

Wednesday, August 3, 2016

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

TDM/Toxicology/DAU

B-302

Development of Latex-Enhanced Immunoturbidimetric Assays for the Detection of Ethyl Glucuronide, the Synthetic Cannabinoids UR-144/XLR-11 and Their Metabolites in Urine Samples

L. Li, J. Darragh, M. Benchikh, G. Shanbag, P. Sinha, M. Rodriguez, R. McConnell, S. FizztGerald. *Randox Toxicology Ltd, Crumlin, United Kingdom*

Background: Latex-enhanced immunoturbidimetric assays due to short reaction times and their application to automated analysers, are valuable analytical tools for the high-throughput screening of samples.

Ethyl glucuronide (EtG) is a metabolite of ethanol which has found application as a biomarker for the detection of recent alcohol consumption. The detection of alcohol consumption has a number of important applications, such as diagnosis and treatment of alcohol intoxication or poisoning, monitoring of individuals in professions with zero-tolerance policies with respect to alcohol, determination of legal impairment and forensic judgement.

UR-144 [(1-pentyl-1H-indol-3-yl)-(2,2,3,3-tetramethylcyclopropyl)methanone] and its fluorinated version XLR-11 (5-fluoro UR-144) are the new generation of synthetic cannabinoids. Both compounds are potent and addictive and as they continue to be sold there is a need for screening tests in the detection process.

The aim of this study was to develop latex-enhanced immunoturbidimetric assays, applicable to a variety of automated clinical chemistry analysers, for the detection of EtG and the synthetic cannabinoids UR-144/XLR-11 and their metabolites in urine.

Methods: In these assays, the corresponding analytes are coated on the latex particles and bind to the specific antibody in the solution, which causes agglutination. When a sample containing the analyte is added the agglutination is inhibited to an extent proportional to the amount of the analyte in the sample. The change in turbidity caused by agglutination is measured as a change in absorbance, which is inversely proportional to the concentration of the analyte in the sample.

The assays are qualitative [cut-off: 500 ng/mL (EtG assay) and 10 ng/mL (UR-144/XLR-11 assay)] and are applicable to a variety of analysers. The results reported were obtained with an RX Daytona analyser.

Results: EtG assay: the assay was standardised to EtG, methyl ethyl glucuronide was also detected with 61.5% cross-reactivity. The limit of detection (LOD) in urine (n=20) was 213 ng/mL. The intra-assay precision (n=20) was <3.5% for 3 different concentration levels of EtG (375 ng/mL, 500 ng/mL and 625 ng/mL). Recovery was assessed with a set of authentic human urine samples (n=32) and 94% were correctly reported as negative and positive with respect to values determined by LC-MS.

UR-144/XLR-11 assay: the assay was standardised to the metabolite UR-144 N-pentanoic acid, UR-144 presented a cross-reactivity(%) value of 25. Other metabolites and synthetic cannabinoids were also detected [cross-reactivity(%) ranging from 28 [UR-144 N-(5-chloropentyl) analog] to 94 (A-796260). XLR-11 metabolites were also detected [cross-reactivity(%) ranging from 33 [XLR11 N-(4-pentyl)analog] to 49 [XLR11 N-(3-fluoropentyl) isomer]. The LOD in urine (n=20) was 2.8 ng/mL. The intra-assay precision (n=20), was <6.5% for 3 different concentration levels of UR144 N-pentanoic acid (5 ng/mL, 10 ng/mL, 15 ng/mL). The mean recovery for all the samples (n=20) at the three concentrations was between 98-105%. All replicates were correctly reported as negative and positive

Conclusion: The results indicate applicability of these latex-enhanced immunoturbidimetric assays to the detection of EtG and UR-144/XLR-11 and their metabolites in urine. The assays are applicable to different automated analysers using ready-to-use reagents, which ensures the reliability and accuracy of the measurements and facilitates the testing procedure.

B-304

The Effect of Sample Storage Conditions on Therapeutic Monitoring of Immunosuppressive Drugs

A. Yaman¹, Y. Aktas¹, R. Turkal², E. Uner¹, O. Sirikci¹, G. Haklar¹. ¹Department of Biochemistry, School of Medicine, Marmara University, Istanbul, Turkey, ²Biochemistry Laboratory, Marmara University Pendik E&R Hospital, Istanbul, Turkey

Background: Conditions of storage and durations between sample collection and analysis may affect the stability of the samples and may lead to false results. We investigated the stability of immunosuppressive drugs cyclosporin, tacrolimus, sirolimus, and everolimus in samples and determined their best storage conditions.

Methods: Whole blood specimens in K₂EDTA tubes sent for drug monitoring (n=10 for each drug) were portioned into 13 aliquots. One sample was analyzed immediately for baseline value, three samples were stored at room temperature (RT), three samples at 2-8°C, and the rest at -20°C, all in dark. The stored samples were analyzed on 1st, 3rd and 7th days with LC/MS/MS (Shimadzu Co., Japan). The samples kept at -20°C were additionally analyzed on 1st, 3rd, and 6th months. The extracts of the samples analyzed for baseline value were kept at 2-8°C and re-analyzed on 1st, 3rd, 7th days. The analytical CV's (CVa) were determined for each drug with quality control materials (ClinChek, Recipe, Germany). The amount of change observed for the conditions tested were calculated as relative bias percentages (RBP) by the formula [(concentration of any time - concentration of baseline)/concentration of baseline] x 100. The medians of RBP for each drug which were less than the CVa were considered stable and not affected by the tested condition (Table 1).

Analyte	CVa %	Storage Condition	Storage Times and RBP		
			1st day	3rd day	7th day
Cyclosporin	6.0	RT	6,7	20,9	7,8
		2-8 °C	3,3	10,8	6,6
		After extraction at 2-8 °C	-11,4	-6,2	-9,7
		-20 °C short term	-0,6	5,3	4,8
			1st month	3rd month	6th month
		-20 °C long term	-1,9	-18,9	-24,9
Tacrolimus	10.5	RT	14,5	-2,7	-3,1
		2-8 °C	15,5	-5,4	-2,9
		After extraction at 2-8 °C	-4,3	-5,9	-17,6
		-20 °C short term	6,8	-8,9	-5,2
			1st month	3rd month	6th month
		-20 °C long term	-0,2	-8,7	-8,9
Sirolimus	7.4	RT	1,4	1,6	7,7
		2-8 °C	16,6	2,0	22,7
		After extraction at 2-8 °C	-7,5	4,9	2,5
		-20 °C short term	-10,5	3,4	19,9
			1st month	3rd month	6th month
		-20 °C long term	11,1	-8,4	-22,2
Everolimus	8.6	RT	7,5	5,3	-0,4
		2-8 °C	2,0	8,3	10,1
		After extraction at 2-8 °C	23,6	21,7	17,6
		-20 °C short term	-2,6	-5,4	1,2
			1st month	3rd month	6th month
		-20 °C long term	1,8	-8,1	-17,3

Results: Best storage conditions determined are given below.

Cyclosporin: 2-8°C for 1 day or -20°C for 1 month

Tacrolimus: After extraction at 2-8°C for 3 days or -20°C up to 6 months

Sirolimus: RT for 3 days

Everolimus: RT for 7 days, 2-8°C for 3 days or -20°C for 3 months

Conclusion: A single common storing condition couldn't be determined for the samples of these drugs. For patients who use more than one immunosuppressive drug, the drawn sample should be portioned and kept under the most appropriate condition to avoid changes caused by instability.

B-305**Evaluation of the ARK Diagnostics Levetiracetam Immunoassay on Beckman AU400 for Therapeutic Drug Monitoring**

C. Abou-Diwan¹, M. Torrey², E. J. Kaleta³. ¹Banner - University Medical Center Phoenix, Phoenix, AZ, ²Laboratory Sciences of Arizona, Tempe, AZ, ³Sonora Quest Laboratories, Tempe, AZ

Background: Therapeutic drug monitoring has been applied to the first generation antiepileptic drugs. The newer antiepileptic agents such as Levetiracetam are often well tolerated, have wider therapeutic ranges, and fewer serious adverse effects. Therapeutic drug monitoring of Levetiracetam is warranted in patients with clinical conditions such as renal failure that may alter the pharmacokinetic characteristics.

Objective: Validate the Ark Diagnostics homogenous immunoassay for the quantitative determination of Levetiracetam levels in plasma, and to compare the immunoassay to a liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay. **Method:** ARK Levetiracetam Assay is a homogeneous immunoassay based on competition between the unlabeled levetiracetam in the patient sample and levetiracetam labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for binding to the antibody reagent. Active enzyme converts the coenzyme nicotinamide adenine dinucleotide (NAD) to NADH that is measured spectrophotometrically as a rate of change in absorbance, by using the Beckman AU 400 automated chemistry analyzers. Validation of this method was performed against a Hitachi 917 chemistry analyzer and an LC-MS/MS method. Performance evaluation included accuracy, linearity, analytical sensitivity, imprecision, carryover, and comparison studies. **Results:** The assay showed excellent correlation to the Hitachi 917 chemistry analyzer ($y = 0.994x - 0.33$; $r^2=0.9967$; $N=40$), and LC-MS/MS ($y = 0.991x - 0.23$; $r^2=0.9980$; $N=16$), with an analytical measurement range of 2.0-100 μ g/dL. The analytical sensitivity was 2.0 μ g/dL. Both within-run (4.6%-4.6%) and between-run (2.6%-5.0%) imprecision were within acceptable limits, and the assay exhibited no carryover (<1%). **Conclusions:** The ARK Levetiracetam immunoassay is suitable for clinical use. This method was successfully used for therapeutic drug monitoring of the novel antiepileptic drug levetiracetam. The method provides rapid turn-around-time (2hours), compared to 2-3 days as a send out test. It is successfully used to optimize therapy for patients with altered renal function.

