.0 to 80.0 µg/mL using guidance from CLSI protocol EP6-A. Assay precision was evaluated using CLSI guideline EP5-A2. A tri-level commercial control and six human serum samples containing phenobarbital at concentrations ranging from 2.5 µg/mL to 77.2 µg/mL were tested. Each sample was assayed in duplicate twice a day for 20 days with at least two hours between runs. The precision ranged from 1.1 %CV to 3.3 %CV for Within-Run and 1.7 %CV to 6.7 %CV for Total-Run. The assay accurately recovered spiked phenobarbital at levels representing sub-therapeutic, therapeutic, and toxic samples. No significant interference was observed with various endogenous substances or compounds whose chemical structure or concurrent therapeutic use would suggest possible cross-reactivity. Abbott ARCHITECT Phenobarbital patient correlation studies: new vs. current on-market assay yielded a regression equation of y=1.00x + 0.42 and a correlation coefficient of 1.00. New assay vs. HPLC yielded y= 0.93x + 0.68 and a correlation coefficient of 0.99. The new reagent has an onboard stability of 40 days and calibration curve stability of 14 days.

Conclusion: The Abbott Phenobarbital Assay enables measurement of phenobarbital in human serum or plasma with high precision across the linear range. The ability to monitor levels of phenobarbital with high accuracy can help ensure appropriate therapy. The assay has applications on the ARCHITECT *c*16000, *c*8000, and *c*4000.

B-424

Development and Validation of a Robust Tandem LC-MS/MS Method for the Quantification of Antidepressants in Serum

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Background: Depression is a rapidly growing issue in the United States, with more than 11% of Americans taking antidepressant medications, allowing for antidepressants to become the most commonly prescribed drug in the United States. There are many drug classes that may be used to treat depression, including the selective serotonin-reuptake inhibitors (SSRIs) such as citalopram (Celexa) and sertraline (Zoloft), as well as the dopamine-reuptake inhibitor bupropion (Wellbutrin). However, treatment success may be variable and can either result in lack of efficacy due to suboptimal drug administration or non-compliance, as well as potential adverse side effects. Success is further complicated by environmental and genetic factors, and the delayed access to an optimal treatment regimen can have deleterious effects. Thus, methods for drug quantification can become important tools in the assessment of drug efficacy to optimize treatment regimens, particularly in scenarios where therapeutic regimens are frequently altered. Here, we present a liquid chromatographic-tandem mass spectrometric (LC-MS/MS) method for the robust, simultaneous quantification of the commonly prescribed antidepressants citalopram, sertraline and bupropion, and its active metabolite, hydroxybupropion, following FDA bioanalytical guidelines.
Methods: Drug-free human serum was spiked with citalopram, sertraline, bupropion and hydroxybupropion, along with their corresponding isotopically-labeled internal standards. Following protein precipitation, samples were injected onto the Prelude turbulent-flow-liquid chromatography system (Thermo Fisher Scientific), consisting of a Cyclone-P column for on-line solid phase extraction and a Hypersil Gold C18 column for chromatographic separation. Sample elution was achieved using acetonitrile (ACN) containing 0.1% formic acid. All four compounds were detected over 4 minutes using a TSQ Vantage Quadrupole mass spectrometer (Thermo Fisher Scientific) with a heated electrospray ionization (HESI) source in positive ionization mode operated in selected reaction monitoring (SRM) mode. Assay validation followed bioanalytical guidelines, and included precision (intra-assay) and accuracy, linearity, and functional sensitivity studies. **Results:** The analytical measuring range

