Wednesday, July 29, 2015

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

TDM/Toxicology/DAU

B-298

Analytical Performance of an Assay on ARCHITECT i System for Measurement of Methotrexate in Human Serum or Plasma

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BACKGROUND: Cancer therapeutic drug methotrexate (MTX) is often monitored in patients to ensure appropriate therapy. An assay on the ARCHITECT *i* System (ARCHITECT Methotrexate) was analytically evaluated for measuring MTX in human serum and plasma. The ARCHITECT Methotrexate assay is under development and has not been cleared by the FDA.

METHODS: The ARCHITECT Methotrexate is a one-step immunoassay. The instrument mixes and incubates a sample (calibrators, controls, sera or plasma) with anti-MTX antibody-coated paramagnetic microparticles and acridinium-conjugated MTX followed by washing and chemiluminescent reaction triggering. Signals obtained as Relative Light Units (RLU) are inversely proportional to the amount of MTX in the sample.

RESULTS: The MTX assay showed a maximal LoQ (Limit of Quantitation) of 0.020 µmol/L. The measuring range was from 0.040 to 1.500 µmol/L, and up to 2500 µmol/L with specimen dilution. The 20-day imprecision study showed a total CV ≤ 7.5% for samples in which MTX levels ranged 0.040 - 12.500 µmol/L and a total CV ≤ 10% for samples in which MTX level > 12.500 µmol/L (n = 80). Deviations from linearity were ± 10% within the range of 0.040 to 1.500 µmol/L MTX. In the therapeutic interference studies, the cross reactions of 20 individual therapeutics at ≥1000 µmol/L were 0%. The cross reactions of Aminopterin and DAMPA, respectively, at 5 µmol/L ranged 43% - 83%. Aminopterin and DAMPA were also tested individually in human serum at 1000 µmol/L in the absence of MTX, and The cross reaction was 61% and 46%, respectively. Each of 11 Endogenous interfering substances, including human anti-mouse antibody and rheumatoid factor, was individually spiked into human serum or plasma samples, and yielded a difference of -7% to 8% away from the control samples. Method comparison of ARCHITECT Methotrexate to tDx/TDxFLx MTX II (TDx) generated a Passing Bablok correlation as [ARCHITECT] = 0.005 + 0.946x [TDx] for samples ranging 0.040 - 0.993 µmol/L (n = 86) and [ARCHITECT] = -0.004 + 1.016x [TDx] for the samples ranging 0.040 - 888.000 µmol/L (n = 119), respectively. Method comparison ARCHITECT Methotrexate to LC/MS/MS ACUITY TQD generated a Passing Bablok correlation as [ARCHITECT] = 0.016 + 0.923x [LC/MS/MS] for the samples ranging 0.040 - 1.438 µmol/L (n = 101). MTX values in specimen stored at room temperature for 24 hours or at 2-8°C for 48 hours were within ± 10% deviation from that of the baseline control ones. The mean concentrations of MTX in specimens collected in K2-EDTA, sodium heparin, and lithium heparin tubes were within ± 10% deviation from that collected in serum tubes (Red Top only) within the Range of 0.040 - 1.500 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 is an analytically accurate, precise, sensitive and robust assay for the measurement of methotrexate in human serum and plasma.

B-299

Use of Urine Drug Screening Positivity Rates to Evaluate the Clinical Impact of Replacing Immunoassay and Gas Chromatography Mass Spectrometry Testing with Liquid Chromatography Tandem Mass Spectrometry

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Background: Urine drug testing (UDT) is often performed to identify potential aberrant drug behaviors (e.g., drug addiction, abuse, misuse, diversion and/or non-compliance). Urine specimens referred to our regional reference laboratory by community-based independent physicians traditionally received UDT using a combination of immunoassay (IA) and gas chromatography mass spectrometry (GC-MS) testing. This tandem IA and GC-MS approach was replaced by a targeted liquid chromatography tandem mass spectrometry (LC-MS/MS) testing protocol in September 2013. Using defined positive/negative cut-off concentrations, this LC-MS/MS method tests for the urinary presence of 63 different licit and illicit drugs and was primarily designed to identify specific opioid and benzodiazepine use. As a quality measure, the efficacy of this new approach to UDT was reviewed post-implementation. UDT positivity rates were used to characterize the prevalence of licit and illicit drugs respectively detected by IA+ GC-MS and LC-MS/MS testing and evaluate the clinical impact of the testing methodology change.

Methods: Urine drug testing results from 134377 and 165209 unique specimens respectively tested by IA+GC-MS (from June 2012 to August 2013) and LC-MS/MS (from September 2013 to December 2014) were retrospectively reviewed. No clinical histories or medication lists were available for either heterogeneous patient cohort. The percent positivity rates for all analytes detected by the IA+GC-MS and LC-MS/MS UDT methods were determined. The relative reported prevalence of analytes contained within both protocols was directly compared. Method specific drug positivity rates were also tabulated to quantify the detection sensitivities for analytes unique to each procedure. IA-based testing included: benzodiazepines; cannabinoids; cocaine; ethanol; opiates; and oxycodone. GC-MS and LC-MS/MS sample pretreatment included: solid-phase extraction and chemical derivatization; and β-glucuronidase incubation and protein precipitation.

Results: Positivity rates for opiate and benzodiazepine IA testing were 22.9 and 8.6%, respectively. LC-MS/MS UDT yielded a relatively higher number of positive test results for the individual opioid and benzodiazepine-related analytes contained in both chromatographic methods. The GC-MS vs. LC-MS/MS positivity rates for selected analytes were: methadone+EDDP, 41.9 vs. 51.4%; oxycodone, 15.9 vs. 16.2%; codeine+morphine+hydromorphone, 11.1 vs. 21.4%; fentanyl+norfentanyl, 3.9 vs. 6.4%; buprenorphine+norbuprenorphine, 0.01 vs. 8.9%; diazepam+nordiazepam+oxazepam+temazepam, 0.01 vs. 7.3%; clonazepam+7-aminoclonazepam, <0.01 vs. 9.0%; and lorazepam, <0.01 vs. 4.9%. Higher LC-MS/MS positivity rates were also generally observed for stimulant, antidepressant and anesthetic drugs common to both methods. The GC-MS testing protocol identified the presence of multiple unique antidepressant, cardiac, antipsychotic and sedative drugs that were not included in the targeted LC-MS/MS method. Their collective positivity rate was ≤4.1%. Evidence of cannabinoid and cocaine-use was respectively detected by IA+GC/MS and LC-MS/MS testing in 30.0 and 41.3% and 7.8 and 10.5% of all tested specimens.

Conclusion: Replacing our combined IA+ GC-MS UDT procedure with a LC-MS/MS protocol increased the detection sensitivity for the targeted licit and illicit drugs but reduced the overall number of drugs that could be detected. Drug positivity rates can be used to identify the impact of a UDT methodology change to patient management. The study's respective drug prevalence can also be used towards the development of LC-MS/MS UDT methods.

B-300

Inappropriate Use of Suboxone® Film to Pass Drug Testing with Cross-Talk to 6-MAM

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Background: Suboxone® is a commercial preparation of buprenorphine and naloxone imbedded in film for administration via sublingual application to achieve better bioavailability than oral administration. Typically, patients receiving Suboxone provide urine specimens with low or undetectable naloxone and buprenorphine. Instead, norbuprenorphine, an active metabolite of buprenorphine, is detectable for

1-2 days. Thus, we were concerned when specimens from three patients each showed the presence of both naloxone and buprenorphine at very high concentrations and no detectable norbuprenorphine. Surprisingly, all three urine specimens also had a low but detectable 6-monacetylmorphine (6-MAM) peak, but no evidence of morphine, which would be expected if heroin had been used. The objective of this study was to investigate suspected specimen adulteration, which was indicated by the presence of the unusual metabolite patterns observed in all three samples.

Methods: All samples were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using a pain management panel capable of detecting and quantifying buprenorphine, naloxone, norbuprenorphine, 6-MAM and morphine, in addition to many additional compounds. Each compound is identified and quantified by two ions using multiple reaction monitoring (MRM) and a unique retention time (RT). The relevant parameters for this study were: buprenorphine, MRM 468.3-396.2 and 468.3-414.3, RT 5.63 min; norbuprenorphine, MRM 414.3-101.1 and 414.3-165.2, RT 4.66 min; naloxone, MRM 328.2-212.0 and 328.1-253.0, RT 3.91 min; and 6-MAM, MRM 328.1-165.1 and 328.1-211.1, RT 3.74 min. Suboxone film was provided by our pharmacy to investigate the possibility of direct addition into the specimens by dipping the film into drug-free urine and assaying the sample by the LC-MS/MS method.

Results: Following immersion of Suboxone film into drug-free urine, we found high concentrations of buprenorphine and naloxone and no evidence of the norbuprenorphine metabolite. Additionally, a small peak associated with 6-MAM was observed, similar to the patient samples as noted above. To confirm cross-talk as a possibility we assayed a naloxone standard (5000 ng/mL) and identified a peak corresponding to 6-MAM. The 6-MAM quantified at a concentration of 85 ng/mL, with appropriate ion ratios; however, the retention time was 3.84 min, shifted from the expected retention time of 3.74 min. It is interesting to note cross-talk the other way was also observed. Injecting 6-MAM (5000 ng/mL) resulted in a detectable signal of naloxone. However, the ion ratios between the two quantifying and qualifying MRMs for naloxone were discrepant: The ion ratio of the ions [MRM 1/MRM 2] for the naloxone control and the cross-talk peak were 1.068 and 0.272 respectively making this non-reportable.

Conclusion: Immersion of Suboxone film into urine samples produces an atypical metabolite pattern of elevated buprenorphine and naloxone with undetectable norbuprenorphine. Naloxone, which is isobaric with 6-MAM, appears to interfere with the MRM's selected for 6-MAM in our assay. This interference is approximately 1.7% of naloxone. Thus, we have identified an unusual metabolite pattern in our assay of buprenorphine, naloxone, and 6-MAM without additional detectable metabolites, which strongly suggests specimen adulteration by directly adding Suboxone to urine.

B-301

Tobramycin Level Monitoring - Discrepancy Between Immunoassays

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Objective and Relevance: Tobramycin, an aminoglycoside, is used primarily to treat gram-negative bacterial infections. Therapeutic drug monitoring is an essential component of clinical therapy. Various immunoassay techniques with applications on many clinical chemistry analyzers have been developed for this purpose. A local, preliminary inter-laboratory comparison demonstrated a bias >30.0% between two such methods. Subsequently, a research plan was devised to investigate performance of tobramycin immunoassays used in Alberta clinical laboratories.

Methodology: Drug-free plasma and serum pools were prepared and spiked with tobramycin stock (100 mg/L) to obtain concentrations of 0.5, 2.0, 5.0 and 10.0 mg/L. Aliquots of each pool (0.5 mL) and aliquots of drug-free serum and plasma were distributed to four Alberta Health Services laboratories. In addition, Validate® linearity testing material (Maine Standards Company, Cumberland Foreside, ME, USA) was purchased, aliquoted and distributed for analysis. Target concentrations (independent of instrumentation) were graciously provided by Maine Standards. The lowest (0.6 mg/L) and highest (11.8 mg/L) concentration Validate linearity samples are prepared gravimetrically and three intermediate concentrations by mixing appropriate proportions of low and high material. Tobramycin analyses were performed as follows: Laboratory A - Beckman Coulter DxC 800 analyzer (Brea, CA, USA) using a particle-enhanced turbidimetric inhibition immunoassay (PETINIA); Laboratories B and C - Siemens Dimension Vista analyzers (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA) by a PETINIA assay; Laboratory D - Roche cobas c501 analyzer (Hitachi High-Technologies Corporation, Tokyo, JP) using a homogenous enzyme immunoassay (HEIA) method. Analyses were carried out within 48h of sample preparation.

Results: The Beckman Coulter DxC800 PETINIA assay gave results within 10.0% of target for all spiked samples with one exception. Serum and plasma samples targeted at 0.5 mg/L produced results less than the assay limit of quantitation (0.5 mg/L), subsequently producing bias greater than 10.0%. Biases on the Siemens Vista PETINIA assay ranged from -12.0 to -40.0% (Laboratory B) and -20.0 to -25.0% (Laboratory C). Results from the Roche cobas c501 HEIA assay demonstrated biases of +20.0% and +40.0% at 0.5 mg/L in plasma and serum respectively and biases ranging from -5.0% to -18.0% in the remaining samples. Analysis of the Validate linearity samples produced similar results. The Beckman PETINIA assay gave results within 7.0% of target for all samples with one exception (0.6 mg/L target: +16.7%). Results from the Siemens PETINIA instruments ranged from -11.8 to -31.4% (Laboratory B) and -33.3 to -41.2% (Laboratory C) of target. The Roche HEIA assay gave results from -17.6 to -26.3% of target with one exception (0.6 mg/L: +16.7%). However, 2013-2014 data from the New York State Therapeutic Substance Monitoring Proficiency Testing Program (n=30 samples) demonstrated average biases from gravimetric target of 15%, -9% and -4% for the Beckman Coulter DxC 800, Siemens Dimension Vista and the Roche cobas c501 analyzers respectively.