B-306**Performance evaluation of mycophenolic acid (MPA) on Siemens Dimension Xpand**

L. Cao, C. Maloy, S. Munson, B. Handy, E. Wagar, Q. Meng. MD Anderson Cancer Center, Houston, TX

Background: Mycophenolic acid (MPA) is an immunosuppressant by inhibiting de novo purine synthesis in T- and B- lymphocytes. It is widely used alone or in combination with calcineurin inhibitors and corticosteroids to reduce acute rejection in transplantation. Although the relationship between MPA exposure and its toxicity is weak, its association with early postoperative efficacy, the great inter-patient pharmacokinetic variability and the influence of concomitant immunosuppressants necessitate the therapeutic drug monitoring (TDM) of MPA. The objective of this study was to assess the analytical performance of MPA on Dimension Xpand.

Methods: MPA was determined with a homogeneous particle enhanced turbidimetric inhibition immunoassay (PETINIA). Basically, MPA in the sample competes with the MPA conjugated on the synthetic particles for monoclonal MPA specific antibodies, and the rate of aggregation of the antigen-antibody complex on the particles is measured using bichromatic turbidimetric analysis at 340nm and 700nm, which is inversely proportional to the MPA concentration in the sample. The evaluation was performed following CLSI guidelines. The performance was evaluated for precision, linearity, lower limit of detection, and accuracy. The within-run and between-run precisions were assessed by analyzing QC material at two levels of concentrations. The correlation between MPA on Dimension Xpand and liquid chromatography-tandem mass spectrometry (LC/MS/MS) was assessed.

Results: The within-run CVs for MPA were 3.2% and 3.3% at the levels of 2.1 μ g/mL and 13 μ g/mL, respectively. The between-run CVs at low and high levels were 3.3% and 3.6%, respectively. The analytical measurement range was determined to be linear between 0.2 - 30.0 μ g/mL. The lower limit of detection was 0.12 μ g/mL using measurable value obtained from zero standard + 2SD (n=20). Comparison of MPA on Dimension Xpand with MPA on LC/MS/MS showed that the slope was 1.156 (95% CI = 1.080 to 1.232) with intercept of 0.28 and correlation coefficient $r = 0.981$ (Deming). The mean bias was 0.80.

Conclusion: Our data demonstrate that the MPA on Dimension Xpand analyzer has an excellent precision of performance with good linearity. There is a good correlation between serum MPA tested on Dimension Xpand and MPA tested on LC/MS/MS. Serum MPA concentrations can be precisely and accurately measured and monitored on Dimension Xpand in patients receiving MPA.

B-307**In vitro formation of ethyl glucuronide in meconium specimens**

I. Shu, A. Baldwin, V. Sagnia, M. Jones, J. Jones, D. Lewis, A. Negrusz. United States Drug Testing Laboratories, Inc., Des Plaines, IL

Background: Among all known ethyl alcohol metabolites detectable in specimens of neonatal origin (fatty acid ethyl esters and ethyl sulfate, etc.), ethyl glucuronide (EtG) in meconium has been sought as the most promising biomarker of prenatal alcohol exposure. However, sources of ethanol alternative to maternal drinking behavior, such as ethanol-producing microorganisms, may be present in meconium and could potentially bias the testing results. The **objective** of our study was to investigate the possibility of *in vitro* EtG formation in authentic meconium following alcohol fortification.

Methods: The method for EtG quantitation in meconium consisted of sample homogenization, strong anion-exchange solid phase extraction, and liquid chromatography-tandem mass spectrometry using D₅-EtG as the internal standard. The limit of detection and the lower limit of quantification (LLOQ) were 6 and 12 ng/g, respectively. According to a calibration model established by a 30 ng/g single-point calibrator, the linearity range was 12-600 ng/g ($y=1.028x-4.109$, $r^2=0.9800$; coefficients of variation 11.2 - 13.4% at 24, 160, and 480 ng/g). Individual 0.1 g aliquots from each of the 20 authentic meconium specimens selected were directly subjected to EtG analysis. At the same time, separate 0.5 g aliquots from the same specimens each was thoroughly mixed with 50 microliters of anhydrous 200-proof ethanol, and then stored at room temperature. At 24- and 48-hours, 0.1 g aliquots of ethanol-fortified meconium samples were taken and analyzed for their EtG content.

Results: At time zero, all 20 meconium specimens showed EtG below the LLOQ, but after 24-hour ethanol exposure, 18 of them presented EtG concentrations significantly exceeding the recently recommended cutoff of 30 ng/g (Table).

Conclusion: The amounts and rates of EtG synthesized *in vitro* following the same level and time of ethanol exposure varied significantly between meconium specimens. Therefore, EtG content in meconium is not an ideal biomarker for maternal alcohol intake during pregnancy.

EtG formation in 10 representatives out of 20 meconium specimens following ethanol exposure			
Sample number	EtG Concentration (ng/g)		
	0 hr	24 hr	48 hr
1	ND	12	14
2	ND	23	35
3	ND	107	226
4	ND	279	318
5	ND	369	802
6	ND	713	653
7	ND	829	2643
8	ND	867	959
9	ND	936	2413
10	ND	1150	2076

ND: Not detected (below limit of detection).

B-308**A Serum Fluoride Method by Ion Chromatography and an Assessment of Patient Population Results**

R. Murphy, L. Blum, J. Schemmer, D. Schiller. NMS Labs, Willow Grove, PA

Background:

Exposures to fluoride can occur through its therapeutic and occupational uses. Fluoride is added to drinking water to prevent tooth decay. It can be administered orally to those children who do not have fluoride added to their water. Fluoride is also found in several adult dentifrices used for oral hygiene. Occupational exposures to hydrogen fluoride and its inorganic salts can occur in among other things the manufacturing of fluorocarbon chemicals, in stainless steel pickling, in aluminum production,

in petroleum alkylation, in uranium chemical production, and in the production of fluorinated chemicals including pharmaceuticals. Accidental or intentional ingestions, inhalation, or dermal contacts of fluorides can be life threatening following acute exposures. Chronic exposures to low level concentrations may lead to fluoride induced toxicities of the skeletal system. The determination of fluoride in serum can be a useful means of assessing exposures to fluoride. Accordingly, the objectives of this study are to present a method for measuring fluoride concentrations in serum by ion chromatography and to assess the population data of 395 patient samples from subsequent analyses.

Methods:

In the current method, fluoride concentrations are determined in serum by Ion Chromatography with suppressed conductivity detection. Patient samples were measured with a Thermo/Dionex ICS-5000 or Dionex DX-500 platform with a hydroxide mobile phase gradient and an AG-15/AS-15 guard/analytical column set. Samples were diluted 10x in deionized water and proteins were removed by ultrafiltration before injection onto the column.

Results:

A method of determining fluoride concentrations in serum by Ion Chromatography was validated in our laboratory. The low affinity for fluoride to ion exchange sites requires a high capacity column with optimized chemistry and a relatively low mobile phase strength. The AS-15 analytical column and AG-15 guard column were found to perform the best in separating fluoride from other early eluting anions such as acetate and lactate. Serum fluoride concentrations from 395 patient samples ranged from <0.05 to 2.7 mg/L with a median concentration of <0.05 mg/L. Of the 395 patient samples, 320 of them were less than the reporting limit value of 0.05 mg/L. The available demographic information revealed 187 males and 206 females were tested with the ages ranging from 1 to 96 years. Age-based values showed 43 patients from 1 - 19 years had serum fluoride concentrations ranging from <0.05 to 0.3 mg/L (median <0.05 mg/L); 286 patients from 20 - 65 years had a range of <0.05 to 2.7 mg/L (median <0.05 mg/L); and 59 patients >65 years had a range of <0.05 to 0.29 mg/L (median <0.05 mg/L).

Conclusions:

A method for analyzing elevated fluoride serum concentrations by Ion Chromatography is presented. Careful selection of the analytical column is key in separating fluoride from potentially interfering anions such as acetate and lactate. Although the majority of patient results reported here are below the reporting limit (0.05 mg/L), elevated levels as high as 2.7 mg/L were observed. Ion chromatography has been shown to be an effective analytical tool in determining serum fluoride levels which can be used to assess fluoride exposure.

B-309

Analysis of Everolimus Blood Levels Using a Unique Quantitative Microsphere System (QMS) on the Siemens Dimension EXL Instrument

Z. D. Webb, P. Akl, A. Hardesty, K. E. Blick. *Un of OK Health Sci Ctr, Oklahoma City, OK*

Background: Everolimus is a macrolide immunosuppressant belonging to the MTOR class of drugs (MTOR, mammalian target of rapamycin). Everolimus is used primarily for maintenance immunosuppressive therapy in renal transplantation to prevent acute rejection of renal allografts. As with many immunosuppressant therapies, everolimus is potentially toxic and has a very narrow therapeutic range making drug monitoring a requirement. Accordingly, we validated the Thermo Scientific QMS everolimus assay on our existing Siemens Dimension EXL chemistry analyzer. Methods: An everolimus comparison study was performed between our QMS/EXL method and a LC-MS/MS reference method using whole blood samples from twenty transplant patients. Also, a linearity range study of our QMS/EXL method was performed by analysis of six samples with known everolimus levels. Within and between run precision studies were based on replicate analysis of quality control materials. EP Evaluator (Data Innovations, Ft Myers, FL) software was used to determine the accuracy, linearity, precision, and range of our QMC/EXL everolimus assay. Results: A comparison regression study was performed between our QMS/EXL method and a LC-MS/MS reference method with everolimus blood levels ranging from 2.7 ng/ml to 13.4 ng/ml and yielded the following regression line: ($n=20$, QMS/EXL = $1.04 \times \text{LC-MS/MS} - 0.687$; $r^2=0.999$). Precision data on quality control samples were as follows: Level 1, mean= 3.87 ng/mL (95% CI, 3.76 to 3.97), SD= +/- 0.225, CV= 5.8%; Level 2, mean=8.07 ng/ml (95% CI, 7.81 to 8.33) SD= +/-0.559, CV= 6.9%; and Level 3, mean= 15.3 ng/ml (95% CI, 15.0 to 15.6), SD= +/- 0.680, CV= 4.5%. Linearity samples covered the assay range of 0.238 to 20.1 ng/mL ($n=6$) were assayed by our QMS/EXL method yielding the following results: regression

line slope = 0.988, y-intercept = 0.110 and an observation error of +/- 0.13 ng/ml or 1.1%, well within limits of our allowable systemic error (SEa) benchmark of 1.23 ng/ml or 10.5%. A maximum deviation of 9.1 % was observed from the target of 100% recovery with six of six mean recoveries with all accurate within our allowable limit of +/- 10%. Conclusions: There is good to excellent correlation between our Siemens Dimension QMS/EXL everolimus method and the LC-MS/MS reference method. Other validation studies on our QMS/EXL method show excellent precision, linearity, accuracy, and a clinically acceptable reportable range. We conclude that our Dimensions QMS/DXL everlimus method is acceptable for use in clinical therapeutic drug monitoring on transplant patients.