of the assay for each analyte was 5-1000 ng/ml. Linearity was assessed from the slope of a $1/x^2$ weighted least squares-fitted linear regression analysis. Three independently prepared and analyzed calibration curves were used to assess linearity. Average slopes for citalopram, sertraline, bupropion and hydroxybupropion were 1.033, 1.036, 1.011, and 1.042, respectively. Further, recoveries across the aforementioned analytical measuring range for citalopram, sertraline, bupropion, and hydroxybupropion were 94.7-104.1%, 94.9-104.2%, 92.5-103.3%, and 92-105.2%, respectively. To assess precision, quality control solutions were prepared at 5 ng/ml (lower limit of quantification), 25 ng/ml (low), 125 ng/ml (mid) and 850 ng/ml (high) levels. Intra-assay precision was determined through analysis of six replicates of each level from a calibration curve. Intra-assay precision ranged from 2.6-3.6% for citalopram, 3.8-11.4% for sertraline, 5.4-9.2% for bupropion and 3.0-6.8% for hydroxyburporion. The functional sensitivity of the assay, which is the computational extrapolation of drug concentration with a %CV=20%, was 0.05 ng/ml, 0.57 ng/ml, 1.54 ng/ml, and 1.4 ng/ml using this method for citalopram, sertraline, bupropion and hydroxybupropion, respectively.

Conclusion: The development and validation of this LC-MS/MS method allows for the robust and high-throughput quantification of antidepressants commonly prescribed to our patient population.

B-425

Detection of 55 Drugs and Pain Management Analytes in Urine Using a Quantitative Liquid Chromatography-Tandem Mass Spectrometry (LC/MS-MS). An All-In-One Screening and Confirmatory Method.

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Background. Screening drug tests are qualitative, and are conducted to identify classes of drugs present in the urine using immunoassay-based methods. They rely on a threshold above which a positive result is produced, and do not detect lower concentrations of a drug. Confirmatory tests are used to verify a positive screening and identify a specific drug. They use gas chromatography-mass spectrometry (GC-MS) or high performance liquid chromatography-tandem MS (LC-MS/MS) methods. Our goal was to validate an all-in-one screening and confirmatory method by LC-MS/MS for 55 drugs and metabolites panel with lower limits of detection than immunoassays and GC-MS.

Methods. 70 urine samples were screened using QuickTox® Drug Dipcards (Branan Medical Corporation, Irvine, CA). Confirmation by GC-MS was a send-out to a large reference laboratory. LC-MS/MS was performed in-house using the Shimadzu (Shimadzu Corporation, Kyoto, Japan) Prominence 20A Liquid Chromatograph, followed by the AB SCIEX QTRAP® 4500 Mass Spectrometry System (AB SCIEX, Framingham, MA). Amphetamines, benzodiazepines, cannabinoids, cocaine, opiates, opioids, oxycodone, fentanyl and analogues and buprenorphine were analyzed in positive mode using the TurboIonSpray® ion source (Electrospray ionization). Barbiturates and marijuana metabolite were analyzed using the negative ion mode for maximum sensitivity. 50 µL of urine was mixed with 20 µL of combined internal standard (IS) solution to each tube. Hydrolysis was performed by adding 10 µL of the β-glucuronidase solution (100,000 units/mL) to each tube and incubation for two hours at 55°C. 110 µL of the curve diluent was added to each tube, mixed and centrifuged to separate any proteins and the supernatant. Samples were then analyzed by LC-MS/MS based on the presence of the specific MRM transitions for each analyte at the correct retention time. Quantitative measurement was accomplished by normalization of the peak area with the area of the IS for each specimen, including matrix specific calibrators, and quality control materials. The instrument software program automatically constructs a calibration curve, using the peak abundance data from the calibrator samples.

Results. Analytical performance of LC-MS/MS was excellent. CVs were <5% within the analytical measurement range. Among all the drugs analyzed, fentanyl showed the lowest estimated limit of detection (LOD) and limit of quantitation (LOQ), 0.003 ng/mL and 0.009 ng/mL, respectively. LOQ for fentanyl at CV<20% was <0.05 ng/mL and was used as clinical cut-off. LC-MS/MS clinical cut-offs ranged from 0.05 (fentanyl) to 20 ng/ml (barbiturates). Recoveries within the analytical measurement range were between 95-105%. Qualitative method comparison between GC-MS and LC-MS/MS using GC-MS cut-offs showed 91% agreement. Quantitative correlation between GC-MS and LC-MS/MS including all drugs was excellent (slope=1.04, r=0.99, p<0.05, n=21). 42% of initial drugs screened by immunoassay were confirmed by GC-MS, however 80% were confirmed by LC-MS/MS using our clinical cut-offs. Turn-around-time (TAT) was between 24-48 hours from receiving the specimen.