Conclusions: Different methods for assessing accuracy of tobramycin immunoassays give inconsistent results. Diagnostic companies should use "gold standard" methods (e.g. tandem mass spectrometry) to validate the accuracy of their tobramycin assays. This will prevent misguided dosage adjustments and potential undesirable clinical consequences for patients treated with tobramycin.

B-302

A Fast, Sensitive, and High-Throughput LC-MS/MS Assay for Benzodiazepines/Z-Drugs/Barbiturates

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Background:

Benzodiazepines, Z-drugs, and barbiturates belong to a group of psychotropic drugs that are often prescribed for the treatment of anxiety, depression, and insomnia. However, as controlled substances, these drugs have the potential for overdosage or abuse. The development of fast and accurate methods for the screening and confirmation analysis of these drugs therefore becomes very critical in toxicological, clinical, and forensic laboratories. LC-MS/MS offers superior sensitivity, selectivity, and robustness for simultaneously detecting benzodiazepines and non-benzodiazepines in complex biological matrices. The current work presents a fast, reliable, and accurate LC-MS/MS method on an IONICS 3Q 120 triple quadrupole mass spectrometer with Restek Biphenyl column for the analysis of a total of 43 compounds, using fast polarity switching.

Methods:

The mixed drug standard solutions and analytical LC column were obtained courtesy of Restek. The mixed drug standard solutions were further diluted with 50/50 mobile phase A (100% H2O, 0.1% formic acid) and mobile phase B (100% Methanol, 0.1% formic acid) to make a series of concentrations ranging from 0.024-50 ng/mL for benzodiazepines, Z-drugs and other anxiolytic/sedatives/muscle relaxants, and 0.24-500 ng/mL for barbiturates. An IONICS 3Q 120 mass spectrometer, equipped with a heated coaxial flow ion source and Hot Source-Induce Desolvation (HSIDTM) interface was used for the best ionization and sampling efficiencies. Electrospray ionization was used for this analysis. The time-managed MRM in MolanaTM software was used to optimize the dwell time for each compound based on the retention times and the number of MRM transitions within given experiments. Fast polarity switching allowed for simultaneous analysis of positive and negative ions within a single run. A Shimadzu Prominence LC system was used. Restek Raptor Biphenyl column (50X2.1mm, 2.7um) gave good separation and nice peak shapes in the chromatogram. The injection volume used was 10 µL. A gradient method was created with a flow rate of 0.6 mL/min and a total LC cycle time of 6.5 minutes.

Results:

The Restek Raptor Biphenyl column (50X2.1mm, 2.7um) gave good separation and nice peak shapes in the chromatogram. A gradient method was created with a flow rate of 0.6 mL/min and a total LC cycle time of 6.5 minutes. All 43 compounds eluted within this 6.5 minutes run time. No carryover was detected in the blank injections immediately following the upper level calibration samples. The calibration curves showed good linearity for all the analytes across the full concentration range with coefficients R2>0.996. All calibration curves used a linear weighting regression of 1/x. The LLOQs for all 43 drugs were in the range of 0.024 to 4 ng/mL, which is much lower than the typical confirmation cutoff concentration (50 ng/mL) for most of

the drugs. At the LLOQs, the accuracy was between 83-116%, and CVs were <14% for all analytes.

Conclusion:

These results clearly demonstrate that this LC-MS/MS method using the IONICS 3Q 120 mass spectrometer and Restek Raptor Biphenyl column can provide a fast, accurate and high throughput solution for clinical pain management to monitor patient drug use and program adherence or for drugs of abuse or other workplace drug testing.

B-303

An Evaluation of the Rapid MEDTOXScan Drug Screening Method for Emergency Department Patients Presenting at Satellite Hospitals

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Objective: While rapid drug screening is routinely available in large medical center core laboratories, satellite hospitals must rely on accurate point-of-care (POC) drug screening methods for emergency department (ED) patients. However, with adoption of such drug screening methods, care must be taken to insure that results obtained correlate closely with those observed in the core laboratory. Indeed, due to variations in methodologies, cross-reactivites, and cutoffs, a clear understanding of the degree of agreement between POC testing and core lab drug screening is essential. Hence, we present a comparison study of a rapid POC drug screen method with our existing core laboratory ultraviolet kinetic method.

Method: We examined 71 ED patient samples (351 individual drug assays) with the PROFILE®-V MEDTOXScan® Drugs of Abuse Test System (MEDTOX, Burlington, NC) a one step, rapid, qualitative immunochromatographic test with results in 10 minutes comparing the latter with the ADVIA® 1800 Clinical Chemistry System (Siemens, Malvern, PA). Eleven drug classes were tested including amphetamine(AMP), barbiturate(BAR), benzodiazepines(BZO), cocaine(COC), methamphetamine(MAMP), opiates(OPI) including methadone(MTD) and oxycodone, phencyclidine(PCP), tricyclics(TCA), and cannabinoids(THC). Data were statistically analyzed using chi-square, Fisher's exact test, and McNemar's exact test.

Results: While we observed 17(4.8%) discrepant results in 351 individual drug assays, statistically there were no significant differences ($p>0.05$) between individual and pooled MEDTOX and ADVIA tests (see table). Also, MEDTOX appeared to be more sensitive than ADVIA for BZO, oxycodone, TCA, and MAMP. Discrepancies between methods were largely due to differences in cutoff levels with cross-reactivity differences also playing a role.

Conclusions: Rapid urine testing for drugs of abuse plays an essential role in ED patient care regardless of the hospital setting. We observed that the MEDTOX method for drug screening on ED patients seen at our satellite hospitals compares favorably with results obtained on our presumably more sophisticated ultraviolet core laboratory method.

Statistical Comparison of MEDTOX Versus Advia						
Drug	Total Discrepant	MEDTOX + Advia -	Advia + MED-TOX -	McNemars's Test	Chi-Square	Comment
Benzodiaz-epines	4/71	4	0	P=0.13	P=0.22	MEDTOX cutoff 150 ng/mL; Advia 200 ng/mL
Oxycodone	3/71	3	0	P=0.25	P=0.34	MEDTOX cutoff 100 ng/mL; Advia 300 ng/mL
Tricyclics	2/71	2	0	P=0.50	P=0.69	Unresolved Discrepancy
Metham-phetamine	4/71	3	1	P=0.63	P=0.67	MEDTOX cutoff 500 ng/mL; Advia 1,000 ng/mL
Amphet-amine	1/71	0	1	P=1.0	P=1.0	Unresolved Discrepancy
Opiate	3/71	0	3	P=0.25	P=0.46	Possible crossreactiv-ity differ-ences
Total Dis-crepancies Individual Assays	Total Cases with Discrepan-cies	MEDTOX + Advia -	Advia + MED-TOX -	McNemar's Test	Chi-Square	
17/351 (4.8%)	17/71 (23.9%)	12	5	P=0.14	P=0.27	

B-304

Monitoring two transitions by LC-MS/MS is not always adequate to identify benzoylecgonine in patient urine samples

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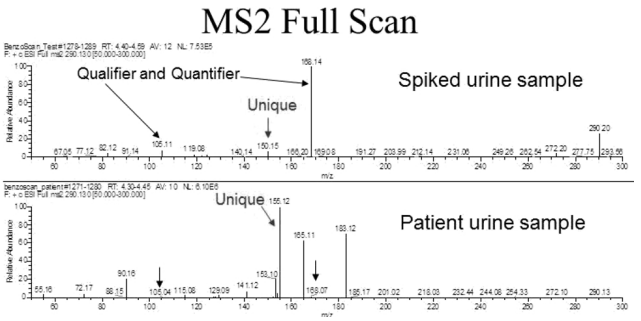
Background: Cocaine has a very short half-life in humans and measuring its major metabolite, benzoylecgonine (BZE) is routinely used to detect cocaine use. LC-MS/MS methods are highly specific and are used to confirm the presence of drugs or metabolites, such as BZE. As such, multiple reaction monitoring (MRM) is frequently used on the MS to monitor fragments resulted from a specific precursor. Typically one precursor ion to two fragment ions is utilized for quantitation and identification. Identification is achieved by calculating the ion ratio of the two ions. In our experience of BZE analysis by LC-MS/MS, some pain management doctors and patients questioned the accuracy of BZE identification. The aim of this study was to explore whether we may improve the accuracy of BZE identification by adding additional transitions. **Design:** Our routine LC-MS/MS method monitors two MRM transitions for BZE, 290→168 as quantifier and 290→105 as qualifier. MS2 Full scan was performed on both a sample spiked with BZE at 45 ng/mL and a patient sample with BZE identified but questioned by the ordering physician. **Results:** The MS2 full scan showed that in the spiked BZE sample, both 290→168 and 290→105 were present. An additional MRM of 290→150 was also present. In the MS2 full scan of the questionable patient sample, both 290→168 and 290→105 were present. However, 290→150 was absent while 290→155 was present. The unique MRMs 290→150 and 290→155 led us to conclude that the patient sample was not BZE. **Conclusion:** Although it is generally believed that monitoring 2 MRM transitions is sufficient for analyte identification it may not be adequate to accurately identify BZE in certain patient samples.



B-305

Reducing excessive inpatient phenytoin level orders - a test utilization review

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Background: Therapeutic drug monitoring (TDM) of phenytoin is critical as it displays non-linear elimination, has a narrow therapeutic index, has many drug interactions, and is 90% protein bound. Phenytoin also has a long half-life (24 ±12 hrs), and will reach steady state only after five to ten days following dose adjustment.

We noticed highly variable ordering patterns for serum phenytoin concentrations at our institution. Some samples were repeatedly drawn within hours and others up to two days following a dose adjustment. We even noted several cases of daily phenytoin (Total and Free) orders continuing for greater than 1 week, despite results indicating therapeutic concentrations or no change in clinical status. Repeated measurements prior to reaching steady state can potentially result in premature phenytoin dose adjustments and unnecessary blood draws.

Objective: Our objective was to develop institutional guidelines for the monitoring of phenytoin concentration in hospitalized patients. Our goal was to decrease unnecessary testing of both total and free phenytoin levels without adversely affecting patient care.

Methods: We began by examining every total and free phenytoin order on inpatients from August to September 2014. Next, we designed and initiated phenytoin monitoring guidelines that were created by a combined effort from the pathology, neurology and pharmacy departments on October 1, 2014. We then assessed the results of the intervention by recording all total and free phenytoin orders on inpatients over a four-month span.

Results: In the eight weeks prior to distributing our guidelines, there were a total of 227 total phenytoin and 139 free phenytoin orders on 42 different patients. After instituting our guidelines, there were a total of 128 total phenytoin and 43 free phenytoin orders on 50 different patients in a four-month span. Using the Mann-Whitney U test, the differences were significant ($p<0.001$) for both total and free phenytoin orders. The number of tests ordered per admission was 4.73 for total phenytoin and 2.90 for free phenytoin before institution of guidelines. Post-implementation, both the average per-admission total and free phenytoin orders decreased significantly to 2.46 and 0.83 ($p<0.05$), respectively. The number of total and free phenytoin results within therapeutic range did not change significantly post-intervention ($p=0.32$, $p=0.28$) nor were there differences in the number of phenytoin dose adjustments per admission ($p=0.078$).

Conclusion: Through a collaborative effort, we were able to successfully decrease the number of unnecessary total and free phenytoin tests ordered. With our data, we estimate that our guidelines will save each patient 12 mL of unnecessary blood loss per admission. We also calculated that our guidelines decreased patient billing by $42,000 over a four-month span. Phenytoin therapy requires monitoring using serum levels, but with a more standardized approach, we can both reduce the cost and improve the quality of care by reducing unnecessary blood draws.

B-306

A Novel Liquid Chromatography Tandem Mass Spectrometry Method for the Simultaneous Quantification of 44 Drugs/Metabolites in Meconium

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Background: Illicit drug use during pregnancy is a major social and medical issue. Meconium is advantageous for detecting prenatal drug exposure. The aim of this study was the development of an LCMSMS procedure for quantifying drugs and metabolites likely to be encountered in meconium.

Method: Patient samples (0.05±0.005 g) were weighed into glass vials. Fifty microliters of a 100 µg/mL internal standard, containing deuterated standards in methanol, was added followed by 1.5 mL of a 0.1M sodium acetate buffer (pH 5.1). The mixture was homogenized for 30 s, transferred to a 1.5 mL tube, and centrifuged at 13,000 rpm for 5 min at room temperature. The supernatant was transferred to a clean glass tube containing 1g of a buffered salts mixture (sodium chloride, sodium carbonate, sodium bicarbonate, 1:1:12 ratio,w/w) and 3 mL of an extraction solvent (methylene chloride, cyclohexane, isopropanol, 9:9:2 ratio,v/v). Tubes were inverted 5 to 10 times. The mixture was centrifuged at 3,500 rpm for 5 min at room emperature. Centrifugation was repeated until the buffy coat wasn't visible. The supernatant was transferred to a clean glass tube and gently evaporated to dryness at 40°C using a gentle stream of air. The extracted samples were reconstituted with 200 µL methanol, vortex mixed, allowed to stand at room temperature for 5 min, and evaporated to dryness at 40°C. Samples were reconstituted with 150 µL of mobile phase A, transferred to autosampler vials, and injected onto a RESTEK Ultra bi-phenyl analytical column (5µm, 50 x 2.1 mm) maintained at ambient temperature. Mobile phase A was 0.1% formic acid in water (1:1, v/v). Mobile phase B was 0.1% formic acid in acetonitrile (1:1, v/v). The acquisition method utilized 10 µL injection volume, 0.6 mL/min flow rate, and a gradient program of 98% A, increased to 95% B over 5.5 min, held for 0.8 min, decreased to 2% B over 0.2 min, and re-equilibrated at 2% B for 1.5 min. Run time was 8 min (injection to injection). The HPLC system consisted of Shimadzu pumps and autosampler. MSMS was performed on a Sciex API 4500 triple quadruple mass spectrometer with an electrospray source monitored in positive and negative ion modes.