B-310

Measurement of plasma Apixaban by LC-MS/MS

W. R. Korn, T. M. Paziuk, D. F. Stickle. *Jefferson University Hospitals, Philadelphia, PA*

Background: Direct oral anticoagulation agents (DOACs: apixaban, dabigatran, rivaroxaban) are of relatively new use, and are intended for use without need for routine concentration monitoring. However, there is arguably need for availability of measurement of DOACs for patients awaiting surgery, to ensure achievement of appropriately low levels after cessation of routine administration. For apixaban, this safety threshold is generally regarded as <30 ng/mL. In this context, we were asked specifically to make available an assay for plasma apixaban at our hospital. Subsequently, an LC-MS/MS assay was developed as a hybrid of methods from the literature (Noguez & Ritchie, 2016, PMID 26660170; Schmitz et al., 2014, PMID 25142183). Because numerous other hospitals are also likely to want to establish apixaban assays in the near future, we report our experience in design and validation studies for measurement of plasma apixaban by LC-MS/MS using the AB Sciex API 3200MD. **Methods:** Apixaban standards (0, 55, 118, 274, 615 ng/mL) were purchased from Hyphen Biomed; d4-rivaroxaban, used as the internal standard (IS), was purchased from Santa Cruz Biotechnology. Plasma samples were prepared as follows: 200 μ l of MeOH containing IS was added to 100 μ l of sample; after vortexing and centrifugation, 100 μ l of supernatant was diluted with 100 μ l of starting LC binary mobile phase mixture (50:50 A:B; A = H₂O, 2 mmol/L ammonium acetate, 0.1% formic acid; B = MeOH, 2 mmol/L ammonium acetate, 0.1% formic acid). 20 μ l of the sample preparation was injected for analysis by LC-MS/MS. LC (Shimadzu UFL Prominence) was performed using a Phenomenex Kinetix C8 column (100 A, 5 μ m, 50 \times 4.6 mm) at 34C, with binary mobile phases having fixed flow rate of 0.4 mL/min. Mobile phase gradients were as follows (time (min), %B): 0, 50%; 1, 50%; 2.5, 98%; 4.5, 98%; 4.6, 50%; 5.0, 50%. MS/MS was run using positive electrospray ionization. Multiple reaction monitoring utilized transitions 461->77 for apixaban, and 440->145 for IS. Retention times were 3.3 min (IS) and 3.4 min (apixaban). Injection-to-injection time was 6.5 min. Quality control samples were prepared in pooled human plasma from apixaban purchased from Cayman Chemical. **Results:** The calibration curve was linear across all calibrators (up to 615 ng/mL; $r > 0.99$). Interassay precisions for quality control samples were 8.4% (30 ng/mL) and 4.7% (200 ng/mL). Accuracy of calibration was verified by 1:1 correspondence between measured results and calculated spiked sample concentrations using apixaban obtained from an independent source (Cayman Chemical). LLOQ was 4 ng/mL. Recovery for spiked samples (comparison of measured results to calculated results) was 101 \pm 2.6%. No analytical interferences were observed across an array of therapeutic drugs, drugs of abuse, pooled plasma, and individual patient plasma samples. No ion suppression effects were observed. **Conclusions:** A plasma apixaban assay by LC-MS/MS was developed using the AB Sciex API 3200MD. Analytical performance characteristics of the assay were judged to be suitable for routine use in the laboratory for measurement and reporting of plasma apixaban.

B-311

Comparison of paired umbilical cord tissue and meconium samples for detection of *in utero* drug exposure

J. Colby. *Vanderbilt University Medical Center, Nashville, TN*

Background & Objective

Prenatal exposure to pharmaceuticals, both illicit and prescribed, has many health consequences for neonates. One of the most severe is the development of neonatal abstinence syndrome, which can require weeks of treatment in a neonatal intensive care unit. Drug-exposed neonates are identified based on clinical presentation and history of maternal drug use, but diagnosis is often confirmed using toxicology testing on specimens from the neonate. Meconium has long been considered the gold

standard for neonatal drug testing, but practical considerations make it an unattractive specimen. Umbilical cord tissue has several advantages over meconium, and limited evidence suggests that it may be used as an alternative specimen. The goal of this study was to compare the detection of drugs in paired meconium and umbilical cord tissue samples collected from babies born at Vanderbilt University Medical Center between 10/1/13 and 4/8/15.

Methods

Patients whose providers ordered both umbilical cord and meconium toxicology testing and whose charts were available for review were included in this IRB-approved study. All toxicology testing was performed by a national reference laboratory using a combination of immunoassay and chromatography-mass spectrometry techniques. Drugs that were only available in either the cord or the meconium panel were excluded from the analysis. Toxicology results were gathered from the laboratory information system and clinical information was collected by chart review.

Results

Paired umbilical cord tissue and meconium results were available for 217 neonates. Of these, 77 pairs were concordant negative and 36 pairs were concordant positive. The remaining 104 pairs of samples had at least one discordant result. When metabolites and parent drugs were grouped together, 66 of these became concordant. For the remaining 38, the results from cord indicated a different drug exposure than the results from meconium. When considering drugs by class, the overall agreement between cord and meconium ranged from 75% to 100%. For the 6 individual drugs where adequate data was available, the concentration of drug measured in meconium did not correlate with qualitative detection in cord.

Conclusions

Accurate drug detection and interpretation of toxicology results is important for identification and treatment of drug-exposed neonates. This study demonstrates different sensitivities of drug detection in umbilical cord tissue and meconium, which indicates that these specimens are not completely interchangeable. These results can be used to help clinicians and laboratorians select the most appropriate test to confirm *in utero* drug exposure, and if testing is performed in multiple matrices, to interpret any discordant results.

B-312

Evaluation and Clinical Validation of a Handheld Digital Refractometer for Urine Specific Gravity Measurement

S. P. Wyness¹, J. J. H. Hunsaker¹, T. M. Snow¹, J. R. Genzen². ¹ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, ²Department of Pathology, University of Utah, Salt Lake City, UT

Objectives: Manual refractometers are used by drug testing laboratories in urine specimen validity testing. They are also valuable in assessing hydration status in sports medicine and athletics. Few comprehensive performance evaluations are available demonstrating refractometer capability from a clinical laboratory perspective. The objective of this study was therefore to conduct a clinical validation of a handheld digital refractometer (Palm Abbe™ model PA202X; MISCO) used for human urine specific gravity (SG) testing.

Methods: Validation experiments followed Clinical and Laboratory Standards Institute® (CLSI) guidelines. Precision studies were conducted using BioRad quality control (QC) material over 20 days. Accuracy studies were conducted over 5 days using residual patient urine specimens spanning the analytical measurement range of four urine solutes: Cl⁻, glucose (GLU), total protein (TP), and creatinine (Cr). Accuracy was assessed by comparison of Palm Abbe SG results to SG from a manual refractometer (URC-NE; Atago) and two methods of alternative evaluation: 1) urine osmolality (Advanced® Model 3250, Advanced Instruments), and 2) a “sum of solutes” score defined as the sum of urine Cl⁻, Cr, GLU, K⁺, Na⁺, TP, and urea nitrogen measured for each specimen on a Roche cobas 8000 system (converted to mg/dL). Linearity was assessed using high patient pools diluted with either low patient pools (measuring range SG 1.0060 - 1.0418) or ddH₂O (measuring range SG 1.0001 - 1.0418). A limit of quantitation (LOQ) study was conducted using diluted urine specimens. Carryover was evaluated using low and high concentrations of QC material and patient urine pools. Previously published reference intervals (RI) were validated using 20 random urine samples from healthy donors. Traditional interference testing was not applicable, however, as all dissolved solutes contribute to SG by definition. Modified “interference” studies evaluating the extent of SG changes (with increasing concentration of NaCl, GLU, and bovine albumin) were conducted. Data was analyzed using EP Evaluator (Data Innovations), StataPro (CLSI & Analyze-it), and SigmaPlot (Systat).

Results: Significant carryover was not observed (% differences ≤ -0.02%). A wash step was still included in our procedures as good laboratory practice. Low imprecision

(average 0.0% CVs) was demonstrated using low and high QC material. Accuracy studies showed good correlation to manual refractometry (Deming regression, $y=1.018x-0.019$, $r^2=0.92$; overall bias, -0.1%). Regression after repeat testing of two outliers resulted in improved linear fit (Deming regression, $y=0.983x+0.016$, $r^2=0.99$; overall bias, -0.1%). Outliers were attributed to inadequate specimen mixing. Linear correlation was demonstrated between SG, osmolality, and sum of solutes. Linearity of Palm Abbe performance was verified with observed error of ≤0.1%. The LOQ study demonstrated acceptable performance at the SG level tested (1.0005). This level was selected as it is lower than our expected target for the low end of the clinically reportable range (1.0010). A random urine RI of 1.0020 to 1.0300 was verified. Increases in SG were observed with increasing concentrations of NaCl, GLU, and albumin ($r^2=0.99$, each).