Conclusion. The quantitative drug analysis method by LC/MS-MS is easy and user-friendly with excellent analytical sensitivities. It requires one-step for extraction of drugs from urine and a direct injection into the LC/MS-MS system. Low volume of urine needed as well as analytical sensitivity is ideal for the Neonate Abstinence Syndrome program.

B-426

Formation of 6-monoacetylmorphine in urine specimens with high morphine concentrations during enzymatic hydrolysis

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Background: 6-monoacetylmorphine (6-MAM), a unique metabolite of heroin, is known as a definitive indicator of heroin intake. 6-MAM in urine is measured by GC-MS or LC-MS methods. Due to variable glucuronide conjugation rates between individuals, some laboratories employ enzymatic hydrolysis using glucuronidase during sample preparation to improve detection consistency. Acetate buffer is the primary choice for preparing enzymatic hydrolysis solution. In our routine LC-MS/MS analysis, we observed a number of low levels of 6-MAM in patient urine specimens with morphine at high concentrations. We hypothesized that the acetate buffer used for enzymatic hydrolysis serves as an acetylating agent leading to the formation of 6-MAM in urine specimens with high morphine concentrations. **Design:** Two sets of studies were performed. In one set, morphine standard and the major morphine metabolite, morphine-3-glucuronide (M3G), were spiked into morphine negative urine samples (n=2) to create five levels of morphine specimens (10,000- 50,000- 100,000- 150,000- 200,000 ng/ml) and M3G specimens (16,250- 81,250- 162,500- 243,750- 325,000 ng/ml) respectively. Internal standards (6-MAM-D3 and Morphine-D3) were added to the samples and the mixtures were incubated with 1M sodium acetate buffer (pH 4.5) at 60°C. Aliquots were taken at timed intervals (0, 2, 4, 6, 18 hours). In the 2nd set, leftover urine specimens (n=4) with elevated concentrations of morphine were incubated with 1M sodium acetate buffer (pH 4.5) at 60 °C for 0 and 18 hours. An additional study was performed to optimize the enzymatic hydrolysis procedure using an alternate buffer (1M citrate buffer, pH 4.5) to determine if 6-MAM would still be formed. The urine specimens used in the 2nd study, as well as the 200,000 ng/mL morphine and 325,000 ng/mL M3G spiked samples from the 1st study were incubated with 1M citrate buffer (pH 4.5) at 0 and 18 hours. Analysis was performed by an LC-MS/MS method. **Results:** Urine samples with elevated levels of both free morphine and M3G (>100,000 ng/mL) incubated for 18 hours using acetate buffer formed measurable amounts (≥ 5 ng/mL) of 6-MAM. All samples with <100,000 ng/mL and all samples incubated <18 hours did not form measurable amounts of 6-MAM. In all samples using citrate buffer, no 6-MAM was at the measurable level. **Conclusion:** False positive identification of heroin may be possible in urine specimens with extremely high levels (>100,000 ng/mL) of morphine when using acetate buffer for enzymatic hydrolysis.

B-427

Development of a Homogenous Enzyme Immunoassay for the Screening of Synthetic Cannabinoids Applicable to Automated Chemistry Analysers

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Background. Synthetic cannabinoids are chemical compounds that mimic the effects of tetrahydrocannabinol, the main active ingredient of cannabis. Originally sold under the brand name “Spice”, this brand name has become a generic term to include the entire class of “legal” smoking blends sold on the internet.The most common of the first wave of synthetic cannabinoid compounds available was JWH018. As these designer drugs continue to be sold there is a need for screening tests, which facilitate the detection process. Immunoassays are antibody-based tests that provide high throughput screening. The application of these tests to automated chemistry analysers is advantageous in clinical laboratory testing settings as it increases the screening capacity. This study reports the development of a homogeneous enzyme immunoassay for the screening of JWH018 and related metabolites in urine, applicable to a variety of automated systems.