Results: Specificity was assessed by retention times and quantifier/qualifier transition peak area ratios. Total imprecision (%CV), assessed at two concentration levels and 80 observations, was <15% for all analytes. Linearities ranged from 0.6 up to 1250 ng/g. Except for m-hydroxybenzoylecgonine, extraction efficiencies/recoveries were all >60%. Matrix effects of native analytes were similar to corresponding deuterated analogues and did not affect quantification. No carryover, endogenous or exogenous interferences were observed. Qualitative correlation between our procedure and a commercial LC-MSMS method showed 100% agreement at cut-offs. Quantitative correlations were also excellent (<20% differences).

Conclusion: We present the development of a LC-MSMS procedure for the simultaneous quantification of 44 drugs/metabolites in meconium employing a small amount of a single sample, deuterated internal standards, and single extraction for all analytes - without derivatization, enzymatic hydrolysis, additional chromatographic resolution, or preliminary immunoassay screening. This novel method is suitable for routine clinical use.

B-307

Improved Sensitivity and Throughput for the Quantification of Buprenorphine, Norbuprenorphine and Naloxone in Human Oral Fluid by LC-MS/MS.

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Background

Buprenorphine belongs to a class of medications called opioid partial agonist-antagonists while naloxone belongs to a class of medications called opioid antagonists. Buprenorphine is primarily metabolized in the liver by dealkylation to form the active metabolite norbuprenorphine. The combination of buprenorphine and naloxone (suboxone) is used to treat opioid dependence by preventing withdrawal symptoms when someone stops taking opioid drugs. However, suboxone produces similar effects to opioid drugs. Consequently, there is an urgent need to develop a sensitive and selective method to simultaneously quantify buprenorphine, norbuprenorphine and naloxone in human oral fluid. Utilizing a highly sensitive triple quadrupole mass spectrometer, our laboratory has developed a quantitative LC-MS/MS method with a limit of quantitation lowered to 20 pg/mL for buprenorphine.

Methods

Oral fluid samples were collected in Quantisal sampling devices from Immunalysis and diluted 2.5 times in a solution containing the internal standards. Considering the 4 times sample dilution in Quantisal, samples were finally diluted 10 times before injection into the mass spectrometer. Samples were analyzed by LC-MS/MS on an ABSciex Triple Quad 6500 System using an Agilent 1260 chromatographic System and a Phenomex Kinetex 2.6 µm Phenyl-Hexyl (50 x 2.1 mm) column. The total run time was 3.5 min and no sample clean-up or extraction was performed. Mass spectral data were obtained in positive electrospray mode. Detection and quantitation were performed by MRM of at least two transitions for each analyte and one transition for each internal standard.

Results

Seven-point calibration curves generated for buprenorphine (0.02-16 ng/mL), norbuprenorphine (0.05-40 ng/mL) and naloxone (0.5-400 ng/mL) with duplicate injections using 1/x2 weighting showed a good linearity ($R2 \geq 0.99$). No significant matrix effects were observed and no interferences were observed from common pain medications. This method was applied to the quantification of buprenorphine, norbuprenorphine and naloxone from patient oral fluids. The liquid chromatography Phenyl-Hexyl column showed stable performance throughout the application and the Triple Quad 6500 mass spectrometer showed high sensitivity and reproducibility. This study demonstrates that the application of LC-MS/MS dilute and shoot method to the analysis of oral fluid samples reduces time and labor spent on sample preparation. In addition, this method provides the means to offer a faster, more sensitive and sample-conserving assay to clinicians.

B-309

Determination of methyl ethyl ketone (MEK) in urine by headspace gas chromatography autosampler and flame ionization detector - GC/FID-HS.

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The determination and quantification of toxic volatiles are extremely important in clinical analysis, which involves the routine monitoring of industrial workers to check the exposure to potentially toxic components and the detection of endogenous metabolites in the body in certain clinical diagnoses.

The main action of MEK in the human body is depression of the central nervous system, producing narcosis potentiating the toxicity of other solvents, particularly carbon tetrachloride hepatotoxicity and neurotoxicity hexane (inhibition of the biotransformation metabolite 2,5- cyclohexanedione).

The MEK is a volatile substance widely used in the footwear industry, furniture, chemicals, paints, wood processing, among others. It is also used as solvent in paint removers, adhesives and polymer coatings. The MEK excreted in the urine can be used as an indicator for evaluation of occupational exposure.

The aim of this study was to validate a simple, rapid and sensitive method for the determination of MEK in urine by headspace gas chromatography with flame ionization detection.

The method consist in a simple extraction of MEK using 2-propanol as internal standard by evaporationand sampling the vapor above the fluid (blood, urine or others) after reached thermal equilibrium gas in a closed vial. The volatilized components present in headspace are aspirated by a syringe and injected into chromatograph.

The extraction involves the addition of 5 ml diluent solution containing the internal standard and 1 mL of the sample, standard, or control into a vial. The vial is shaken for 30 seconds on vortex. Chromatographic separation was performed on PerkinEkmer BAC 1: 450°C: 30m x 320µm x 1.8 µm column and mobile phase Nitrogen 99.999%. The chromatographic running time is approximately 6.0 minutes.

The parameters evaluated in the validation were selectivity, linearity, accuracy, precision, repeatability and reproducibility, detection limit, quantification limit and matrix effect. The calibration curves for all compounds were linear with $r^2 > 0.9993$, The linear analytical range of the procedure was between 1.0 and 6.0 mg/L. Accuracy (99.7-101.9%), intra-assay precision (0.8-5.17%) and inter-assay precision (2.8-4.3%) were acceptable. The determination limit was 0.1mg/L and the quantification limit was 0.2 mg/L. The method was applied to the measurement of MEK in urine of a pool contaminated with MEK. In conclusion, the GC / FID-HS method has been developed successfully for monitoring occupational exposure ofindustrial workers and the quantitative analysis of MEK.

B-310

Triazole Antifungal Drug Monitoring in Serum using LC-MS/MS

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Background: Invasive fungal infections are increasing in both number and scope with immunocompromised and critically ill patients. Therapeutic drug monitoring (TDM) of some antifungals can assist clinicians with dose optimization and improve outcomes. While not all antifungals benefit from TDM, those with concentration-dependent toxicity or variations in absorption, metabolism, or other pharmacokinetic factors are candidates for TDM. The University of Wisconsin Hospital and Clinics Toxicology group has developed an assay which will monitor three triazole antifungals (posaconazole, voriconazole, and itraconazole) and one active metabolite of itraconazole (hydroxyitraconazole) in serum using liquid chromatography tandem mass spectrometry (LC-MS/MS).

Methods: Patient serum is mixed 1:3 with acetonitrile containing 350 ng/mL of all four deuterium labeled compounds as internal standards. The sample is centrifuged and an aliquot is passed through a Supelco Hypersil phospholipid column. This sample is then diluted with 400 µL of acetonitrile and 5 µL is injected onto the LC-MS/MS. Separation occurs using a Kinetex C18 column (100x3.0 cm) with 10 mM ammonium formate/0.1% formic acid as Buffer A and 0.1% formic acid in acetonitrile as Buffer B. The gradient is 70% to 0% Buffer A over seven minutes. The drugs are quantitated on an API4000 tandem mass spectrometer with twelve different ion transitions monitored using multiple reaction monitoring (MRM) corresponding to two MRMs per drug or metabolite and one for each internal standard. All stock standards were purchased from Toronto Research Company (Toronto, Ontario Canada) and Cerilliant (Round Rock, Texas).

Results:

The analytical measurement range (AMR) for posaconazole is 0.1-4 µg/mL with a limit of detection (LOD) of less than 0.025 µg/mL. The intra and inter-assay precision was less than 2.2% and 2.8% respectively. Correlation of this method with a reference lab had a regression of y=1.0642x +0.0827, and correlation coefficient (R^2)=0.991.

The AMR for voriconazole is 0.2-8 µg/mL with a LOD of less than 0.05 µg/mL. The intra and inter-assay precision was less than 0.6% and 1.6% respectively. Correlation of this method with a reference lab had a regression of y=1.003x +0.131, and R^2=0.997.

The AMR for itraconazole is 0.2-8 µg/mL with a LOD of less than 0.05 µg/mL. The intra and inter-assay precision was less than 0.8% and 3.5% respectively. Correlation of this method with a reference lab had a regression of y=1.0054x +0.0451, and R^2=0.992.

The AMR for hydroxyitraconazole is 0.2-8 µg/mL with a LOD of less than 0.05 µg/mL. The intra and inter-assay precision was less than 1.8% and 3.0% respectively. Correlation of this method with a reference lab had a regression of y=0.9948x -0.0338, and R^2=0.987.

Standard addition experiments with high hemolysis and bilirubin showed no interference. However, grossly lipemic samples showed a bias to under report the antifungal concentrations by as much as 15% which could be due to under pipetting serum.

Conclusion: A new antifungal assay was developed to better monitor drug therapy in immunosuppressed and critically ill patients. This allows faster turnaround time and quicker response to invasive fungal infections.

B-311

The Application of QuEChERS, a Novel Sample Preparation Technique for the Quantitative Determination of Benzodiazepines and Anabolic Steroids in Whole Blood

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Background: In clinical laboratories, the commonly used sample preparation techniques include immunoassay, liquid-liquid extraction (LLE) and solid phase extraction (SPE). QuEChERS (acronym for Quick, Easy, Cheap, Effective, Rugged and Safe) is a novel sample preparation technique that was originally reported by the scientists at the USDA in 2003 for the determination of multi-class pesticide residues in fruits and vegetables. Since then, QuEChERS has been adopted quickly and widely to determine various analytes of interest in different matrices, such as veterinary drugs in animal tissues, mycotoxins in grains, polycyclic aromatic hydrocarbons in seafood, bisphenol A in canned foods and beverages, and cannabinoids in medical marijuana

and cannabis foods. In this study, QuEChERS methodology will be applied for the analysis of clinical therapeutic drugs, such as benzodiazepines and anabolic steroids in whole blood.

Method: Add 2 mL extraction solvent (e.g. acetonitrile) with internal standards (optional) to a 15-mL centrifuge tube with pre-packed extraction salts (e.g. magnesium sulfate and sodium chloride), add 1 mL whole blood to the centrifuge tube, shake and centrifuge. After centrifugation, the proteins, blood cells and some un-dissolved extraction salts remain in the bottom of the centrifuge tube, while the target analytes are extracted into the upper, clear solvent layer. For sample cleanup, transfer 1 mL of the blood extract to a 2-mL centrifuge tube containing SPE sorbents (e.g. PSA and C18). The matrix co-extractives, such as organic acids and lipids, are retained on the sorbents which results in a purified extract for instrumental analysis.

Results: Matrix matched calibration curves were constructed for analyte quantification. The responses for 10 representative benzodiazepines and 12 steroids were linear with R2 ranged from 0.9963 to 1.0000 over the concentration range of 10 - 500 ng/mL. The matrix effect was evaluated by comparing the slopes of the matrix matched calibration curves to those of the calibration curves of solvent standards. The matrix effect was found to be insignificant, from -22 to 18%, which indicated that the QuEChERS method effectively removed the matrix interferences that may cause significant ion suppression or enhancement. Excellent analyte recoveries (81.4- 105%) and relative standard deviations (RSD% ≤ 10%) were obtained. This method was applied to 6 real whole blood samples, no target analytes were detected above the limit of quantitation of 10 ng/mL.

Conclusion: A novel sample preparation technique, QuEChERS, has been demonstrated to be simple, fast, and effective for the quantitative determination of benzodiazepines and anabolic steroids in whole blood. This provides clinical laboratories a successful alternative sample preparation method for drug assays in whole blood samples.

B-312

Measurement of synthetic cannabinoids in human urine by liquid chromatography-tandem mass spectrometry

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Background:

Synthetic cannabinoids (i.e., K2, Spice) were originally designed as research tools to aid in the investigation of the endocannabinoid system due to their ability to bind to cannabinoid type 1 (CB1) and cannabinoid type 2 (CB2) receptors. Often referred to as herbal incense or potpourri, synthetic cannabinoids are gaining in popularity among recreational drug users as an inexpensive and "legal" alternative to marijuana. Synthetic cannabinoids do have some effects common to that of marijuana and its primary psychoactive compound, Δ^9-tetrahydrocannabinol (THC), with a higher degree of intoxication associated with their consumption. Users of synthetic cannabinoids however may demonstrate more serious side effects such as hypertension, hallucinations, tachycardia, sinus bradycardia, chest pain, dysrhythmias, seizures and even death. The metabolism of synthetic cannabinoids occurs via cytochrome P450 enzymes and generally includes either hydroxylation and/or dehalogenation with excretion in the urine as glucuronide conjugates.