Conclusions: The Palm Abbe digital refractometer was a fast, simple, and accurate way to measure urine SG. Our extensive CLSI-based validation studies demonstrated acceptable clinical performance of this handheld instrument.

B-313

Validation of a LC/MS-MS Method for Pain Management Confirmatory Drug Testing of 55 Drugs and Metabolites

W. E. Nell¹, L. R. Moss², M. E. Hinsdale¹, C. S. Muhammad³, A. Bryant², S. H. Wong². ¹Wake Forest Baptist Medical Center, Winston-Salem, NC, ²Wake Forest School of Medicine, Winston-Salem, NC, ³Sulaimani University, School of Medicine-Kurdistan, Sulaymaniyah, Iraq

Background: Urine drug testing is routinely performed in pain management and other healthcare settings to ensure patient compliance and detect problems such as illicit or non-prescribed drug use, diversion, misuse, and abuse. We developed a LC-MS/MS assay for an academic hospital laboratory in consultation with pain management physicians to simultaneously detect/quantitate 55 drugs and metabolites including tapentadol, buprenorphine, carisoprodol, zolpidem and the following groups: opiates, synthetic opioids, benzodiazepines, amphetamines, barbiturates, anticonvulsants, sedatives, and illicit drugs. **Methods:** Fifty-five compound standards and fifty-two internal standards were monitored using scheduled multiple reaction monitoring on an SCIEX API 5500 mass spectrometer with electrospray ionization in a positive/negative ion switching mode with two ion transitions per analyte for identification and quantitation. Reversed-phase HPLC separation was performed using a biphenyl column (100x2.1 mm, 2.7 μm) with a binary mobile phase (A: 0.1% formic acid in water; B: 0.1% formic acid in acetonitrile) by gradient (5-100% B) with a 0.6-0.8 mL/min flow rate. Seven calibrators were prepared in drug free urine between 1-5000 ng/mL (exact range varied by compound). Samples were prepared by diluting 100 μL patient urine or calibrator with 60 μL of 1:3 internal standard:β-glucuronidase solution, incubating for 30 minutes at 55°C, centrifuging, and diluting the supernatant 1:10 with 90:10:0.1 of water:acetonitrile:formic acid (v/v) in a fresh vial before injection. **Results:** The method validation test did not show any significant sample carry-over, matrix effects, or sample interferences. The compounds at the lowest cut-off had inter-day precision ranges between 4.3-18.5% with 49 of 55 compounds being ≤10%. All but two compounds had an intra-day precision ≤10% at their respective low cut-off concentrations (CV range=2.3-17.3%). The lowest cut-off for each compound was less than or equal to the cut-off used by the send-out reference lab, which utilized a reflex testing algorithm to screen urine samples by immunoassay and confirm positive screens by GC-MS or LC-MS/MS methods. In a qualitative method comparison of 71 patient samples comprising the 33 compounds tested by the reference lab, our LC-MS/MS method was 96.1% (199/207) in agreement with the positive reference lab results; the 8 negative compounds included 2 illicit, 1 benzodiazepine, 5 opiates. We detected and quantified 29 additional positive results (14 were above the screening cut-off) that were not reported by the reference lab. One hundred additional positive results among the 22 compounds not tested by the reference lab were detected by our LC-MS/MS method. When 20 CAP toxicology survey samples were tested, our LC-MS/MS method detected 100% of the drugs reported and the correlation between concentrations by Deming regression was acceptable ($R^2=0.9966$; $y=1.014x-0.0591$). **Conclusions:** Our lab developed a robust, single injection LC-MS/MS method capable of detecting and quantifying 55 drugs and metabolites in urine with minimal sample preparation, acceptable precision, and overall agreement to the results of the reference lab currently used by our hospital as well as to CAP toxicology survey samples. Ongoing method validations using alternative sample matrices are being pursued to monitor compliance using oral fluid and clinical efficacy using serum.

B-314**Quantitation of 32 Drugs in Serum by LC-MSMS**

P. Rutter, M. Jarrells, R. Whitley. *University of Kentucky, Lexington, KY*

Background: Blood drug testing is an effective tool for compliance monitoring, dose correlation and clinical tolerance. The aim of this study was the development of a fast and cost-efficient LC-MSMS method for simultaneously detecting and quantifying thirty-two drugs in serum or plasma. The panel includes opiates, amphetamines, cocaine, benzodiazepines, cannabinoids, analgesics, methadone and barbiturates. Deuterated analogs were used as internal standards.

Method: Fifty microliters of patient serum/plasma was placed into a polypropylene tube. Fifty μL of a 100 $\mu\text{g}/\text{mL}$ internal standard mixture, containing deuterated standards in methanol, was added followed by 200 μL of acetonitrile. The solution was vortex mixed for 15 s and centrifuged at 13,000 rpm for 5 min at room temperature. The supernatant was transferred to a disposable glass tube and evaporated to dryness at 37°C for about 20 min using a gentle stream of air. The extracted sample was reconstituted with 200 μL of mobile phase A, vortex mixed, transferred to an autosampler vial, and injected onto a RESTEK Ultra bi-phenyl analytical column (5 μm , 50 x 2.1 mm) maintained at 40°C. 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 quadrupole mass spectrometer with an electrospray source monitored in positive and negative ion modes.

Results: Specificity was assessed by retention times and unique quantifier/qualifier transition peak area ratios. Total imprecision (40 runs, two concentration levels in duplicate per run) averaged 11% CV with low-level drugs, and 9% CV with medium-level drugs. LOD (s:n ratio of 3:1) ranged from 0.1 to 11.0 ng/mL, and LOQ (s:n ratio of 10:1) ranged from 0.3 to 40.0 ng/mL. Linearities ranged from 0.5 to 2000 ng/mL. Extraction efficiencies for native and deuterated analytes were greater than 87%. Matrix effects of native analytes were similar to corresponding deuterated analogs and did not affect quantitation. No carryover, endogenous or exogenous interferences were observed, with analyte stability at room temperature for 24 h. Qualitative correlation between our procedure and a commercial LC-MSMS method showed 100% agreement at cutoffs. Quantitative correlations showed less than 25% differences.

Conclusion: We present the development and validation of a LC-MSMS procedure for the quantitative determination of 32 drugs/metabolites in serum or plasma at therapeutic levels employing a small amount of a single sample, deuterated internal standards, and a single extraction - without derivatization, additional chromatographic resolution, or preliminary immunoassay screening. This novel method is suitable for routine clinical use.

B-315**Determination of Benzodiazepines Clonazepam, Clobazam and N-desmethyloclobazam in serum by liquid chromatography electrospray ionization tandem mass spectrometry for therapeutic drug monitoring.**

M. E. R. Diniz, L. N. Dias, B. F. P. Paulo, E. Mateo, A. C. S. Ferreira. *Hermes Pardini Institute (Research and Development Sector), Vespasiano, Brazil*

Benzodiazepines are among the most frequently prescribed drugs worldwide and its therapeutic monitoring is very important to optimize the individual dosage of each medicine. The Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) is a reference technique for therapeutic drug monitoring TDM. Therefore, a LC-MS/MS method using a simple liquid-liquid extraction was developed and validated for a therapeutic monitoring of clonazepam, clobazam and clobazam metabolite (N-desmethyloclobazam) in serum. For this, 200 μL of serum samples were spiked with internal standard temazepam and extracted with ethyl acetate. Detection was performed using a Waters Alliance-Quattro Micro tandem mass spectrometer triple quadrupole operate in a positive mode. Chromatographic separation was obtained on a Symmetry C18 column with an isocratic mobile phase containing methanol, water, acetonitrile and formic acid at a flow rate of 400 $\mu\text{L}/\text{min}$. The method had a chromatographic running time of approximately 6.0 min. The limit of detection (LOD) was 1.0 ng/mL⁻¹ for clonazepam, 4.0 ng/mL⁻¹ for clobazam and 35.0 ng/mL⁻¹

for N-desmethyloclobazam. The linear analytical range of the method was between 10.0 and 160.0 ng/mL⁻¹ for clonazepam, 25.0 and 525.0 ng/mL⁻¹ for clobazam and 100.0 and 5000.0 ng/mL⁻¹ for N-desmethyloclobazam. The precision studies were less than 10% for all analytes. The analytes were determined with satisfactory sensitivity, accuracy, repeatability and linearity. In conclusion, the LC-MS/MS method has been applied successfully for the quantitative analysis and therapeutic monitoring of these benzodiazepines.

B-316**Determination of benzoylecgonine in urine using a Solid Phase Extraction and Gas Chromatography Mass Spectrometry for occupational evaluation.**

B. F. P. Paulo, F. V. Andrade, E. Mateo, A. C. S. Ferreira, M. E. R. Diniz. *Hermes Pardini Institute (Research and Development Sector), Vespasiano, Brazil*

The Cocaine is one of the most stimulant drugs. In recent years, abuse of cocaine has become one of the serious social problems. Benzoylecgonine is the main biotransformation product of cocaine in urine and is one of the biological markers of cocaine exposure. In this work, we developed a GC-MS method for determination of benzoylecgonine in human urine using a Strata-X solid phase extraction cartridge followed by BSTFA derivatization. Chromatographic separation was performed on an HP-5MS column (30m x 0.25 mm x 0.25 μm) and helium flux of 2.0 mL/min. The column temperature was held at 160 °C for 2 minutes, it was increased to 300 °C at 20 °C/min and maintained at 300°C for 1 minute. The injection was done in splitless mode and 0.75 min with the injector at 260 °C. Analysis was performed on a GC-MS PerkinElmer Clarus SQ 8T, EI mode, operated at 230 °C. The data is collected by selective ion monitoring mode of the ions of *m/z* 240 (quantifier) and two ions qualifiers for benzoylecgonine and *m/z* 243 (quantifier) and two ions qualifiers for benzoylecgonine-d3. The limit of detection was 15.0 ng/mL and the method was linear between 25.0 and 500.0 ng/mL. The medium range of recovery obtained was between 93.8 and 107.4%. The intra-day was less than 2.6% and inter-day precision was less than 4.1%. In conclusion, the GC-MS method has been developed and validated successfully with a good precision and recovery and has been applied for occupational evaluation of the cocaine exposure.