Methods. The assay is based on competition between drug in the sample and drug labelled with glucose-6-phosphate dehydrogenase (G6PDH) for a fixed amount of antibody in the reagent. In the absence of drug in the sample, JWH018-labelled G6PDH conjugate is bound to antibody, and the

enzyme activity is inhibited. However, when free drug is present in the sample, the antibody binds to free drug and the unbound JWH018-labelled G6PDH then exhibits maximal enzyme activity. Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that can be measured spectrophotometrically at 340nm. The assay is qualitative utilising a 15ng/ml cut-off and in this evaluation the RX daytona analyser was used.

Results. The assay was standardised to the major urinary metabolite JWH018 N

pentanoic acid, 55 synthetic cannabinoidswere also detected with %cross-reactivity (%CR) values ≥5%, including the 5 hydroxypentyl metabolite and 4 hydroxypentyl metabolite of JWH018 (%CR: 62% and 24% respectively) and AM2201 N- 4-hydroxypentyl metabolite (%CR: 114%). The limit of detection in urine (n=20) was 5.2ng/ml (measuring range 0-20ng/ml). Inter-assay precision (n=20) was calculated as 3.07% at 10ng/ml, 1.89% at 15ng/ml and 2.06% at 20ng/ml. Total assay precision was assessed by running a negative (10ng/ml) and positive (20ng/ml) spiked urine samples, 2 replicates per run, 2 runs per day over 20 days. All replicates were correctly reported as negative and positive (n=80). Recovery was assessed at 10, 15 and 20ng/ml and all samples (n=20) showed recovery of between 115-124%. 101 samples were assessed against LC/MS and exhibited 92% agreement. When 245 samples were assessed with this assay and a commercially available biochip based immunoassay an agreement of 95% was obtained.
Conclusion. The results show that the developed homogeneous enzyme immunoassay for the screening of synthetic cannabinoids, exhibits optimal analytical. The assay presents a broad cross-reactivity profile, which increases the screening capacity. Moreover, the application to automated chemistry analysers improves the screening for designer drugs in clinical laboratories.

B-428

ARK™ Voriconazole Assay for the Roche/Hitachi Modular P Automated Clinical Chemistry Analyzer

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Background: Despite the availability of newer antifungal agents, invasive fungal diseases remain a leading cause of morbidity and mortality in immunocompromised patients. Voriconazole (VRZ) is an extended-spectrum triazole indicated for treatment of invasive fungal diseases. Here an ARK enzyme immunoassay for therapeutic drug monitoring (TDM) of VRZ is described.

Methods: The ARK Voriconazole Assay is a liquid stable homogeneous enzyme immunoassay, consisting of two reagents, 6 calibrators (0.00, 1.00, 2.00, 4.00, 8.00 and 16.00 µg/mL) and 3 controls (1.50, 5.00 and 10.00 µg/mL). The performance of the ARK assay was evaluated on the Roche/Hitachi Modular P analyzer. Limit of quantitation, linearity, precision, recovery, specificity and method comparison were studied.

Results: The assay measured concentrations as low as 0.50 µg/mL and was linear to 16.00 µg/mL. Total precision ranged 5.1% to 6.3%CV and within-run precision ranged 4.3% to 4.8%CV in a 20-day study (CLSI guideline EP15-A2) that evaluated precision for the 3 quality controls in a synthetic proteinaceous matrix and for similar concentrations in spiked serum samples. The assay accurately recovered spiked VRZ samples throughout the assay range. The assay did not crossreact with the antifungal fluconazole, itraconazole, and posaconazole. Voriconazole N-oxide (major metabolite) was tested at 10 µg/mL and no crossreactivity was observed. No interference from endogenous substances, anticoagulants and potentially co-administered drugs occurred at the elevated concentrations studied. Seventy-four specimens at concentrations throughout the range (0.5 to 8.1 µg/mL) were assayed and gave the following Passing Bablock regression results when compared to LC/MS/MS values: ARK = 1.01 LC/MS/MS + 0.07 (r2=0.98).