Methods:

Deuterated stable isotopes were added to 500µL of urine as internal standards. Ammonium acetate buffer (0.5M, pH = 5.0) and beta-glucuronidase were then added and this mixture was incubated at 50ºC for 30 minutes. The synthetic cannabinoids and internal standards were extracted by solid phase extraction using Bound Elut SPEC C18 3mL (15mg) columns (Agilent Technologies, Santa Clara, CA.). The samples underwent separation via liquid chromatography using a Kinetex™ 5µm C18 50x4.6mm column (Phenomenex, Torrance, CA) on a TLX4 high-throughput liquid chromatography system (Thermo Fisher Scientific, Waltham, MA), followed by analysis on a tandem mass spectrometer (6500 QTRAP, AB SCIEX, Foster City, CA) equipped with an electrospray ionization source in positive mode. Ion transitions were monitored by multiple reaction monitoring (MRM) mode.

With the exception of JWH-073, metabolites that are structural isomers were not chromatographically separated. Chromatographic separation was deemed unnecessary as the clinical interpretation is identical and independent of which isomer is detected. The above method includes detection of JWH-018 N-(4/5-hydroxypentyl), JWH-073 N-(3-hydroxybutyl), JWH-073 N-(4-hydroxybutyl), JWH-122 N-(4/5-hydroxypentyl), JWH-210 N-(4/5-hydroxypentyl), JWH-250 N-(4/5-hydroxypentyl), AM2201 N-(4-hydroxypentyl), RCS-4 N-(4/5-hydroxypentyl), UR-144 N-(4/5-hydroxypentyl) and XLR11 N-(4-hydroxypentyl).

Results:

Baseline separation of all 10 analytes and their internal standards was achieved within 7 minutes. Method performance was demonstrated using precision, linearity, recovery and analytical sensitivity and specificity studies. Precision and linearity studies were performed using urine fortified with synthetic cannabinoid standard solutions. Intra-assay precision coefficients of variation (CVs) ranged from 2.0% to 4.7%. Inter-assay precision CVs ranged from 5.3% to 16.2%. Linearity was demonstrated for each analyte with the slopes of the equations ranging from 0.9842 to 1.0328 and correlation coefficients (R^2) ranging from 0.9989 to 0.9997. Recovery studies demonstrated averages of 96% to 102% from expected values. The assay is reported qualitatively with cutoffs ranging from 0.1 to 0.8ng/mL depending upon the analyte. To assess accuracy, comparison to an external reference laboratory was performed and demonstrated an overall agreement of 99%. Additionally specimens were shown to be stable under ambient, refrigerate and frozen conditions for up to 35 days.

Conclusion: This method provides for the simultaneous and reliable analysis of multiple synthetic cannabinoids in urine.

B-313

Performance Characterization of ARK Gabapentin Immunoassay on an Advia 1200 Analyzer

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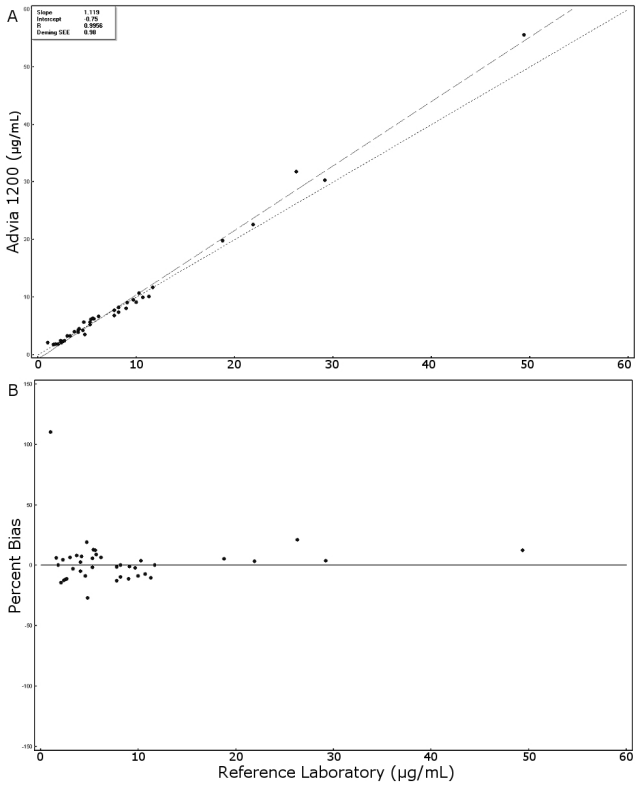
Background: Gabapentin is an anticonvulsant used for the treatment of epilepsy and neuropathic pain, and various off-label applications such as anxiety disorders and restless leg syndrome. Therapeutic drug monitoring of gabapentin is helpful for optimizing individual therapy, managing comedications, and assessing compliance. Our objective was to evaluate a gabapentin immunoassay (ARK Diagnostics, Sunnyvale, CA) on a Siemens ADVIA 1200 (Siemens Healthcare Diagnostics, Deerfield, IL). **Methods:** Linearity was assessed by spiking a left-over patient serum sample to a gabapentin level of 40 µg/mL followed by serial dilution with saline and analyzing the resulting specimens in triplicate. Intra-day precision was evaluated by analyzing three quality control materials included in the ARK reagent package for 10 replicates a day while inter-day precision was assessed by analyzing the three quality control materials once a day for 35 days. Accuracy was assessed by comparing results of split patient samples by the ARK immunoassay to a liquid chromatography tandem mass spectrometric method offered by an independent clinical laboratory (n=40). **Results:** The assay was linear from 0.8 to 40 µg/mL with recoveries ranging from 80.4% to 113.1%. No carryover was observed up to 101.0 µg/mL. Intra- and inter-day precision were less than 14.6% for all concentrations tested. The assay compared favorably with the reference laboratory method with Deming regression parameters of slope 1.119 (95%CI 1.084 to 1.153), intercept -0.75 (-1.18 to -0.31) µg/mL, standard error of estimate 0.98 (Figure 1A), and mean bias

3.2% (Figure 1B). **Conclusion:** The gabapentin ARK Diagnostics immunoassay on Advia 1200 was validated for clinical use.



B-314

The Use of Biochip Array Technology for the Multi-Analytical Detection of Drugs of Abuse in Oral Fluid to Significantly Expand Current Test Menus

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Background: Drug detection involves initial screening of samples for drugs, and in medical applications the screening tests results are directly used for medical evaluation. For legal purposes, the screening procedure eliminates all negatives, and positive results are regarded as presumptive and require

confirmation using confirmatory methods such as high performance liquid

chromatography and mass spectrometry. Laboratory tests of oral fluid for drugs of abuse have continued to expand in the workplace as well as in legal and medical settings. The collection of oral fluid is simple and non-invasive and can be easily observed. Immunoassays are highly selective antibody-based tests that provide high throughput screening of a range of drugs and their metabolites in different matrices. To expand the screening capacity even further, biochip array technology has been used to provide multiplex screening of different drug classes from a single sample.This is relevant when the volume of sample available for analysis is limited. Biochip array technology employs competitive chemiluminescent immunoassays for drug testing. The immunoassays can be applied to several dedicated analysers.The automation facilitates the integrity, reliability and accuracy of the drug testing process. The aim of this study was to expand the test menu of drugs of abuse in oral fluid through the development of biochip arrays enabling the detection of 23 classes of drugs of abuse at a selected cut-off concentration relevant for an oral fluid testing laboratory.

Methods: Competitive chemiluminescent immunoassays were employed. The capture antibodies are immobilised and stabilised on the biochip surface defining microarrays of discrete test sites. The immunoassays were applied to the Evidence analyser. Two biochips were used for the semi- quantitative detection of 23 drug classes including: amphetamines, barbiturates, benzodiazepines, methadone, opiates, PCP, cocaine, oxycodone, propoxyphene, cannabinoids, fentanyl, buprenorphine, tramadol, meprobamate, synthetic cannabinoids, dextromethorphan, tricyclic antidepressants, meperidine, methylphenidate, mitragynine and ketamine. The Quantisal Oral Fluid Collection Device was used following manufacturer's instructions.

Results: The limit of detection (LOD) for all the drugs of abuse studied was determined by assessing 20 negative sample replicates and was calculated as the mean +3 standard deviations. For all drugs tested the calculated LOD was below the selected cut-off i.e. methamphetamine 0.59ng/mL (cut-off:20ng/mL) benzodiazepines 0.18ng/mL (cut-off:10ng/mL) fentanyl 0.07ng/mL (cut-off:1ng/mL) buprenorphine 0.05ng/ml (cut-off:1ng/mL). The % agreement with LC/MS was assessed for five drugs of abuse in authentic oral fluid samples and it was found to be 100% for buprenorphine (n=18), cocaine (n=33) and oxycodone (n=18), 94.4% for tramadol (n=18) and 91.7% for cannabinoids (n=60). In addition, samples containing all 23 drugs spiked below at and above the required cut-off were assessed and all samples were correctly classified as positive and negative. Samples collected with other collection devices (OraSure and Oral-Eze) were also assessed and the drugs studied were detected.

Conclusion: This study indicates applicability of biochip array technology to increase the screening capacity of drugs of abuse in oral fluid. Two biochip arrays utilising 25 µl sample volume each allowed the simultaneous detection of 23 classes of drugs of abuse present in oral fluid samples in 3 types of collection buffer.

B-315

Detection of cocaethylene and levamisole in cocaine users by ultra performance liquid chromatography-tandem mass spectrometry

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Background: Cocaine, a psychostimulant, is highly abused and accounts for one of the main reasons for drug related ER visits. To enhance its simulative properties, cocaine is often co-abused with alcohol. Cocaethyelene (CE), a metabolite of cocaine and alcohol, is a psychostimulant as well and leads to prolonged euphoria because of its longer half-life compared to cocaine. CE has well documented cardiotoxic effects, and thus, the risk of death is 18 times greater when cocaine and alcohol are abused together than each drug on its own. To enhance cocaine-induced euphoria, several adulterants are used, one such adulterant is levamisole (LEV). It is historically a veterinary antihelminthic and has been recently determined as cocaine adulterant and to cause severe adverse reactions in cocaine users. Because of the highly toxic effects of CE and LEV, our objective was to determine their prevalence in cocaine-positive patient samples at the University of Texas Medical Branch – Galveston.

Method: Cocaine positive urine samples from drug screens performed between December, 2014 and February, 2015 at the University of Texas Medical Branch – Galveston, TX were tested for LEV, CE, cocaine (COC), benzoylecgonine (BE), and alcohol metabolites (ethyl glucuronide (EtG) and ethyl sulfate (EtS)) using ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). All standards and internal standards were purchased from Cerilliant (Round Rock, TX). Each standard analyte had its respective internal standard, except for LEV, which had BE internal standard. Six calibrators ranging from 25-2000 ng/mL were used for LEV, BE, CE, and COC, along with a negative control and 2 levels of positive controls prepared in drug free urine. Five calibrators ranging from 250-3000 ng/mL were used for EtS and EtG along with a negative control, 2 levels of positive controls (UTAK laboratory), and an additional positive control prepared in drug free urine. Controls and samples were diluted 1:4 with 0.1% formic acid/water spiked with internal standard and analyzed.

Result: Linearity for LEV, COC, BE, and CE ranged from 10-40,000 ng/mL with R^2>0.998 and cut off value of 50 ng/mL. Linearity for EtS and EtG ranged from 200-50,000 ng/mL with R^2>0.998 and cut off value of 500 ng/mL. Within the 11 week period, 1280 samples were screened for cocaine at our institute and only 7.4% were positive for cocaine. Further analysis of the BE positive samples showed that only 50% were positive for COC and 30% were positive for CE, the metabolite of COC and alcohol. In addition, 52.5% and were positive for EtG and 50% were positive for EtS. LEV was positive in 80% of the BE positive samples. The average age for cocaine positive patients was 45 yr. 61% were male and 43% were Caucasians.

Conclusion: This study highlights the prevalence of LEV and CE in the study population and indicates need for LEV/CE screen in suspected cases of drug abuse due to the associated adverse effect of both substances.

B-316

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

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Background: Epilepsy is a medical condition that produces seizures affecting a variety of mental and physical functions. Oxcarbazepine (OXC) and eslicarbazepine acetate are the second and third generation antiepileptic drugs (AED) respectively. These prodrugs are metabolized to 10-monohydroxy derivative (MHD), the active agent. Serum levels usually range 3 to 35 µg/mL. Here an ARK enzyme immunoassay for therapeutic drug monitoring (TDM) of MHD is described.

Methods: The ARK™ Oxcarbazepine Metabolite Assay is a liquid stable homogeneous enzyme immunoassay, consisting of two reagents, 6 calibrators (0.00, 2.00, 5.00, 12.00, 25.00 and 50.00 µg/mL) and 3 controls (3.00, 10.00 and 30.00 µg/mL). The performance of the ARK assay was evaluated on the Roche/Hitachi Modular P analyzer. Precision, limit of quantitation, recovery, specificity and method comparison were studied.