B-317**Tobramycin Immunoassay Discrepancies - Comparison to Tandem Mass Spectrometry**

C. E. Shalapay¹, E. Ang², J. M. Boyd³, P. D. Colbourne¹, E. J. Dunn², A. Hollier⁴, A. McKeage³, M. Melnyk⁴, A. A. Venner⁵, C. Walker⁵, J. C. Wesenberg⁵, D. F. LeGatt¹. ¹University of Alberta Hospital, Edmonton, AB, Canada, ²DynacareNext, Brampton, ON, Canada, ³Calgary Laboratory Services, Calgary, AB, Canada, ⁴Queen Elizabeth II Hospital, Grande Prairie, AB, Canada, ⁵David Thompson Health Region, Red Deer, AB, Canada

Objective: Tobramycin, an aminoglycoside, is primarily used to treat infections caused by aerobic gram-negative bacteria. Various tobramycin immunoassay methods exist on many clinical analyzers. A previous investigation of immunoassays used in Alberta revealed discrepancies in the results. This study aimed to investigate the accuracy of these assays compared to a tandem mass spectrometry (LC/MS-MS) assay.

Methodology: Fresh drug-free plasma and serum pools were prepared from two volunteers. Four pools of each sample matrix were spiked with tobramycin to obtain concentrations ranging from 1.0 to 10.0 mg/L. Aliquots (0.5 mL) of drug-free and spiked pools were prepared and distributed to four Alberta Health Services laboratories and to a reference laboratory for LC/MS-MS analysis. Samples were shipped refrigerated and analysed within 48 hours. 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) with a PETINIA assay; Laboratory D – Roche Cobas c501 analyzer (Hitachi High-Technologies Corporation, Tokyo, JP) using a homogenous enzyme immunoassay (HEIA); Laboratory E – Thermo TSQ Quantum Ultra (ThermoFisher, Mississauga, ON, CA) with a LC/MS-MS method.

Results: Beckman Coulter DxC 800 results ranged from 3.7% to 15.1% and 15.6% to 31.8% higher than LC/MS-MS in serum and plasma, respectively. Biases in serum and plasma on the Siemens Vista assay were from -10.0% to 22.2% and -4.5% to

-12.0% respectively (Laboratory B) and -10.0% to -22.2% and -8.0% to 13.6%, respectively (Laboratory C), compared to the LC/MS-MS assay. The Roche Cobas c501 assay gave results from -17.0% to 20.0% and -8.0% to 22.2% compared to LC/MS-MS in serum and plasma, respectively. For the Beckman PETINIA assay, 25% of the spiked samples (2/8) gave results >20% higher than LC/MS-MS. For the Siemens PETINIA and Roche HEIA assays, one spiked sample gave results >20% lower and >20% higher than LC/MS-MS, respectively. For the 5.0 and 10.0 mg/L spiked samples (serum or plasma), clinically discrepant results were realized when comparing the Beckman PETINIA assay to both the Siemens PETINIA and ROCHE HEIA methods. Specifically, if these samples were peak/post-dose collections with a target range of 5.0–10.0 mg/L, the 5.0 mg/L spiked serum sample gave a concentration within target by the Beckman assay (6.1 mg/L) and below target by the Siemens and Roche assays (4.4 mg/L); the 10.0 mg/L spiked sample gave a concentration exceeding target by the Beckman assay (11.0 mg/L), while the Siemens and Roche assays gave concentrations within target of 8.8 and 9.5 mg/L, respectively.

Conclusions: Although most immunoassay results were within $\pm 20\%$ of LC/MS-MS values, some results were clinically discrepant. This, in turn, could lead a physician or pharmacist to take different clinical courses of action in regards to dosing. Diagnostic companies have an obligation to clinical laboratorians, physicians, pharmacists and above all, patients, to improve the accuracy of their tobramycin immunoassays through standardization with a non-immunogenic reference method like LC/MS-MS.

B-318

False Positive Carbamazepine Results by Gas-Chromatography Mass Spectrometry (GC-MS) and by VITROS 5600 Carbamazepine Assay Following a Massive Oxcarbazepine Ingestion

U. Garg, L. Johnson, A. Wiebold, A. Ferguson, C. Frazee, S. Thornton. *Children's Mercy Hospitals and Clinics, Kansas City, MO*

Background: Carbamazepine (CARB) and Oxcarbazepine (OCBZ) are used as monotherapy and adjunctive therapy for the treatment of partial and generalized seizures. Both drugs are metabolized to their active metabolites. CARB is metabolized to 10,11-epoxide CARB, and OCBZ is metabolized to 10-hydroxy-10,11-dihydrocarbamazepine (DHC). For therapeutic drug monitoring of CARB, both parent drug and metabolites are measured as both compounds accumulate to significant concentrations. Because conversion of OCBZ is very rapid and the parent drug is present in very low concentrations, only DHC is measured for therapeutic drug monitoring. Reference ranges for CARB and DHC are 4-12 and 15-35 $\mu\text{g/mL}$ respectively. A 23 month old patient with a seizure disorder presented to our emergency department after a suspected unintentional ingestion of his seizure medication. He had significant central nervous system depression and seizure-like movements. At the time of presentation, it was not clear if the patient was on Tegretol (CARB) or Trileptal (OCBZ) or if the patient had ingested another drug. Broad spectrum urine drug screening by gas-chromatography mass spectrometry (GC-MS) and CARB by immunoassay were ordered.

Methods: Broad spectrum drug screening was performed by an alkaline liquid-liquid extraction followed by GC/MS analysis. CARB assay was performed using Ortho Diagnostics VITROS 5600 Integrated System. HPLC linked to a UV detector was used for the assay of CARB, OCBZ and their metabolites.

Results: Urine drug screening by GC-MS showed the presence of CARB and OCBZ. CARB concentration measured by immunoassay was 7.9 $\mu\text{g/mL}$. Since CARB was reported on both GC-MS drug screening and VITROS 5600 immunoassay, it was initially thought that the patient ingested both drugs. Further investigation suggested that the patient ingested a large amount of OCBZ (estimated 4.5 grams) and had no access to CARB. This raised the possibility of a false positive carbamazepine by both GC-MS and immunoassay. To establish the absence or presence of CARB in the sample, a HPLC assay that detects CARB, OCBZ and their metabolites was performed. The HPLC assay did not show the presence of CARB or its metabolite. However, OCBZ and DHC were detected at concentrations of 20.4 and 49.3 $\mu\text{g/mL}$ respectively. To further investigate the interference of OCBZ and/or DHC, plasma samples were prepared with different concentrations of OCBZ and DHC (12.5, 25, 50, 100 and 200 $\mu\text{g/mL}$), and analyzed by VITROS 5600 CARB assay. CARB values for the samples spiked with OCBZ were <3, 4.3, 8.9, 16.3 and >20 $\mu\text{g/mL}$ respectively. CARB values for all the samples spiked with DHC were <3 $\mu\text{g/mL}$.

Conclusion: The false positive CARB result by GC-MS was likely due to thermal conversion of DHC to CARB in the injection port. OCBZ interferes with VITROS 5600 CARB assay. The finding is particularly important in overdose situations when OCBZ concentrations can be high.

B-319

A new high sensitive and specific assay for the determination of acetaminophen on clinical chemistry analyzers

L. Nuerenberg, C. Fache, P. Schu, M. Grimmeler. *DiaSys Diagnostic Systems GmbH, Holzheim, Germany*

OBJECTIVE

Acetaminophen (also known as Paracetamol or APAP) is the most frequently used analgetic drug to treat mild or moderate pain caused e.g. by headache, muscle aches or toothaches. It also is frequently used to treat colds and fevers. In contrast APAP toxicity is the foremost cause of acute liver failure in the Western world and accounts for most drug overdoses. Due to its hepatotoxicity it is, by far, the most common cause of acute liver failure in both the United States and the United Kingdom. Currently available assays for the determination of an acetaminophen overdose in clinical chemistry laboratories do show clear problems with icteric or hemolyzed samples. Current tests also do interfere with the APAP antidote (N-Acetylcysteine, NAC).

METHODOLOGY

Here we present a new ready-to-use, two component reagent to measure acetaminophen on clinical chemistry analyzers (CCA). The new reagent is based on a colorimetric test, coupling the APAP specific enzymatic reaction to a new colorimetric chemistry principle. The new reagent shows an excellent performance using two point end kinetics and a low sample volume of only 4 μL . It is also not affected by so far known interferences like NAC, hemolyzed or icteric samples.

RESULTS

The new test shows a wide linear range from 3 to 600 mg/L APAP and allows for the robust determination of acetaminophen values in serum or plasma samples on routine CCA without prior dilution. Using a Hitachi 917 analyzer system, the test demonstrated an extraordinary precision of < 1% within run at a cut off concentration of 5 mg/L analyte. No significant interferences, at an acetaminophen concentration of 5 mg/L, with bilirubin (conjugated 30 mg/dL and unconjugated 12 mg/dL), NAC 1200 mg/L, hemolyzed samples 800 mg/dL, glucose 1000 mg/dL and intralipid 1000 mg/dL are given for the assay.

CONCLUSIONS

The new acetaminophen test is a very sensitive and highly specific method, which clearly demonstrates diagnostic utility for monitoring regular acetaminophen treatment. It also is very suitable for the diagnosis of patients with an APAP overdose or patients undergoing NAC antidote treatment. For this reasons the new chemistry test of acetaminophen offers a simple, robust and very reliable possibility to diagnose acetaminophen in the clinical laboratory routine.