Conclusions: The ARK Voriconazole Assay measures VRZ in human serum or plasma with excellent precision at very low concentrations which is essential for long-term monitoring of patients. Ability to measure trough levels of VRZ with high accuracy and fast turn-around time makes this method clinically useful for VRZ TDM.

B-429

Ultrafast, high-throughput quantitative analysis of Carboxy-THC in urine using Laser Diode Thermal Desorption coupled to tandem mass spectrometry

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Background: The 11-Nor-9-Carboxy-THC (THCC) is a major metabolite of THC in urine. The confirmation of THCC in patients is often conducted in urine as a non-invasive procedure to determine the presence of drugs of abuse. The detection and quantification of THCC are traditionally performed by gas (GC) or liquid (LC) chromatography coupled to mass spectrometry (MS) or tandem mass spectrometry (MS/MS) methods.

The laser diode thermal desorption (LDTD) ion source uses an infrared laser diode to thermally desorb neutral species of THCC molecules from a dried sample. These neutral species are carried into a corona discharge region, where they undergo efficient protonation and are directed into the mass spectrometer. The total analysis time is performed very rapidly (9 seconds).

The objective of this work is to validate this analysis method and test different real samples using the LDTD-MS/MS. A cross validation study against the LC-MS/MS approach for the analysis of THCC was done in order to evaluate the performance of the alternative LDTD-MS/MS developed method.

Methods: A calibration curve and quality control materials were prepared in blank urine samples. To 500 uL of calibrators, QC and patient specimens, 50 µL of internal standard (THCC-d9, 5ug/mL in MeOH) and 200 uL KOH (3N) were added. The mixture was vortex-mixed and incubated at 38°C for 15 minutes for the glucuronide hydrolysis. A liquid-liquid extraction was then performed by adding 200 uL of HCl (6N) and 1000 uL of Hexane:EtAc (95:5). After vortexing and centrifugation at 5000 rpm for 2 min, 6 uL of the organic layer was deposited in the LazWell Plate (pre-coated with EDTA 200 ug/mL). The LDTD laser power was ramped to 45% in 3 seconds, and shut down after 1 second. Negative ionization mode was used, and API-5500 QTrap system was operated in MRM mode with MS transitions of 343->245 and 352->254 for THCC and THCC-d9 respectively.

Results: The calibration curves show excellent linearity with r2 = 0.9991 between the quantification range of 5 to 5000 ng/mL. Inter-run, intra-run accuracy, and precision ranges between 95,8 % and 103,1 % with RSD from 8,5 to 13,5%, respectively. No matrix effect or carryover was observed. The stability of THCC in and out (dry sample) of solution was evaluated for a period of 3-days. This method was cross validated with results from a traditional LC-MS/MS method for 49 real patient specimens. A good correlation between LC-MS/MS and LDTD-MS/MS data is obtained with r2 = 0.9956. All negative samples correlated accordingly.

Conclusion: LDTD technology provides unique advantages in developing an ultra-fast method for analysis of 11-Nor-9-Carboxy-THC in urine. This method has demonstrated accurate, precise and stable results with a run time of 9 seconds.