Results: Total precision ranged 5.4% to 6.9%CV and within-run precision ranged 6.5% to 8.6%CV in a 5-day study using quality controls and spiked serum samples. Acceptable quantitation and recovery was observed from 0.70 to 35.00 µg/mL. The assay crossreacted 20% to 30 % with structurally similar oxcarbazepine and carbamazepine and its metabolites (cis-10, 11-dihydroxy carbamazepine, dihydro-carbamazepine, and carbamazepine epoxide). The assay did not crossreact with other AEDs tested (gabapentin, lamotrigine, levetiracetam, topiramate, and zonisamide). Thirty specimens (5.90 to 26.10 µg/mL) were assayed and gave the following Passing Bablock regression results when compared to UPLC values: ARK = 1.05 UPLC - 0.78 (r2=0.94).

Conclusion: The ARK Oxcarbazepine Metabolite Assay measures oxcarbazepine MHD in human serum with excellent precision and recovery. Ability to measure trough levels of oxcarbazepine MHD with high accuracy and fast turn-around time makes this method clinically useful for TDM.

B-317

ARK™ Lacosamide Assay for the Beckman AU480 Automated Clinical Chemistry Analyzer

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Background: Epilepsy is a medical condition that produces seizures affecting a variety of mental and physical functions. Lacosamide (LCM) is a new generation antiepileptic drug (AED). Typical serum levels are 7.9 ± 4.9 µg/mL (31.4 ± 19.5 µmol/L). Here an ARK enzyme immunoassay for therapeutic drug monitoring (TDM) of lacosamide is described.

Methods: The ARK™ Lacosamide Assay is a liquid stable homogeneous enzyme immunoassay, consisting of two reagents, 6 calibrators (0.0, 1.0, 2.0, 5.0, 10.0 and 25.0 µg/mL) and 3 controls (1.50, 7.0 and 15.0 µg/mL). The performance of the ARK assay was evaluated on the Beckman AU480 Automated Clinical Chemistry Analyzer. Precision, limit of quantitation, recovery, specificity and method comparison were studied.

Results: Total precision ranged 4.4% to 7.7%CV and within-run precision ranged 4.2% to 5.7%CV in a 5-day study for quality controls and spiked serum samples. Acceptable quantitation and recovery was observed from 0.6 to 20.0 µg/mL. The assay crossreacted 1.3% with o-desmethyl lacosamide (10.0 ug/mL in the presence of 5.0 µg/mL LCM) and 2.7% with o -desmethyl lacosamide (20.0 µg/mL in the presence of 10.0 µg/mL LCM). Forty nine specimens (1.1 to 14.4 µg/mL) were assayed and gave the following Passing Bablock regression results when compared to UPLC values: ARK = 0.99 UPLC - 0.02 (r2=0.93).

Conclusion: The ARK Lacosamide Assay measures lacosamide in human serum with excellent precision and recovery. Ability to measure trough levels of lacosamide with high accuracy and fast turn-around time makes this method clinically useful for TDM.

B-318

Evaluation of a Reagent Kit Under Development for the Therapeutic Drug Monitoring of Cyclosporine and Tacrolimus in Whole Blood by LC-MS/MS

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Background: Immunosuppressive agents cyclosporine and tacrolimus are calcineurin inhibitors which play a critical role in interleukin-2 promoter induction after T-cell activation. An assay for the monitoring of cyclosporine and tacrolimus in human whole blood samples as an aid in the management of kidney, liver and heart allograft transplant therapy is described using the Waters® MassTrak™ TDM Immunosuppressants Kit*. This kit is currently under development and not available for sale.

Methods: The MassTrak TDM Immunosuppressants Kit uses a LC-MS/MS system to perform the analysis. The samples (50µL of calibrators, controls or human whole blood) are manually extracted using a simple protein precipitation technique. Following centrifugation the supernatant was injected onto the ACQUITY TQD LC-MS/MS system for multiple reaction monitoring detection and quantification. Evaluation of the reagent kit was performed at Fujirebio Diagnostics Inc.

Results: The measuring range was 25 to 1500ng/mL (up to 3000ng/mL with specimen dilution) for cyclosporine and 1 to 30ng/mL (up to 60ng/mL with specimen dilution) for tacrolimus. The 20 day precision study demonstrated a 2.9-5.5% total CV over the range 33.6-1432.8ng/mL for cyclosporine and a 3.4-5.8% total CV over the range 3.0-20.7ng/mL for tacrolimus using three controls, four panels on one system and one reagent lot. Tacrolimus was shown to be linear over the range 0.0 to 36.6ng/mL and cyclosporine deviation from linearity ranged from -6% to 5% over the range 10.3-2267.7ng/mL. The limit of quantification was determined to be 6.9ng/mL for cyclosporine and 0.2ng/mL for tacrolimus, using three systems and two reagent lots.

Interference studies demonstrated mean levels of cyclosporine and tacrolimus were within 90-110% of that in unspiked control samples when endogenous (including; bilirubin, creatinine, uric acid, hematocrit) and exogenous (including; insulin, intralipid, K_2EDTA, Vitamin B12 and other immunosuppressant agents) potential interfering compounds were tested. Cyclosporine spiked recovery ranged from 91-99% for samples over the range 49.3-1137.1ng/mL. Tacrolimus spiked recovery ranged from 94-101% for samples over the range 3.0-25.1ng/mL.

Accuracy of the assay was assessed by analysing International Proficiency Testing (IPT) samples for cyclosporine and tacrolimus from Bioanalytics, UK (ASI Ltd). Cyclosporine and tacrolimus MassTrak TDM Immunosuppressants Kit determinations (n=40) were compared with the schemes target concentrations. Linear ordinary fit analysis for cyclosporine demonstrated a correlation coefficient of r = 0.999 and the Deming fit was described by the equation MassTrak = 1.06 IPT + 0.68 with no significant constant bias, however, proportional bias was detected ($p<0.05$) although this was within the allowable bias goals of the assay (10%). Linear ordinary fit analysis for tacrolimus demonstrated a correlation coefficient of r = 0.997 and the Deming fit was described by the equation MassTrak = 1.00 IPT + 0.09 with no significant constant or proportional bias ($p>0.05$).

Conclusions: Initial studies indicate the MassTrak TDM Immunosuppressants Kit, currently under development, will provide an accurate, precise, sensitive and robust assay for the measurement of cyclosporine and tacrolimus in human whole blood by LC-MS/MS analysis.

** The MassTrak TDM Immunosuppressants Kit is under development and not available for sale*

B-319

Performance comparison of Six Different Rapid Screening Drug Test Cards / Cups

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Although the use of mass spectrometry for urine drug testing provides the most definitive and accurate answer, urine drug test cup is still of interest because results are readily available in a few minutes and testing can be done with minimal training. We evaluated the performance of six commercially available Rapid Urine Drug Testing kits, and compared their screening results to the drug screens performed on the Chemistry analyzer, and with confirmatory results from Gas Chromatography - Mass Spectrometry (GC-MS) if available.

We concluded that Alere iScreen Dx, Discover™ Multi-Panel Drug Test Cup, MEDTOX® urine drug test cassette, BioRad TOX/See™ Rapid Urine drug test all have acceptable drug screen performance (as compared to our screening performed on the Chemistry analyzer and / or confirmatory results from GC-MS). Unexpectedly, we found that the non-waived products (MEDTOX® and BioRad TOX/See™) do not necessarily outperform waived products (Discover™). While all products we tested utilize the same testing principles, certain product (e.g. ABMC Rapid Drug Screen cassette) has more frequent internal QC failure as well as issues where urine fails to migrate to the test strip. QuikScreen© 12 urine drug test kit also had more discrepancy when compared to screening performed on the Chemistry analyzer and / or confirmatory results from GC-MS.

Table 1 represents concordance of four rapid urine drug testing kits with screening results on chemistry analyzer / confirmatory result from GC/MS if available.

MEDTOX®, Discover™ and TOX/See™ showed the best performance. The advantage of MEDTOX® is that it uses a reader to read the results, thereby remove subjectivity from individual. Discover™ cup is a waive product, therefore is subjected to less regulatory requirement. This makes it simpler to implement as an alternative to performing immunoassay screen on the Chemistry analyzer.

Table 1: Performance of four rapid urine drug testing kits

Drug Class	Alere iScreen Dx	Discover™	MEDTOX®	BioRad TOX/See™
Amphetamines	88%	100%	97%	97%
Benzodiazepines	75%	96%	88%	75%
Cannabinoids	100%	96%	97%	94%
Cocaine Metabolite	94%	100%	97%	100%
Methadone	88%	96%	100%	100%
Opiates	81%	100%	94%	81%
Oxycodone	81%	82%	90%	91%
Overall	87%	96%	95%	91%

B-320

Should mass spectrometry be used for urine drug screens in peri-partum patients?

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Background: Immunoassay methods have been used for urine opiates screens in all patients including those from the labor and delivery (L & D) department at the University of Texas Medical Branch - Galveston. However, due to the high cut off value (300 ng/mL), false negative results were observed in pregnant women from the L & D who used opiates. The babies delivered by this group of mothers have increased risk for some medical problems, including fetal growth restriction, abruptio placentae, fetal death, preterm labor, and intrauterine passage of meconium, just to name a few. The purpose of this study was to compare the current enzyme immunoassay method with the tandem mass spectrometry method to determine the appropriateness of its use in peripartum women as a screening method.

Methods: This is an ongoing prospective study. 405 urine samples were tested for opiates by the enzyme immunoassay method from Jan 21 to Feb 21, 2015 at our institute. 39 urine samples were randomly selected to test for 9 opiate drugs and metabolites (morphine, codeine, hydrocodone, hydromorphone, oxycodone, oxymorphone, norhydrocodone, noroxycodone, and 6-monoacetylmorphine) by the UPLC-MS/MS quantitative method. For the UPLC-MS/MS quantitative method, the calibrators were prepared in drug free urine using certified reference materials (Cerilliant) at 6 levels ranging from 25 - 2000 ng/mL for 8 high concentration analytes and 2.5 - 200 ng/mL for 1 low concentration analyte. Two levels of control samples were used. Controls and samples were prepared via beta-glucuronidase hydrolysis.

Results: Preliminary results are as follows: the mean age was 42.5 years (range: 1 day-93.2 years). Out of 405 samples, 52.4% were female, 31.4% were African Americans, and 66.3% were Caucasians. The enzyme immunoassay result distribution was as follows: 67.4% (<= 50 ng/mL), 6.7% (50 to 300 ng/mL), 25.9% (>= 300 ng/mL). 39 patients (10 patients with test result <=50 ng/mL, 9 patients with result between 50 to 300 ng/mL, and 20 patients with test result >=300 ng/mL) were randomly selected for UPLC-MS/MS analyses. Among the 39 selected samples, 25 tested positive by the UPLC-MS/MS method, and of that 20 samples were tested positive by immunoassay and 5 were tested negative. The remaining 14 samples were negative from both methods. The sensitivity of the enzyme immunoassay method is 80%, specificity is 100%, positive predictive value is 100%, and negative predictive value is 73.7%.

Conclusion: For pregnant women, UPLC-MS/MS method should be used for urine drug screen/quantification because of its superior analytic sensitivity that will allow us to identify more individual who use the drugs when compared to results obtained by enzyme immunoassay. Thus, the use of UPLC-MS/MS for screening will enhance the identification of mothers using the drugs and therefore, lead to prompt management of associated conditions.

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One-step extraction and quantitation of toxic alcohols and ethylene glycol in plasma by capillary gas chromatography (GC) with flame ionization detection (FID)

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Background: Clinical analysis of volatile alcohols (i.e. methanol, ethanol, isopropanol, and metabolite acetone) and ethylene glycol (EG) generally employs separate gas chromatography (GC) methods for analysis. The added turn-around time to analyze patient samples using separate methods on separate columns can impact patient care in the emergency department. Here, a single robust method for combined analysis of volatile alcohols and EG is described.

Methods: Volatile alcohols and EG were extracted from 200 µL of patient, calibrator, and QC samples with 2:1 (v:v) acetonitrile containing internal standards (IS) 1,2 butanediol (for EG) and n-propanol (for alcohols). Samples were vortexed for ~5 - 10 seconds and centrifuged for 2 minutes at 10,000 rpm on a benchtop centrifuge. 1 µL of the supernatant was injected (with 2:1 split ratio) onto a Restek-200 bonded stationary phase capillary column (30m × 530µm) with a fused silica guard column (10m × 520µm). Chromatographic separation was conducted on an Agilent 6890 GC FID and required modulation of both the gas flow (2 mL/min for 1 minute up to 15 ml/min for elution) as well as the temperature (45°C for 3 minutes to elute alcohols, up to 250°C at 70°C/min to elute EG). The total run time was 7.9 minutes. Six-point calibration curves were fitted with a quadratic regression curve to allow for non-linear recovery of EG at the low concentrations. QC material included Biorad Liquicheck level 1 and 2 serum volatile alcohols and an in-house prepared EG stock.