B-320

New Emit II Plus Buprenorphine Assay with 5 ng/mL Cutoff

A. Dominowski, H. Yau, G. Siefing, N. Morjana. *Siemens Healthcare Diagnostics Inc., Newark, DE*

Background: Buprenorphine is a semisynthetic opioid analgesic compound with a chemical structure similar to morphine. It is used as a substitution drug for treatment of opioid addiction but has a high potential for abuse and addiction. A new assay for measurement of buprenorphine has been developed by Siemens Healthcare on the Viva-E® Drug Testing System. The Emit® II Plus Buprenorphine assay has a cutoff of 5 ng/mL. The assay consists of ready-to-use liquid reagents that provide qualitative and semiquantitative results.

Methods: Precision was evaluated at the cutoff and multiple levels according to CLSI EP5-A2. Analytical recovery was studied by spiking buprenorphine into human urine at levels that span the assay range (0-25 ng/mL). On-instrument stability was assessed by testing the assay controls over time. Specimens (127) were analyzed and the results compared to those of LC-MS/MS. Cross-reactivity with structurally related drugs was assessed at a concentration of 100,000 ng/mL. The effect of common interferences was assessed by spiking the interferents into human urine in the presence of buprenorphine at the control levels of 3 and 7 ng/mL.

Results: Qualitative repeatability CVs (rate) for all levels ranged from 0.38 to 0.58%, and within-lab CVs ranged from 0.77 to 0.94%. Semiquantitative repeatability CVs (ng/mL) ranged from 1.68 to 4.38%, and within-lab CVs ranged from 2.23 to 6.92%. The assay's limit of detection was found to be 0.7 ng/mL. Semiquantitatively, the assay quantified buprenorphine-spiked samples between 2 and 25 ng/mL within $\pm 10\%$ of nominal values. The assay's measuring interval was between 0.7 and 25 ng/mL. At the 5 ng/mL cutoff, the percent agreement of specimens between the Viva-E analyzer and LC-MS/MS was 94%. Discordant samples were within $\pm 25\%$

of the cutoff by both methods. The assay reagents demonstrated similar detection of buprenorphine and norbuprenorphine in urine with minimal cross-reactivity (<0.01%) to the structurally related opioids. Potentially interfering substances gave acceptable results relative to the 5 ng/mL cutoff. The reagents were stable onboard the Viva-E analyzer for a minimum of 4 weeks.

Conclusion: The Emit II Plus Buprenorphine assay on the Viva-E analyzer is a suitable screening method for urine specimens at the cutoff level of 5 ng/mL for both qualitative and semiquantitative analysis of buprenorphine.

B-322

Metabolic Patterns of Drugs and Drug Metabolites Observed in Urine, Serum or Plasma

F. Wu¹, M. H. Slawson², K. L. Johnson-Davis³. ¹Department of Pathology, University of Utah Health Sciences Center, Salt Lake City, UT, ²ARUP Laboratories, Salt Lake City, UT, ³Department of Pathology, University of Utah Health Sciences Center, and ARUP Laboratories, Salt Lake City, UT

Background: Urine and serum specimen types are commonly used to assess patient adherence for prescribed drugs for pain management or for drug abstinence programs. Random specimen collections are typically used to determine whether patients are positive for the prescribed drug(s) and negative for the drug(s) not prescribed. Drug testing is performed by qualitative or quantitative analysis; however, hydration status and urine output volume can impact the drug concentration in various matrices. Quantitative analysis of parent and metabolites are routinely performed to assess adherence over time. However, there is minimal information to characterize the positivity rate of parent drug and metabolite(s) for tramadol, tapentadol, meperidine, fentanyl and methylphenidate, in urine and serum/plasma, to assess patient adherence.

Methods: Deuterated internal standards were added into an aliquot of the specimen. Drugs and metabolites were recovered from the urine, serum or plasma by solid phase extraction. The elution solution was evaporated and reconstituted in water. The solution was then injected into an ultra-high pressure liquid chromatograph coupled to tandem mass spectrometer (Waters Acquity UPLC TQD, [UPLC-MS/MS]). The instrument was operated in an ESI interface using multiple reaction monitoring (MRM) mode. There were four mass spectrometry methods that were validated for clinical use. Cutoff concentrations used to distinguish between positive and not detected varied between drugs. The following concentrations were used as cutoffs: 50 ng/mL (tramadol and tapentadol), 100 ng/mL (tramadol and tapentadol metabolites), 2 ng/mL (meperidine), 5 ng/mL (normeperidine), 10 ng/mL (methylphenidate) and 100 ng/mL (ritalinic acid) in urine and serum/plasma, respectively. For fentanyl and norfentanyl, 1.0 ng/ml was used as a cutoff for urine specimens, and 0.1 ng/ml was for serum or plasma specimens. Retrospective data were evaluated from the laboratory information system at ARUP laboratories (Salt Lake City, UT), a national clinical reference laboratory. The results were evaluated for positivity rates of the drugs and patterns of parent drug and drug metabolite(s).

Results: For urine specimens, the positivity rate was the highest for methylphenidate (61.8%, n=3948), followed by tramadol (43.8%, n=7972), fentanyl (40.9%, n=11136), tapentadol (35.7%, n=1948), and meperidine (9.5%, n=1678). Among positive samples, both parent drug and metabolite(s) was detectable in 95.2% of meperidine samples, 94.4% of tramadol samples, 93.6% of fentanyl samples and 86.6% of tapentadol samples. For serum or plasma specimens, the positivity rate was the highest for methylphenidate (74.2%, n=775), followed by fentanyl (57.3%, n=211), meperidine (55.7%, n=61), tapentadol (50.4%, n=129), and tramadol (30.1%, n=752). Similar metabolic patterns were found in serum or plasma. Of positive results, both parent drug and metabolite(s) were found in 98.3% of fentanyl samples, 79.8% of methylphenidate samples, 79.4% of meperidine samples, 70.8% of tapentadol samples, and 43.4% of tramadol samples.

Conclusion: Our data demonstrates the metabolic patterns of 5 drugs from a random urine or serum/plasma collection in patients. The data presented can be used to guide clinicians to determine drug adherence by assessing the positivity rates of the parent drug and corresponding metabolites. Of note, the lack of metabolites in serum is not indicative of noncompliance.

B-323

Red Blood Cell Methotrexate Polyglutamates are Stable for Over a Decade When Stored at Sub-Zero Temperatures (-80 Degrees C)

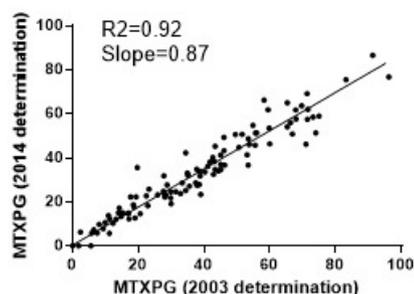
K. J. Brady, R. Apilado, Y. Qu, D. Stimson, C. Ibarra, T. Dervieux. *Exagen Diagnostics, Vista, CA*

Objective: The establishment of long-term frozen stability of analytes is an important aspect of quantitative bioanalysis in therapeutic drug monitoring (TDM). In the present study we sought to establish the long-term stability of red blood cells (RBC) long-chain methotrexate polyglutamates (MTXPG3) from a group of 108 rheumatoid arthritis subjects previously enrolled to establish the performance characteristics of this TDM test (Arthritis Rheum. 2004 50:2766-74).

Methods: RBC MTXPG3 concentrations were originally measured in 2003 using a validated HPLC assay coupled with a post column photo-oxidation technique and fluorimetric detection (Clin Chem. 2003 49:1632-41). All patients enrolled in this study (from December 2002 to April 2003) consented for the long-term storage of their specimen. A total of 104 specimen were stored at -80 C in our biorepository and available for re-analysis in February 2014 using the same analytical method. Results were expressed as nmol/L packed RBCs. Analysis consisted of linear regression with slope.

Results: As presented in the Figure there was a good concordance between RBC MTXPG levels determined in 2003 and those determined in 2014. RBC MTXPG levels determined in 2003 were 38±2 nmol/L (average±SEM) compared to 33±2 nmol/L 11 years later. Regression slope was 0.87 thereby indicating an acceptable bias of 13%. Regression coefficient was 0.923.

Conclusion: This is the first study establishing the long-term stability of RBC MTXPGs over a decade of storage at subzero temperature.



B-324

Prevalence of Gabapentin Abuse Among Clinical Patients

K. Quigley, V. Adhlakha, P. Reddy, A. Miller. *ARIA Diagnostics, Indianapolis, IN*

Background:

Prescription medication abuse has drastically increased in the US over the last three decades. While toxicological analysis of clinical specimens is common practice among physicians, expanded testing to identify inconsistencies associated with non-prescribed drugs is less common.

During routine urine toxicology testing it was noted that a significant amount of the patient population was testing positive for Gabapentin when not prescribed by the physician. At the request of a single physician, a clinical investigation was conducted to assess the rate of misuse among his patients to determine the potential dangers associated with non-reported Gabapentin use.

Methods:

The purpose of this study was to determine the prevalence of Gabapentin misuse among a subset of clinical patients. LC/MS/MS testing was conducted on urine specimens when requested by the treating physician. The range of testing includes opiate/opioids, drugs of abuse, Gabapentin, and Pregabalin, in addition to other substances. The method used for testing underwent a complete validation study where accuracy, precision, linearity (AMR), interference, carryover, ion ratio, retention time, LLOQ, matrix effect, suppression and specificity were assessed.

Using data mining, the laboratory identified all of those patients whose urine drug analysis indicated illicit Gabapentin use.

Results:

A total of 323 patients were tested over a five month period. Of these patients, 240 (74.30%) were compliant with their prescription drug regimen: positive for prescribed substances, negative for non-prescribed substances. In addition, 13 patients (4.03%) were non-compliant, testing negative for prescribed Gabapentin. A total of 70 patients (21.67%) were positive for non-prescribed Gabapentin.