B-430

High-Throughput Quantitative Analysis of 5 Antifungal Drugs in Human Serum

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Research laboratories traditionally rely on HPLC and more recently LC/MS/MS for quantitative analysis of antifungal drugs (AFs). The objective of this study was to develop a rapid and very robust online SPE/MS/MS method for high throughput and accurate quantitation of 5 antifungal drugs (voriconazole, ketoconazole, fluconazole, itraconazole, and posaconazole) in human serum. This method employs protein precipitation followed by dilute and shoot on the SPE/MS/MS system, enabling analysis of all 5 AFs at 14 seconds per sample producing >20x savings in analysis time and solvent consumption compared to typical analytical methods. Samples were prepared by spiking AFs into drug-free human serum followed by adding internal standard mix, a protein crash with acetonitrile and then diluting samples 10-fold with a basic dilution. Samples were then analyzed via SPE/MS/MS using a reversed-phase C18 cartridge at 14 seconds per sample. A simple protein precipitation methodology followed by (dilutes and shoot) and analysis by SPE/MS/MS allows for the accurate and precise measurement of these analytes in human serum over a linear range of (0.2 - 25 mcg/mL). Standard curves consisting of each AF spiked into serum had excellent linearity within the measured range with an R2 value greater than 0.995. This methodology is capable of throughputs greater than 240 samples per hour providing a high-throughput and very efficient mode of analysis. Carryover was assessed by analyzing the AUC of the blank calculated as % of the mean peak area of the 0.2 mcg/mL samples. No significant carryover (< 5.0%) was determined for all of the AFs. QC standards for each AF were run over a series of days to establish both intra- and interday precision and accuracy values. The accuracies determined were within 10% and coefficient of variation values were all less than 10% for concentrations within the measured range. The reproducibility of the method was evaluated by measuring >2000 sequential injections of all five AFs spiked into serum. The instrument response was stable for each of the five analytes with coefficient of variation ranging from 2.3-6.9 % showing the robustness of the system, SPE cartridge lifetime and consistency of quantitation for the analytes in the panel.A panel of five antifungal drugs including: voriconazole, ketoconazole, fluconazole, itraconazole, and posaconazole were quickly, accurately, and precisely measured in serum using a simple protein precipitation protocol and an SPE/MS/MS system. Samples were analyzed at 14 seconds per sample, providing a high-throughput method capable of analyzing more than 240 samples per hour. This methodology provides comparable results to HPLC, and LC/MS/MS, but at > 20x the speed and efficiency of typical methods.

B-431

Measurement of the antiepileptic drugs, lacosamide and lamotrigine, in human serum by liquid chromatography-tandem mass spectrometry

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Background: Lacosamide and lamotrigine are antiepileptic drugs for whom the monitoring of drug levels allows for maximization of their seizure suppressing effects while minimizing the prevalence of adverse side effects. Lacosamide (VIMPAT®) is approved for adjunctive therapy to treat partial-onset seizures in epileptic patients 17 years of age and older. Lamotrigine (Lamictal) is approved for treatment of bipolar I disorder and a variety of seizure disorders including Lennox-Gastaut syndrome, primary generalized tonic-clonic seizures and partial-onset seizures. Both lacosamide and lamotrigine are completely absorbed after oral administration with negligible first-pass metabolism. Peak plasma concentrations occur within one to five hours after oral dosing, and the elimination half-life (in adults) is approximately 13 hours for lacosamide and 25 to 33 hours for lamotrigine. Both are believed to modulate the activity of voltage-gated sodium channels, in turn stabilizing neuronal activity.

Methods: Stable drug isotopes (lacosamide-$^{13}C,D_3$ and lamotrigine-$^{13}C,^{15}N_4$) are added to 50uL of serum as internal standards. Protein is precipitated from the mixture by the addition of acetonitrile. The supernatant is then further diluted with deionized water. Lacosamide, lamotrigine and their respective internal standards are then separated from the other serum constituents by liquid chromatography (TLX4, Thermo Fisher Scientific, Waltham, MA) using a Kinetex™ 5µm C18 50x4.6mm column (Phenomenex, Torrance, CA) followed by analysis on a tandem mass spectrometer (QTRAP 6500, AB SCIEX, Framingham, MA) equipped with an electrospray ionization source in positive mode. Ion transitions monitored in the multiple reaction monitoring (MRM) mode are m/z 255.9 → m/z 211.0 for lamotrigine, m/z 255.9 → m/z 144.9 for lamotrigine ion pair, m/z 261.0 → m/z 144.9 for lamotrigine-$^{13}C,^{15}N_4$, m/z 251.0 → m/z 108.0 for lacosamide, m/z 251.0 → m/z 90.9 for lacosamide ion pair, and m/z 255.0 → m/z 108.0 for lacosamide-$^{13}C,D_3$. Calibrators consist of six standard solutions ranging from 0 to 50ug/mL.