The method was evaluated for precision, accuracy, reproducibility, linearity, selectivity and limit of quantitation (LOQ), followed by correlation to existing GC methods, using patient samples, Bio-Rad QC, and in-house prepared QC material.

Results: The method yielded inter-day precision values ranged from 6.5 - 8.8% CV (L2 Biorad QC) and 6.8 - 11.3% CV (L1 Biorad QC). Linearity was verified in the range ~0.5 - 50 mmol/L for each analyte and the LOQ was calculated to be between 0.25 and 0.44 mmol/L for each analyte by calculating the noise in the lowest calibrator ($10 \times \sigma$) divided by the slope of the line. Correlation of the new method against current GC methods showed good agreement between them (slopes ranging from 1.03 - 1.12, and y-intercepts ranging from 0 - 0.85 mmol/L; $R2 > 0.98$; $N = 35$). Recovery was shown to be ~100% at all levels tested (low-mid range-high) for all analytes, although EG was more variable at low concentrations. Carryover was assessed to be negligible for volatile alcohols in the measuring range; however EG showed clinically significant carryover at >10 mmol/L; therefore injection of CLRW was required to eliminate contamination. Potential interfering substances include toluene (elutes with EG) and benzene (elutes with methanol); however the method is able to resolve 2,3 butanediol, diethylene glycol, and propylene glycol in addition to the peaks quantified.

Conclusion: Here we describe a simple procedure for simultaneous analysis of EG and volatile alcohols that comes at low cost and with a simple liquid-liquid extraction requiring no derivitization to obtain adequate sensitivity for clinical specimens.

B-325

Evaluation and Application of a Commercial Ethylene Glycol Enzymatic Assay in the Clinical Management of Ethylene Glycol Poisoning

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Background: Ethylene glycol toxicity remains an important toxicological problem in medical practice. Ethylene glycol is the most frequently encountered glycol toxin in the United States. Early diagnosis and treatment can prevent significant morbidity and mortality. Gas Chromatorgraphy-Flame Ionization Detection (GC-FID) is the preferred method for ethylene glycol detection. However, these instruments and the needed expertise are not readily available in most clinical laboratories.

Objective: Validate the Catachem rapid enzymatic assay for the quantitative determination of ethylene glycol levels in plasma. Use this method in combination with an in house GC-FID in the clinical management of ethylene glycol poisoning.

Methods: The Catachem Ethylene Glycol procedure is based on the affinity of the bacterial enzyme Glycerol Dehydrogenase (EC 1.1.1.6.) to catalyze the oxidation-reduction reaction of Ethylene Glycol in the presence of NAD. This two point kinetic procedure is read at 340nm, which was detected spectrophotometrically by using the Beckman AU 400 automated chemistry analyzers. Validation of this method was performed against a previously validated GC-FID method. Performance evaluation included accuracy, linearity, analytical sensitivity, imprecision, carryover, endogenous and exogenous interference studies, and parallel studies.

Results: The enzymatic assay showed excellent correlation to an in house GC-FID method (y = 0.964x + 0.3; r2=0.9966; N=40), with an analytical measurement range of 10-150 mg/dL. The analytical sensitivity was 10mg/dL. Both within-run (1.6%-3.1%) and between-run (4.1%-5.2%) imprecision were within acceptable limits. The assay showed cross reactivity with propylene glycol and various butanediols at elevated concentrations. Interference from hemolysis and lipemia was within acceptable limits (±5%). The assay is affected by icterus.

Conclusion: This method was successfully used for rapid rule out of ethylene glycol toxicity. It is successfully used to monitor response to therapy following hemodialysis of patients confirmed of having ethylene glycol toxicity. This assay has contributed to a significant decrease in turnaround times as well as a decrease in labor costs. The assay can be adapted in clinical laboratories with no GC-FID capabilities.

B-327

Monitoring of micafungin use in clinical practice using high-performance liquid chromatography with fluorescence detection

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Background:

Micafungin is an antifungal drug. It has a unique mechanism of action that works by way of concentration-dependent inhibition of 1,3-beta-D-glucan synthase resulting in reduced formation of 1,3-beta-D-glucan in the fungal cell walls. The decreased production of 1,3-beta-D-glucan leads to osmotic instability and thus cellular lysis. Micafungin has been approved for the treatment of candidemia, acute disseminated candidiasis, Candida peritonitis, abscesses and esophageal candidiasis. Previous reports of micafungin pharmacokinetics showed substantial variability in blood and plasma concentrations. Clarification of the effective blood concentration of micafungin by evaluating its clinical effect enables rapid and accurate treatment. Therapeutic drug monitoring (TDM) of micafungin in plasma can be useful for the evaluation and diagnosis of the drug inhibitory concentration, so subsequent medication could be adjusted according to the TDM results.

Methods:

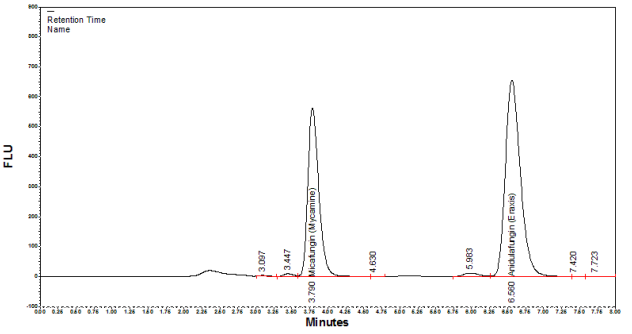
The high-performance liquid chromatography with fluorescence detection system was used for the separation and quantitation of micafungin from plasma samples. The separation of micafungin and internal standard anidulafungin was achieved through the use of reversed-phase high-performance liquid chromatography. Sample preparation involved a single dilution step, solvent extraction, protein precipitation, and ultra-centrifugation. Micafungin and anidulafungin were extracted from plasma samples using methanol and analyzed on a Zorbax Eclipse Plus C18 column with fluorescence detection set at excitation and emission wavelengths of 273 and 464 nm, respectively.

Results:

The standard curve was linear through the range of 0.1-80 µg/mL using a 0.1-mL sample volume. The intra- and inter-day precisions were all less than 5% and accuracies ranged from 94.8 to 105.1%. Average recoveries were 94.1 ± 0.7% and 92.8 ± 1.2% for micafungin and anidulafungin, respectively. Figure 1 represents a typical chromatogram of plasma sample containing 23.3 µg/mL of micafungin.

Conclusion:

The method is very applicable to monitor and optimize micafungin therapy in order to ascertain clinical efficacy and minimize adverse effects.



B-328

Detection of everolimus from dried blood spots using liquid chromatography tandem mass spectrometry

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Background: Monitoring immunosuppressants levels in blood is critical to ensuring proper drug dosage; however, this can be difficult for transplant recipients who live far away from the central laboratory. A potential solution to this problem is the use of dried blood spots (DBS), which can be collected by the patient and mailed to the lab for analysis. Thus, removing the requirement for a phlebotomist and transport of a liquid blood sample. Everolimus is a new immunosuppressant that has been shown to be useful in liver and heart transplantation and has less severe side effects than other immunosuppresants currently in use.

Objective: Establish a method to measure everolimus from dried blood spots using liquid chromatography-tandem mass spectrometry (LC-MS/MS)

Methods: Fifteen microlitres of whole blood spiked with everolimus (2.5, 5, 10 and 35 ng/mL) was spotted onto Whatman 903 protein saver cards. Several extraction solvents were evaluated (water, acetonitrile, methanol:acetonitrile, methanol). Everolimus-d4 was added along with the extraction solvent as an internal standard. The extracts were vortexed for 10 min after which the supernatant was removed and evaporated to dryness under air. It was then reconstituted in 50:50 methanol:water and injected into the mass spectrometer.

Extracts were analyzed using an Agilent 6410 triple quadrupole mass spectrometer coupled with an Agilent 1100 HPLC. Separation was achieved in a 2.8 min run using a valco valve to facilitate switching between a precolumn (POROS R1/20 2.1 mm x 20 mm) and analytical column (Phenomenex Luna C18 (2) 5 µm x 10 x 2.00mm). Following injection, the sample was loaded onto the precolumn (for 0.8 min) using mobile phase A (50:50 methanol:water). The valvo valve then switched allowing mobile phase B (2 mM ammonium acetate, 0.1% formic acid in methanol) to elute the analytes off the precolumn and onto the C18 analytical column (0.7-2 min). Flow rate was 0.5 mL/min and the injection volume was 50 µL. Multiple reaction monitoring (MRM) data for everolimus and everolimus-d4 was collected between 0.7 and 2 min. The source parameters were: gas temperature 250°C, gas flow 10 L/min, Nebulizer gas 50 psi, capillary voltage 4000V. MRM transitions for everolimus and everolimus-d4 were optimized using the Agilent Optimizer software.

Results: Four solvents were evaluated for extraction of everolimus from DBS. Our preliminary results indicate that methanol was the best at extracting everolimus from DBS and will be used for future experiments. Recovery of everolimus from four spiked concentrations (range: 2.5-35 ng/mL) ranged from 85-117% with an average of 101.6% ± 13%. Comparison with the results from the DBS extraction with the protein precipitation and dilution method currently in use in our lab gave an R2 of 0.9978.

Conclusion: We have developed a method to use DBS as specimen of choice for measuring everolimus on LC-MS/MS. Our preliminary conclusion indicates that DBS can be used for measurement of everolimus. We are running 40 more specimens to support our initial conclusion. The completed study will be presented at the national meeting.

B-329

High-Throughput Urine Analysis to Detect Buprenorphine and Ethanol Use by Multi-channeling LC-MS/MS

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Background: In order to maximize sample throughput and minimize solvent consumption, two forensic LC-MS/MS methods used to detect buprenorphine and/or ethanol use were developed for a multichannel UHPLC system utilizing positive-displacement pumps interfaced to a tandem mass spectrometer.

Methods: Urine specimens and corresponding calibrators and QCs to be analyzed for buprenorphine (Bup) and norbuprenorphine (Norbup) were hydrolyzed by incubation with β-glucuronidase solution and then mixed with cold methanol containing Bup-D_3 and Norbup-D_4 internal standards. Urine specimens and corresponding calibrators and QCs to be analyzed for ethyl-glucuronide (EtG) and ethyl-sulfate (EtS) were diluted 1:10 with water containing internal standards EtG-D_3 and EtS-D_5. After centrifugation, 10 uL injections of supernatants from each preparation were made.

Results: The desired quantitation range from 5 to 500 ng/mL for Bup/Norbup and the desired range from 100 to 5000 ng/mL for EtG/EtS were consistently linear (r^2 > 0.995 with 1/X weighting) whether the calibrators were injected into one channel or across all channels. For both methods, internal standard peak areas showed less than 25% coefficient of variation (CV) among calibrators, QCs and specimens (n = 20) on any of the four channels. Retention time variations throughout these batches were less than 3% CV. Results were within +/- 15% of those determined on a conventional multichannel system using reciprocating pumps. Comparatively, the multichannel system with positive-displacement pumps reduced solvent consumption by at least 65%. A maximum throughput of 34 urine samples per hour was achieved when batches were submitted across three channels. Since the data windows of both methods were a little more than 1/3rd of the total run times, adding the fourth channel did not increase sample throughput. However, using all four channels provided assurance that the total throughput would not be compromised in the event of one channel shutting down because of a leak or a column reaching its maximum pressure.

Conclusion: The multichannel UHPLC system achieved the desired sample throughput and solvent consumption.

B-330

Milrinone therapeutic drug monitoring in pediatric cardiac surgery

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Background: Milrinone is a potent selective phosphodiesterase type III inhibitor which stimulates myocardial function and improves myocardial relaxation. It is used extensively post-cardiac surgery in both adults and children. Although therapeutic monitoring is crucial to maintain therapeutic outcome, little data is available. A proof-of-principle study has been initiated in our institution to determine desirable milrinone levels (ie. therapeutic index) in children following cardiac surgery. The objective of this study was to develop an LC-MS/MS method to quantify milrinone in serum of pediatric patients and then to determine its pharmacokinetic parameters.

Methods: A liquid-liquid extraction procedure was used to prepare samples for analysis. Milrinone was measured using LC-MS/MS. Calibrants and internal standard were prepared in blank patient serum and charcoal stripped serum, respectively. Performance of the method was assessed by linearity, LoD, dilution recovery, accuracy, and precision. To determined the pharmacokinetic profile of the drug, patient samples were acquired post-surgery; the first sample was drawn within 2 hours post surgery and then every 6-8 hours for 24-36 hours. Pharmacokinetic analysis will be conducted using Non-Linear Mixed Effects Modeling (Non-MEM).

Results: Calibration curves followed a regression in the linear range of 50 - 800 ug/L. The lower limit of quantification is 5.9 ug/L based on triplicate runs of a low concentration sample that did not exceed 20% coefficient of variance (CV). Drug dilution recovery and accuracy was <120% and 150 ug/L. Within day CV was 5.7% at 95, 4.4% at 339, and 8.3% at 679 ug/L. Between day CV was 11.7% at 77, 8.8% at 318, and 8.2% at 587 ug/L. Data from the pharmacokinetic analysis will be presented.