Of those patients who tested positive for non-prescribed Gabapentin, 66 patients were on prescription drug regimens that included other substances: Opiate/Opioid (55.7%), Opiate/Opioid and Cyclobenzaprine (8.57%), Opiate/Opioid and Benzodiazepines (18.57%), other substances (8.57%). Four patients were not prescribed medications but tested positive for other substances in addition to Gabapentin.

Discussion/ Conclusion:

Gabapentin (Neurontin) is a known analogue of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) and is used for its anticonvulsant properties. While most widely used as an antiepileptic, in recent years it has also been used to treat neuropathic pain. Little is understood regarding the mechanism of action, however it is thought to have some effect on voltage activated calcium channels. Side effects include dizziness, ataxia, somnolence, nervousness and fatigue.

Little information exists regarding the significance of Gabapentin abuse among clinical patients. Until recently, it was considered to have little potential for abuse however this review has shown that a significant amount of patients are taking Gabapentin without physician consent. This could be due to the fact that recent studies have revealed that Gabapentin may potentiate the "high" obtained from other central nervous system acting drugs.

While Gabapentin is relatively safe and has a low potential for serious adverse effects, even in large doses, negative effects may occur when concomitant use with other CNS depressants occurs. Our study demonstrated the importance of performing full confirmatory testing in order to effectively identify at-risk patients.

B-325

Automating the Initial Chromatogram Review of a Liquid Chromatography Tandem Mass Spectrometry-based Broad Spectrum Urine Toxicology Screening Procedure with Peak Integration Software

A. S. Ptolemy, S. Standish, M. Hoseh, G. Waite, D. Bailey, H. Li. *Dynacare, London, ON, Canada*

Background: Our regional reference laboratory performs qualitative broad spectrum urine toxicology screening via a targeted liquid chromatography tandem mass spectrometry (LC-MS/MS) procedure. This protocol uses defined positive/negative cut-off concentrations to screen urine for the presence of 63 different licit and illicit drugs. Volume for this test has grown ~30% since it was introduced in September 2013. This growth combined with our current process of requiring a medical laboratory technologist (MLT) to manually review the quality of all chromatographic results prior to release has impacted the tests turn-around-time. The commercially available ASCENT® software from Indigo bioAutomation is an algorithm-based peak picking and chromatographic data review system. This software deploys an exponentially modified Gaussian equation best fit model for standardized peak integration and offers the potential to automate the initial chromatogram review. **Objective:** Validate the ASCENT® software and its user-defined quality assurance peak integration rules by evaluating the reporting accuracy of the automated procedure relative to the current manual process. **Methods:** The validity of the ASCENT®-based protocol was assessed through parallel batch specimen processing and a comparison of the software-reviewed and MLT-reviewed data. Accuracy of the automated process was assessed by evaluating: (a) the relative agreement of all positive/negative results for all tested analytes within $N > 1500$ patient specimens; and (b) the correctness of external proficiency testing challenge results. Although this test is reported qualitatively, the relative agreement of the derived analyte concentrations for all positive results was also compared. Within the tested patient specimens, the positivity rates for all analytes ($N=63$) included within the screen respectively ranged from 0 to 75.3%. The software's ability to accurately integrate low prevalence analytes was verified via its review of standard, quality control (QC) and spiked patient material with known analyte concentrations spanning their respective cut-offs and linear measuring ranges.

Results: The derived concentrations of the calibration and QC standards in all tested batches were within our defined quality acceptance criteria when reviewed by either the MLT or software. A 99.9% (97009/97146) overall agreement between the software-reviewed and MLT-reviewed positive/negative patient results was observed. 82.5% (113/137) of the discordant results had analyte concentrations within ± 20 ng/mL and/or 20% of their respective positive/negative cut-off concentrations. Classifications of the remaining 17.5% (24/137) of discordant results were attributed to: (a) gross

analyte concentration differences between the MLT-reviewed result and software-reviewed result ($N=6$); (b) MLT-review reporting error ($N=3$); and (c) software-review reporting error due to deficiencies in the implemented quality assurance peak integration rules ($N=15$), which were corrected prior to implementation. The software-reviewed results of all external proficiency testing challenges ($N=5$) were 100% accurate. The median percent concentration difference of all positive screening results ($N=5868$ from $N=53$ analytes) within the tested patient specimens was 4.1% (range: 0.01 to 36.2%). **Conclusion:** The automated ASCENT® software-review of our LC-MS/MS-based broad spectrum urine toxicology screening chromatograms had the equivalent accuracy and quality of our initial MLT-review process. Laboratories seeking opportunities to automate LC-MS/MS-based testing may consider using the outlined validation procedure to evaluate the ASCENT® software as a potential process enhancement.

B-326

Validation of an LC-MSMS method for nicotine reveals that the tobacco alkaloid anabasine is of limited clinical utility in differentiating active smokers from patients taking nicotine replacement therapy

M. Feldhammer¹, D. Menasco², W. Zhang¹, J. C. Ritchie¹. ¹Emory University, Atlanta, GA, ²Biotage, Charlotte, NC

Intro: The need to provide accurate and quantifiable data in the detection of patient adherence to smoking cessation programs necessitates the utilization of high-resolution instrumentation. Reliance on immunoassay-based approaches can lack the sensitivity and specificity to provide a complete clinical picture in the context of various nicotine replacements and smoke cessation therapies. Surprisingly, during our validation of a new LC-MS/MS assay, we determined that this utilization resulted in a very high false negative rate when compared to patients' self-reported smoking status. Our results therefore call into question the clinical utility of anabasine as a biomarker of active tobacco use.

Methods: Patient urine samples were obtained from our orthopedics and transplant centers. Samples analyzed by automation were purchased from UTAK. Interfering matrix components were removed using supported liquid extraction (SLE) columns either by vacuum manifold or on the Extrahera (Biotage®) automation station. The extracted analytes (nicotine, cotinine, trans-3'-hydroxycotinine and anabasine) were chromatographically resolved over 7 minutes on a Waters 50 x 2.1, 1.7 μ m BEH-C₁₈ column prior to injection and analysis by tandem mass spectrometry (Waters Corp.). Linear responses were evaluated across the typical range reported for routine tobacco users. Patients smoking status was self reported to Emory clinicians and obtained by analyses of the electronic medical record. Patients were categorized as either active smokers (nicotine > 30 ng/mL; cotinine > 50 ng/mL; trans-3'-hydroxycotinine > 120 ng/mL; anabasine > 3 ng/mL) or non-smokers if they did not fulfill the above criteria. [3].

Results: The quantification limits of the method were 1-5 ng/mL and the limits of detection ranged from 0.6-5 ng/mL. The linear range for all analytes was confirmed over 5-5000 ng/mL. Interday and intraday precisions for all analytes had a C.V. of <15% at the low end of the quantification limit. Method comparison studies were performed with either GC-MS (within institution method) or by LC-MS/MS (ARUP) correlation coefficients were determined to be between (0.95-0.99). Under the auspice of automation, recoveries were determined to be 107, 95, 53, and 107 percent for nicotine, anabasine, trans-3'-hydroxycotinine, and cotinine, respectively. Moreover, sample processing times (completed in batches of 24) decreased by 50% using the Extrahera™ automation station when compared to manual extractions. Most surprisingly we determined that 64% of our patients that self declared as active smokers and tested positive for nicotine had anabasine levels below <3 ng/mL.

Conclusions: Nicotine, cotinine, trans-3'-hydroxycotinine and anabasine can be simultaneously accurately quantified in human urine by LC-MS/MS to distinguish between smokers and non-smokers. Moreover, workflow efficacy improved significantly when automation was integrated into the pipeline. Interestingly the clinical utility of anabasine as a means of detecting active tobacco use is highly questionable, as up to 64% of admitted smokers had no detectable levels of the alkaloid.

B-327**Validation of Analytical method for quantification trans-trans muconic acid (t,t-MA) urinary as a biomarker exposure to benzene**

E. K. Sugawara, J. R. Joaquim, J. S. Ratero, J. Monteiro, D. R. Ramadan, S. Tufik. *AFIP, São Paulo, Brazil*

Background: The primary purpose of biological monitoring is to protect the health of employee, preventing them from excessive exposure to chemical products. The t,t-MA is a benzene biotransformation product and has been recommended as exposure biomarker adopted by the Brazilian legislation for the monitoring of occupational exposure to solvent. This study was conducted in order to validate the urinary t,t-MA detection method, for their use in biomonitoring of employees exposed to the product.

Methods: We used high performance liquid chromatograph Hewlett-Packard® HP 1100 brand model consisting of autosampler, quaternary pump and UV detector with variable wavelength. Detection was accomplished at 227 nm. For the chromatographic separation employed Hypersil GOLD aQ® analytical column (125 x 4 mm, 5µm) and maintained at 30 °C during the analysis. The mobile phase was water:methanol (94:6, v/v) with a flow rate of 1.0 ml/min. The linearity was observed in the expected concentration range. Urine was used as a biological matrix for the study and t,t-MA standard.

Results: The linearity was studied using enriched samples of t,t-MA from 0.1 to 3.00 mg/L and the urine samples were evaluated by six times, each. The limit of detection (L.D.) and the limit of quantification (L.Q.) were defined as the lowest concentration, whose the coefficient of variation did not exceed 20% and 15%, respectively. The intra-assay precision was demonstrated by average coefficient of variation (CV%) for urine sample analyzed from seven different concentration and by six times, each one, in the same moment of the study. To determine inter-assay CV% mean analyzed three different concentrations for three days. The accuracy of the method was verified by analyzing samples of known concentration and expressed in percentage. The retention time (RT) of 3.3 min was obtained and the total analysis time was seven min. The use of solvents such as ether extraction and acetonitrile in the mobile phase composition, ensure simplicity and low cost in the exam. Linearity was studied in the concentration range 0.1 to 3.0 mg/L with a coefficient of determination (R²) of 0.99374. The CV% average intra-assay accuracy and precision obtained for the range of concentration of 0.2 to 3.0 mg/L was 2.86%. For the concentration range between 0.5 and 1.5 mg/L mean inter-assay CV% was 5.10%. The method's accuracy is between 87% and 109%. The samples used in determining these parameters were prepared from urine obtained through donation and previously submitted to the proposed method, proving the absence of the substance.