Results: Method performance was assessed using accuracy, precision, linearity and specimen stability. Accuracy of the method was assessed by comparison to external reference laboratories performing analysis of these drugs by tandem mass spectrometry. Comparison to the external laboratories demonstrated excellent agreement between the methods with overall differences of less than 3%. Precision studies were performed using commercially available quality control material (AEDII, UTAK Laboratories Inc., Valencia, CA) and an in-house prepared serum pool fortified with lacosamide and lamotrigine standard solutions. Intra-run precision coefficients of variation (CVs) ranged from 1.9% to 2.1% for lacosamide and 1.4% to 2.8% for lamotrigine. Inter-run precision CVs ranged from 3.3% to 4.7% for lacosamide, and 3.1% to 7.1% for lamotrigine. Linearity studies were performed using serum fortified with standard drug solutions. Linearity was demonstrated over the assay range (0.2 to 50ug/mL) for each analyte, yielding the following equations: observed lacosamide value = 1.0331*(expected value) - 0.1118, $R^2 = 0.9999$; observed lamotrigine value = 1.0194*(expected value) - 0.0590, $R^2 = 1.0000$. Specimens were stable when stored at ambient, refrigerate and frozen temperatures for up to 32 days.

Conclusion: This method provides for the simultaneous and reliable analysis of lacosamide and lamotrigine in human serum.

B-432

Development of a comprehensive drug screen in urine using liquid-chromatography quadrupole time of flight mass spectrometry.

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Background: Recently, there has been much interest in using high resolution mass spectrometry (HRMS) for drug screening applications as it offers several advantages over tandem-based methods (LC-MS/MS). Importantly, HRMS instruments are often used in an untargeted manner. This is especially attractive for broad spectrum drug screening because it allows for potential identification of unknown or unexpected compounds. Despite the interest in these techniques, few studies have investigated the performance of high resolution instruments in comprehensive drug screening. The objective of this study was to develop a broad spectrum drug screen for urine using LC-HRMS and to determine its performance in identifying drugs and metabolites in 100 routine clinical samples.

Methods: All chromatographic separations were performed on a Phenomenex Kinetex 2.6-µm C18 column (3 x 50 mm) thermostatted at 30 degrees C. A binary mobile phase consisting of (A) 0.05% formic acid in 5 mM ammonium formate (aq.) and (B) 0.05% formic acid in acetonitrile/methanol (50:50 v/v) was ramped linearly from 2% to 100% B over 10 minutes. To determine the performance of our assay, 100 patient comparison urine samples were diluted 1:5 with 12.5% acetonitrile/methanol (50:50 v/v) in water and spiked with fentanyl-d5 internal standard. Data were collected on an ABSciex TripleTOF® 5600 System operating in full-scan positive ion mode with IDA-triggered acquisition of product ion spectra. Data were analyzed using the MasterView function of PeakView software (AB Sciex) and Analyst TF (AB Sciex, Version 1.5.1). Criteria for positive identification of a drug included chromatographic retention time, accurate mass, isotope pattern, and library match. Results from the HRMS analysis were compared to several other methods (LC-MS/MS, LC-Orbitrap, gas chromatography-mass spectrometry, immunoassay and patient prescription history) to determine whether a compound was a true positive or false positive.

Results: Retention times were established for 210 compounds with coefficient of variations that ranged from 0 to 9%. The lower limit of detection was 25 ng/mL or less for 83% of the compounds. Using a targeted approach for data analysis and an in-house spectral library, our HRMS method found 523 positive hits in the patient comparison samples. Of these candidate hits, 509 (97%) were verified by another method meaning that only 14 were deemed to be false positives. The HRMS missed 52 compounds that were identified by one or more of the reference methods.

Conclusion: Overall, the HRMS method identified compounds with high confidence; 97% of the compounds found by HRMS were verified by another method. The majority of the 52 missed compounds could be attributed to differences in lower limits of detection. In general, the targeted, LC-MS/MS method had slightly better sensitivity than the HRMS method. However, the ability to detect unknown or unexpected compounds still makes HRMS very appealing. Therefore, a combination of LC-MS/MS and HRMS may be the most effective approach for broad spectrum drug screening.