Conclusion: This simple and quick method proved to be sensitive, specific, and precise for therapeutic monitoring of milrinone in patients post-cardiac surgery, a population not well studied. Pharmacokinetic profiling will help to determine both the pharmacokinetic parameters of this drug as well as the therapeutic range. These results will help improve dosing and monitoring of pediatric patients post-surgery.

B-331

Quantitation of 78 Compounds in Urine by LC-MS/MS

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Introduction

Forensic toxicologists face an ever-expanding list of compounds for analysis. The need to reliably quantitate large-panel assays is continually increasing. Large panel assays are required in order to speed sample analysis time, lower analytical costs and obtain results quicker while keeping good data quality. Herein we developed a fast, cost efficient liquid chromatography tandem mass spectrometry (LC-MS/MS) method which meets laboratory requirements for limit of quantitation for quantitative analysis of 78 compounds in human urine.

Methods

The 78 compounds consist of opiates, amphetamines, sedatives, drugs of abuse and others. Thirty deuterated analogs were used as internal standards. We developed a very simple sample preparation method in which urine samples were diluted by 50 fold. LC-MS/MS analysis was conducted on a Thermo Scientific™ TSQ Quantiva™ triple quadrupole mass spectrometer with a HESI ionization probe in polarity switch mode. Two SRM transitions were collected for analytes and one SRM transition was collected for internal standards. Nine minutes of LC gradients on Ultimate™ 3000 RS LC pump were used to separate compounds, which results in analysis of 6 samples per hour. All data acquisition and quantification for this method was performed using TraceFinder™ software version 3.2.

Results

We evaluated the following method performance:

LOQ: by two sets of calibration standards from 5 ng/mL to 5000 ng/mL. The acceptance criterion was difference within +- 20% and ion ratio within specified range.

Precision: by 10 replicate injections of QC samples

Linearity range: by serial dilutions from 5000 ng/mL

In conclusion, we developed a fast, cost efficient method for quantitative analysis of 78 compounds in human urine. The method meets laboratory requirements for limit of quantitation. A short 9 minutes LC-MS/MS method allows analysis of 6 samples per hour.

B-332

Utilization of comprehensive urine drug screens in Southern Alberta

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Background: Comprehensive drug screens are ordered for several indications including clinical assessment, employment, addiction, rehabilitation, or pain management monitoring. With the rise of recreational drug use, physicians may be tempted to order full drug screens in order monitor their patients. This can create an environment of unnecessary utilization by health care providers. Calgary Laboratory Services performs all clinical comprehensive urine drug testing for Southern Alberta. The toxicology department performs 50000 tests per year, approximately 12000 of which are comprehensive drug screens.

Objective: To investigation utilization of comprehensive urine drug screens in Southern Alberta by clinical health care providers

Methods: Comprehensive drug screens at Calgary Laboratory Services are performed in two steps. Samples are first screened by immunoassay for opiates, cocaine, amphetamines, barbiturates, methadone, EDDP, oxycodone and benzodiazepines. They then undergo liquid-liquid extraction and are run by gas chromatography-mass spectrometry (GC-MS) in total ion monitoring mode. Separate GC-MS confirmation assays for opiates, cocaine and cannabinoids are available if indicated.

Data was obtained for all comprehensive urine drug screens performed at Calgary Laboratory Services between 2010 and 2014 from our laboratory information system (Cerner Millennium). All data was deidentified according to CLS privacy policies. The data obtained included patient age and sex, sample collection time, ordering location, and comprehensive drug screen results. All data analysis was performed in Microsoft Excel 2007.

Results:A total of 51,866 comprehensive drug screens were reported on 12,228 patients during the 5 year period. This number increased from 9239 in 2010 to 11074 in 2014 (increase of 20%). Over half of the workload was from three ordering locations: two methadone clinics and the addictions clinic at our main tertiary care centre

which accounted 28%, 20%, and 18% of total comprehensive drug screen volumes, respectively. The ordering practices were further investigated by looking at how often comprehensive urine drug screens were ordered on the same patient. Of the samples run between 2010 and 2014, 76% had another sample submitted on the same patient at least once. When analyzed by number of weeks until the testing was repeated, we determined that testing was most often repeated on a patient within 1 week of the previous sample being submitted (15%). This declined in a time dependent fashion over the following weeks (2 weeks: 12%, 3 weeks: 7%, 4 weeks 8%). Amazingly, most repeat testing within 1 week was on patients from the addictions clinic at our major tertiary care centre, not from the methadone or other rehabilitation clinics.

Conclusion: Data collected from comprehensive urine drug screens performed in Southern Alberta from 2010-2014 show that 15% of repeated tests were ordered within 1 week of initial testing. This is an important observation as there is usually no clinical indication for a comprehensive drug screen to be repeated within 1 week. We plan to implement a guideline to restrict comprehensive urine drug screens to once per month, which will result in a savings of $425,000 per year.

B-333

Effects of sodium arsenite on the some laboratory signs and therapeutic role of thymoquinone in the rats

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OBJECTIVE: Serious health problems in humans are caused by arsenic (As) exposure, which is wide spread in the environment. Sodium arsenite (SAs), capable of inducing macromolecular damage is evaluated for its damaging effect in the blood vessels, liver and kidneys of Wistar rats. This study was undertaken to investigate the ameliorative effects of thymoquinone on SAs-induced oxidative and inflammatory damages in the serum of male Wistar rats.

MATERIALS AND METHODS: 27 Wistar Albino rats divided into three groups of nine rats each were administered to controls saline (10 mg/kg), SAs (10 mg/kg), and SAs plus thymoquinone (10 mg/kg/day) for two weeks orally. Biochemical parameters (albumin, total protein, alanine amino transferase, aspartate amino transferase, urea, creatinine, uric acid, triglyceride, total cholesterol, HDL-cholesterol) were analyzed by otoanalyzer; nitric oxide levels spectrophotometrically, and cytokines (interleukin-6, monocyte chemoattrectant protein-1, macrophage migration inhibitory factor) were measured by ELISA method in the rat serum samples.

RESULTS: Inflammatory cytokines and some biochemical variables were found to be increased in the SAs group compared to control group. On the other hand, thymoquinone supressed these laboratory signs, which are thought to be the characteristic signs of SAs toxicity, most probably by its ameliorative effects including anti-inflammatory and antioxidant properties.

CONCLUSIONS: In conclusion, As causes tissue damage by disintegrating metabolic responsiveness and regulations, vitiating antioxidant systems, decomposing immune competent cells, and finally inducing DNA damages. Therefore, it may be concluded from the present study that supplementation of TQ significantly protects deleterious effects from SAs-induced toxicity by reducing inflammatory and oxidative damages, as indicated by levels of serum biomarker.

B-334

Therapeutic Drug Profiles in Human Breast Milk: To Feed or Not to Feed?

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BACKGROUND: The last two decades has seen a substantial increase in the rates of women breastfeeding. 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. The objective of this study is to investigate the risk of drug exposure of three drugs in nursing infants. Here, we present drug profile cases of lithium, methotrexate and tacrolimus in breast milk.

METHODS: 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 phases; milk composition changes across the feed, thus suggesting that drug concentration can also be variable based on physicochemical properties of the drug. We worked to create a simplified drug extraction method using organic solvents to facilitate efficient drug extraction from both the lipid and aqueous phases of breast milk. Methods were then developed to measure these drugs using LC-MS/MS.

Breast milk samples were obtained from lactating women receiving lithium, methotrexate or tacrolimus. Samples were obtained pre-dose and then at various time-points throughout the dosing interval. A unique feature of this study is that both foremilk and hindmilk were collected. Breast milk samples were aliquotted and stored at -20oC until sample preparation, extraction and analysis.

RESULTS: Time-concentration profiling of methotrexate and its metabolite in breast milk were determined following a once-weekly subcutaneous dose of 25 mg of methotrexate. Foremilk and hindmilk samples were measured and peak drug concentration was found between 1-12 hours post-dose, with low but detectable levels from 48-96 hours post-dose. Tacrolimus breast milk pharmacokinetics was assessed following an 8 mg dose. Peak milk tacrolimus concentration was found between 12-20 hours post-dose. Higher tacrolimus concentrations were present in the lipid-rich hindmilk samples. Lithium time-concentration profiling was established and it was found that lithium selectively accumulates in the aqueous phase of breast milk with peak concentration found between 1-8 hours post- 900 mg oral dose. Data showing the potential risk to the nursing infant will be presented.

CONCLUSION: These cases highlight the importance of determining drug concentrations in breast milk from nursing mothers given the increasing use of medication in nursing women. These results demonstrate that measurable levels of drugs are observed in breast milk. Combining these results with milk-volume consumption, metabolism and clearance, this data can be used to determine the relative infant dose and hence the risk of adverse effects to the nursing infant. The data generated from this study will help guide clinical decisions for drug use in nursing mothers.

B-336

Determination of methanol in urine and plasma by headspace gas chromatography autosampler and flame ionization detector - GC / FID-HS.

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The determination and quantification of toxic volatiles are extremely important in clinical analysis, which involves the routine monitoring of industrial workers to check the exposure to potentially toxic components and the detection of endogenous metabolites in the body in certain clinical diagnoses.

The main risks of a high exposure to methanol are severe recurrent metabolic acidosis with increased anion gap, caused by accumulation of formic acid and, in late stages, also lactic acid. The acidosis and metabolite formic acid cause to the central nervous system depression/toxicity, and visual disturbances that may be permanent. Complete blindness is possible and the target are central nervous system and retina.

The methanol is widely used as an industrial solvent in the manufacture of other chemicals such as paint and varnish removers and is present in automotive antifreeze.

The aim of this study was to validate a fast, easy and cheap method for the determination of methanol in urine and plasma by headspace gas chromatography with flame ionization detection.

The method consisted in a simple extraction of methanol and the internal standard 2-butanol off the sample by evaporation of this analyte, and sampling the vapor above the fluid (blood, urine or others) after reaching thermal equilibrium gas in a closed vial of 22 mL. The volatilized components present in headspace were aspirated by a syringe and injected into chromatograph for separation.

To improve the extraction a salt of ammonium sulfate was added to turn the solution saturated, a technique called salting-out. A simple dissolution of an inorganic salt in water can decrease the solubility of an organic substance in water and consequently increase its volatility.

Chromatographic separation was performed on a PerkinElmer BAC 1: 450°C: 30m x 320µm x 1.8 µm column and mobile phase Nitrogen 99,999%. The chromatographic running time was approximately 4.1 minutes.

The parameters evaluated in the validation were selectivity, linearity, accuracy, precision, repeatability and reproducibility, detection limit, quantification limit and

matrix effect. The calibration curves for all compounds were linear with r^2>0.9993. The linear analytical range of the procedure was between 0.1 and 25 mg/dL. Accuracy (93.5-102.0%), intra-assay precision (0.7-1.2%) and inter-assay precision (4.8-8.35%) were acceptable. The determination limit was 0.06mg/dL and the quantification limit was 0.1 mg/dL. The method was applied to the measurement of methanol in plasma and urine of a pooled contaminated with methanol. In conclusion, the GC / FID-HS method has been developed successfully for monitoring industrial workers and the quantitative analysis of methanol.

B-337

Laboratory Investigations on Falsely Elevated Tacrolimus Concentrations on the Dimension Xpand

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Background: Tacrolimus is the most widely used immunosuppressant. Monitoring of blood tacrolimus levels is essential to achieve therapeutic efficacy and avoid toxicity. We measure tacrolimus on the Dimension Xpand using an antibody-conjugated magnetic immunoassay (ACMIA). We recently encountered three cases with unexpectedly elevated tacrolimus levels and further investigations were conducted.

Methods: Whole EDTA blood samples were collected from patients with unexpectedly elevated tacrolimus results. Plasma samples were also prepared from these patients. Samples were run by ACMIA on Dimension Xpand, chemiluminescent microparticle immunoassay (CMIA) on the Architect analyzer, and by liquid chromatography/ tandem

mass spectrometry (LC-MS/MS).

Results: Case 1 was a 58-year-old man who was on tacrolimus 1 mg twice daily after liver transplantation. Tacrolimus levels however were noted to be >30 ng/mL for a period of 3 week hospitalization. There were no notable symptoms and signs of toxicity. Tacrolimus level was 8.5 ng/mL as measured by LCMSMS from the same sample in a reference laboratory. When this patient was off tacrolimus for 1 week, the tacrolimus level on Xpand was still >30.0 ng/mL while it was <1.0 ng/mL by LC-MS/MS. Case 2 was a 63-year-old man with a history of myelofibrosis who received a stem cell transplant. Tacrolimus was given 1 mg twice daily. After tacrolimus had been discontinued for 2 weeks, his blood tacrolimus levels were still high varying from 10.0 ng/ml to greater than 30.0 ng/mL. An aliquot of sample with tacrolimus concentration of 12.5 ng/ml from Dimension Xpand was sent to a reference lab and the tacrolimus concentration was reported as <1.0 ng/mL. Case 3 was a 70-year-old man, with chronic myelogenous leukemia who received allogeneic stem cell transplantation and 1 mg tacrolimus twice daily. Although he has been off tacrolimus for 1 week, his blood tacrolimus levels were still high ranging from 12.0-22.0 ng/mL. Of a sample with tacrolimus value of 15.2 ng/mL on Dimension Xpand, LC-MS/MS and Architect gave the results of 3.8 ng/mL and 4.0 ng/mL, respectively. Interestingly, his plasma tacrolimus level was also high (12.8 ng/mL) although no evident hemolysis was determined, whereas tacrolimus levels were <1.0 ng/mL measured by LC-MS/MS and Architect from the same plasma sample. Measurements of tacrolimus from samples with serial dilutions also indicated the presence of potential interference.

Conclusion: Falsely elevated tacrolimus levels can occur due to immune interference when measured by Dimension Xpand using ACMIA assay. These falsely elevated results can potentially impact patient management and outcome. Unexpectedly elevated tacrolimus results should be investigated for potential interference. Measurement of tacrolimus from plasma is an alternative method to quickly rule out the interference while LC-MS/MS remains the standard of measurement.

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A retrospective study of urine drugs of abuse screening positivity rates by immunoassay at a national reference laboratory

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Background: According to the results from the 2013 National Survey on Drug Use and Health, the rate of illicit drug use among adults aged 18 to 25 years old was 21.5% and 7.3% for adults age 26 and older. The purpose of this retrospective study was to evaluate the positivity rate of one of our in-house urine drug screen panels in comparison to the national average for drug use in adults. The urine drugs of abuse panel studied consists of screening by immunoassay; positive immunoassay results are then confirmed by mass spectrometry. This panel can detect several drugs and drug classes, which include: amphetamine, barbiturates, benzodiazepines, cocaine, ecstasy (MDMA), marijuana (tetrahydrocannabinol; THC), methadone, methamphetamine, opiates (morphine, codeine, dihydrocodeine, hydrocodone, hydromorphone, oxycodone and oxymorphone), phencyclidine (PCP) and propoxyphene. Alcohol screening was also conducted if it was included in the client-selected orderable.

Method: Reagents from Microgenics were used for the oxycodone assay, while Syva EMIT® II Plus reagents were used for the rest of the drugs in the screen. The screen was performed on a Beckman AU5810 random access automated clinical analyzer. Percent positivity for each immunoassay was determined. Agreement with previously validated GC-MS or LC-MS/MS confirmatory methods was also evaluated, in order to assess the true positivity percent versus the percent of false-positive results (positive by screen but negative by confirmation). False-negative results were not investigated.

Results: There were 8825 de-identified screening results for each of the drugs in the panel, except for alcohol (N = 2296). The gender demographics consisted of 45.4% males; overall mean age was 42 yrs old (± 16 SD); range 0-97 yrs. The percent of samples that were preliminary screen positive was 10.0% for amphetamine/methamphetamine/MDMA, 12.8% for benzodiazepines, 43.7% for opiates (including oxycodone), and 20.3% for THC. The percent of preliminary screen positive samples for the following immunoassays: alcohol, barbiturates, cocaine, methadone, propoxyphene, and PCP were relatively low, < 3%. Overall confirmation results demonstrated that 1222 (14.6%) of the samples that tested positive by screen were false-positives and tested negative by confirmation testing. The false-positive rate for amphetamine/methamphetamine was ~14%, ~34% for opiates (excluding oxycodone), 25% for propoxyphene, and 100% for PCP and MDMA immunoassays. In addition, the percent of false-positive samples for the following immunoassays: alcohol, benzodiazepines, cocaine, methadone and THC were ~0-1%. The false positive rates for barbiturates and oxycodone were < 3.0%.

Conclusions: Based on the results from this retrospective study, the positivity rate for THC and cocaine was near the national average; however, our positivity rate for illicit and hallucinogen drug use was significantly less. The discrepancy is likely due to our patient population for testing and limitations for testing other illicit drugs and hallucinogens in this screening panel. Some immunoassay tests were more prone to false-positives, such as, PCP, opiates, MDMA, and propoxyphene. Definitive testing is necessary, especially for immunoassay tests that are prone to false-positive or false-negative results, when quantitative results are important for interpretation (e.g. opiates), and when results are inconsistent with clinical expectations.

B-339

Improved, simple, accurate Tacrolimus assay* without Manual Extraction for the Dimension RxL and EXL systems

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Background: The new Dimension® Integrated Chemistry Systems Tacrolimus (TAC) assay* provides confidence in patient results with improved productivity for complete transplant patient care.

Methodology: The TAC assay is based on ACMIA technology. The principle and operation of the TAC assay are as follows: a new pretreatment reagent is added to a reaction vessel on the Dimension system. Next a sample of whole blood containing tacrolimus is added. On board the blood and pretreatment reagent react to assure the lysis of the whole blood. A new Anti-tacrolimus antibody-β-galactosidase conjugate is added next and allowed to react with tacrolimus from the patient sample. Finally, pre-decorated chrome particles coated with a tacrolimus analog are added and allowed to bind the unreacted conjugate. The tacrolimus bound conjugate does not bind to the chrome but remains in the supernatant when a magnetic field is applied to the above reaction mixture. The tacrolimus bound conjugate is detected by transferring the supernatant from the reaction vessel to a photometric cuvette, where the enzyme tag is detected using a sensitive chromogenic substrate. Time to first result is 15 minutes.

Results: The new TAC antibody has improved cross-reactivity to the MI metabolite,13-O-Desmethyl tacrolimus (1% cross-reactivity vs. 15%

with TACR). MI was identified as the most abundant metabolites in the

literature. An extremely close relationship was observed between the Dimension TAC assay and the LCMS/MS reference method: TAC = 1.04 (LC/MS)-0.30; r =0.97 (n=315, range = 1.3 to 24.9 ng/mL), as well as to the Abbott Architect predicate assay: TAC = 0.99(ARCH) - 0.42, r = 0.98 (n=308, range = 2.4 to 24.2 ng/mL) A few discordant samples which were observed in the previous generation method TACR versus LCMS show excellent agreement with the new TAC assay. The new antibody and conjugate provide more sensitivity and better precision. From a precision profile the limit of quantification has been determined to be 1.0 ng/mL and the maximum within-lab reproducibility (%CV) has been found 8.8% at 1.8 ng/mL tacrolimus.

Conclusion: The new Dimension tacrolimus assay offers the quality along with the enhanced productivity of a fully automated assay for complete care of transplant patients. It allows confident management of tacrolimus dosing at any therapeutic target level in accordance with clinical practice guidelines. *Under FDA review. Not available for sale in the USA. Product availability varies by country.

B-340

Studies on Profiling of Drugs in the Clinical Urine Samples using Automated ToxPrep Method on ToxPrepTM Workstation

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Background: It is a well known fact that chronic pain affects a large portion of the human population and patients are prescribed drugs as a form of pain relief and for treatment. However, some patients do not follow their prescription which can lead to the under-treatment and over-treatment risks to them. As a result, clinics have started setting up point of care laboratories to provide in office drug profile in patient's blood or urine samples that should provide accurate results in a timely manner. In order to meet the ever growing demand for such clinics, urine testing has been found the most common testing matrix used for monitoring drugs and patient treatment.

Methods: The ToxPrep method involves detection of drug compounds with and without hydrolysing the samples using glucuronidase with subsequent addition of reagents followed by analysis of the prepared sample with LC-MS.

Results: In the present studies, automated ToxPrep protocol on ToxPrep™ Workstation was used by running clinical urine samples on a 96 well plate. Linearity, QC of samples, dilution study, sensitivity, and carry-over were studied for 85 clinical urine samples for 25 drugs including Cocaine, Codeine, Morphine, and Methadone. The linearity for individual compounds performed resulted an average R^2=0.999, and coefficient of variance (CV) for precision and accuracy were below 5%.

Conclusion: The measurable, simple, serial dilution study with this method and sensitivity of the linear curve was clinically acceptable with no carry over from well to well. This automated method for patient sample processing is applicable from medium to high volume patients.

B-341

Broad Spectrum Urine Drug Screening: the challenges of assessing qualitative cut-off performance characteristics in an LCMSMS System.

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BACKGROUND: Methods employing chromatographic separation and identification by tandem mass spectrometry are valuable for front-line or confirmatory testing of urine samples for a broad range of drugs and drug metabolites. The cut-offs adopted by most laboratories for positive screening results are derived from the Substance Abuse and Mental Health Services Administration (SAMHSA) regulations. In a pediatric centre, however, there is additional need to reliably detect many drugs below these cut-offs, as well as to assess the qualitative performance of our method at the cut-offs in use. The objective of this study was to determine how best to assess the linearity and qualitative cut-off performance characteristics in an LCMSMS System used for screening.

METHODS/RESULTS: To address these needs two evaluations were performed: (1) Forty-five drugs or drug metabolites were assessed for linearity below the SAMHSA-derived positive cut-offs in use at the Hospital for Sick Children in Toronto, Ontario. (2) The performance of our method at the cut-off of 50 ng/mL for 3,4-MDA and 3,4-MDMA was assessed by application of the CLSI Guideline for Evaluation of Qualitative Test Performance (EP12-A2).

In the linearity study, analyte pools containing between 8 and 10 spiked analytes were prepared at seven concentrations, ranging from 1.56 ng/mL to 100 ng/mL of each analyte, and were analyzed in duplicate on an LCMSMS QTrap 3200 system. The linearity of the peak area under the curve of each analyte in response to concentration was evaluated by application of CLSI Guideline EP6. Differences in linear performance were observed specific to drug, drug metabolite, and between mass-transitions. Additional analysis carried out queried the influence of normalization of analyte peak area to the area of the internal standard (D5-Diazepam), the concentration at which minimum requirements were met for the library matching algorithm, and the success rate of software peak identification without user intervention.

In the qualitative cut-off study, the capacity of our broad spectrum urine drug screen to correctly partition samples into a qualitative screen Positive or screen Negative designation was evaluated. Using a concentration of 50 ng/mL 3,4-MDA or 3,4-MDMA for the C_{50}, that is, the concentration at which the positive rate would be expected to be approximately 50%, replicates of samples at concentrations at and bracketing the C_{50} (e.g. ±20%) were analyzed. Previously established area under the curve count cut-offs failed to correctly partition samples as Negative at the -20% bracket and partitioned all samples as Positive at the C_{50}. However, normalization of the data to the internal standard and establishing a new normalized area cut-off at the C_{50} improved the method partitioning performance.

CONCLUSION: As a result of this evaluation, there is confidence that our method correctly partitions Positive versus Negative samples with concentrations ± 20% from the SAMHSA cut-off of 50 ng/mL for 3,4-MDA and 3,4-MDMA. This work highlights the challenges with determining performance characteristics in an LC-MS/MS system used for screening.

B-344

The Development of an LC-MS/MS Screening Method for 104 Targeted Compounds in Whole Blood, using Library Searching on a QTRAP Mass Spectrometer

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Background:

In order to detect a large variety of drugs in whole blood, many forensic laboratories incorporate multiple screening assays to cover different drug classes. Each assay screens for a single compound class, and frequently the assay cannot distinguish between specific analytes within a class. Our objective was to develop a single LC-MS/MS assay capable of accurately identifying >100 target compounds.

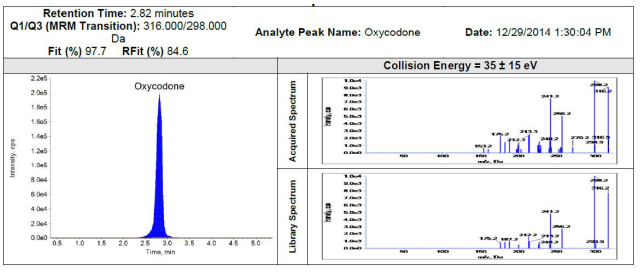
Method:

Our method employs MRM (Multiple Reaction Monitoring) measurements on an AB SCIEX 3200 QTRAP LC/MS/MS system to detect 104 target compounds in less than 10 minutes. The QTRAP enabled simultaneous MRM detection and 'on-the-fly' acquisition of a full-scan MS/MS spectrum for every detected compound. All acquired MS/MS spectra were searched against a spectral reference library, to increase confidence in compound identifications compared to traditional MRM-based methods (see Figure).

500 uL of whole blood containing internal standard was vortex mixed with 3ml of acetone, the sample was centrifuged, and the clean supernatant was collected and dried under nitrogen gas, then reconstituted with MeOH prior to analysis by LC-MS/MS. LC separation was achieved using a Phenomenex Kinetex PFP (50x2.1mm, 2.6um) column.

Results:

A cross-method comparison with an outside laboratory demonstrated that our method (i) provided more specific information about compound identity, (ii) provided superior sensitivity, and (iii) detected more compounds. The external testing only detected the presence of a compound class, for example "opiates", whereas the QTRAP screening method identified specific opiate compounds such as Oxycodone, Noroxycodone, Dihydrocodeine, etc. In certain cases the superior sensitivity of the QTRAP screening method detected the presence of compounds that were missed by the external testing. The established cut-off level was 10 ng/mL for the majority of the basic drugs, 1 ng/ml for the fentanyl group and PCP, 250 ng/mL for Trazodone, Pregabalin, and Gabapentin, and 1000 ng/mL for Carisoprodol and Meprobamate.



	Compound Name	Library Hit	Peak Area	Purity (%)
1	Oxycodone	Oxycodone	1,870,000	83.5