Conclusion: The method was quick and efficient in determining urinary t,t-MA. The efficiency and selectivity combined with the technical robustness can be employed to t,t-MA dosage in the control of occupational exposure to benzene.

B-328**Validation of a Broad Spectrum Drug Screening Method Using High Resolution LC-TOF**

I. Metushi, R. Fitzgerald. *UC San Diego Health, San Diego, CA*

Introduction: Urine drug screening is among the most widely used procedures in clinical toxicology laboratories. Immunoassay is the primary method used for drug screening in many laboratories followed by confirmation through tandem mass spectrometry (LC-MS/MS). LC-MS/MS isolates the protonated analyte [(M+H)⁺] of interest followed by fragmentation in the collision cell and monitoring of one or more product ions. However, one of the major limitations of LC-MS/MS is that it difficult to perform non-targeted screening. Recently, high resolution mass spectrometry (HRMS) such as time of flight-mass spectrometry (TOF-MS) has been proposed as an alternative for non-targeted drug screening. We validated a UPLC-TOF HRMS method for drug screening by assessing reproducibility (within run and between run precision), patient comparison studies and carry over effects. **Methods:** Sixty one drugs were divided into six groups at three different concentrations (100 ng/mL, 1000 ng/mL and 5000 ng/mL) and within run (five injections), between run (twenty injections over 20 days) and patient comparison studies were performed. Identification criteria for a positive result involved accurate mass (within 5 ppm), one fragment (within 10 ppm) and retention time match (within 0.2 min). Compound separation was achieved using Waters and detected through TOF-MS (Xevo G2 TOF; Waters Corp). A MRM-based LC-MS/MS (UPLC-Xevo TQ-S) was used to confirm positive results and served as the reference method. **Results:** Within run studies revealed that out of 5 injections, norpropoxyphene, EDDP and tramadol were not identified at a low concentration of 100 ng/mL; however, detection greatly improved at the

higher concentrations of 1000 ng/mL and 5000 ng/mL. The remaining drugs were all identified 5/5 times at all three concentrations. Between run precision studies revealed that, as in the case of within run precision, norpropoxyphene and tramadol were not identified at 100 ng/mL. In addition, other drugs such as morphine, norbuprenorphine, propoxyphene, EDDP and MDA were identified 17/20, 14/20, 17/20, 6/20 and 18/20 times respectively at a concentration of 100 ng/mL. Upon increasing the concentration of these drugs to 1000 ng/mL and 5000 ng/mL the detection rate improved significantly. Initial patient comparison studies for 23/61 drugs in the panel were performed. The LC-MS/MS method identified a total of 244 compounds while the TOF-HRMS method identified 190 and 188 compounds respectively when samples were run in duplicate. The drugs with the highest false positive rate of identification were oxycodone, EDDP, hydrocodone, lorazepam and methamphetamine. Drugs with the highest false negative rate identification were: fentanyl, morphine, norfentanyl, hydroxalprazolam, amphetamine, norfentanyl, methamphetamine and nordiazepam. TOF-HRMS was able to identify a number of drugs that were not part of the normal confirmation panel through LC-MS/MS. **Conclusion:** Our UPLC-TOF HRMS method for drug screening offers the advantage of performing non-targeted drug screening (compared to MRM based LC-MS/MS) and has the potential for higher sensitivity and specificity than conventional immunoassays. Current validation studies show that at low drug concentrations, some of the drugs such as norpropoxyphene and tramadol were missed, mainly because due to the lack of fragment identification. However, upon increasing the drug concentrations the identification status improved significantly.

B-330**Non-FDA-Approved Benzodiazepine Analogs: Clonazolam and Etizolam. Reactivity with the ThermoFisher Immunoassay, Elimination Half-Life, and Incidence in Benzodiazepine Positive Urine Samples.**

X. M. R. van Wijk¹, A. Arens¹, D. Lung², A. H. B. Wu¹, K. L. Lynch¹.
¹University of California, San Francisco, San Francisco, CA, ²San Mateo Medical Center, San Mateo, CA

Background: Benzodiazepines are widely used for treatment of anxiety and insomnia, however, this class of drugs is also commonly abused. Many different benzodiazepines and analogs have been produced that are not FDA-approved. We recently encountered two of these, clonazolam and etizolam, in clinical toxicology cases in the San Francisco Bay Area. The objective of this study was to determine the reactivity of these two benzodiazepine analogs with the immunoassay that we use in the clinical lab and determine the incidence in urine samples that were analyzed for drugs-of-abuse. We also determined the elimination half-life in a case of dual ingestion of clonazolam and etizolam.

Methods: For immunoassay cross-reactivity studies, 0-800 ng/mL of clonazolam or etizolam was prepared in drug-free urine. Samples were analyzed in triplicate using the ThermoFisher CEDIA® High Sensitivity Benzodiazepine Assay. For incidence studies, we collected 211 urine samples that were previously determined positive on the ThermoFisher Benzodiazepine Assay at the Zuckerberg San Francisco General. Samples were analyzed using a previously validated liquid chromatography high resolution mass spectrometry (LC-HRMS) method. Also, serum samples from a case of dual ingestion of clonazolam and etizolam were analyzed using this LC-HRMS method. Preparation of serum samples consisted of protein precipitation by acetonitrile, drying of the sample, and reconstitution in 2% mobile phase B. Urine samples were diluted 1:9 using 2% B. HRMS data was acquired with an ABSciex TripleTOF®5600 system in positive ion mode, collecting full scan data with IDA triggered acquisition of product ion spectra. Chromatographic separations were performed on a Phenomenex Kinetex C18 column (50 x 3.00 mm, 2.6 µm). Mobile phase A was 0.05% formic acid in 5 mM ammonium formate. Mobile phase B was 0.05% formic acid in 50% methanol 50% acetonitrile. The elution gradient was ramped linearly from 2% to 100% B over 10 minutes. Data analysis was done using PeakView® and MasterView® software (version 2.0, AB Sciex).

Results: Clonazolam and etizolam both showed significant cross-reactivity with the ThermoFisher CEDIA® High Sensitivity Benzodiazepine Assay. When using our current cut-off of 200 ng/mL, a concentration greater than 113 (95% CI: 107-120) ng/mL clonazolam, or 195 (187-204) ng/mL etizolam, was calculated to lead to a positive test result. However, none of the 211 analyzed urine samples, which were previously determined positive on the ThermoFisher Benzodiazepine Assay, were found to contain clonazolam, etizolam, or their metabolites. In a clinical toxicology case of dual ingestion of clonazolam and etizolam, the highest concentration found in serum was 10.2 and 281 ng/mL, respectively. The elimination half-life was 216 (95% CI: 136-521) and 285 (227-386) minutes, respectively.

Conclusion: Clonazepam and etizolam significantly cross-react with the ThermoFisher CEDIA® High Sensitivity Benzodiazepine Assay. Although recently encountered in clinical toxicology cases, clonazepam and etizolam were not detected in 211 benzodiazepine positive urine samples. The elimination half-life for etizolam was similar to previously reported values. An elimination half-life for clonazepam was not reported earlier.

B-331

Up in Smoke: Uncovering a Lack of Evidence for Proton Pump Inhibitors as a Source of THC Immunoassay False Positives

J. L. Powers¹, N. N. Rasmussen², D. Hurst³, F. G. Strathmann¹. ¹University of Utah, Salt Lake City, UT, ²ARUP Laboratories, Salt Lake City, UT, ³University of North Carolina, Greensboro, NC

Background: False positives in immunoassays can result from cross-reactivity of antibodies. This becomes an issue in interpretation of drug screens, and it is recommended that false positive screens be followed up with more specific confirmatory testing. Since 2005 the drug package insert for pantoprazole has included a statement of reports of false positive urine screening tests for tetrahydrocannabinol (THC) in patients receiving proton pump inhibitors (PPIs). However, no data is given nor a specific screen mentioned. A search of literature and the internet did not result in concrete data. There is an adverse event report of a positive THC meconium screen from an infant whose mother was taking pantoprazole, but the urine screen was negative. Recently, a case report was published claiming a false positive THC from a young patient who presented with cyclic vomiting syndrome and was given ondansetron, pantoprazole, and diazepam. The urine drug screen obtained 2.5 h after administration of pantoprazole was positive, but confirmatory testing via gas chromatography-mass spectrometry was negative. However, testing in the absence of pantoprazole was not done and there was no mention of the assay or cutoff used. A higher number of false positive THC urine screens in infants compared to adults has been attributed to metabolite differences. **Methods and Results:** Although the number of THC positive screens in our lab that do not confirm is low, we wanted to investigate the possibility of PPIs contributing to these. A THC-negative urine sample was spiked with pantoprazole (10,000 ng/mL) and analyzed using the EMIT II Plus Cannabinoid assay. This detects the 11-nor- Δ^9 -THC-9-COOH metabolite (THC-COOH) with a 20 ng/mL cutoff. A negative result was obtained. Since pantoprazole is metabolized in vivo and almost 80% of an oral dose is excreted as metabolites, we also investigated urine samples from 32 patients currently taking a PPI (dexlansoprazole, n=4; esomeprazole, n=3; lansoprazole, n=2; omeprazole, n=17; pantoprazole, n=6). These samples were obtained under IRB approval from volunteers or from samples previously submitted for drug testing to our lab for which current drug history was also available. These also resulted in no positives at the 20 ng/mL cutoff. Investigation of raw data identified one sample close to the cutoff value; it confirmed as positive for THC by a liquid chromatography-tandem mass spectrometry method with a 5 ng/mL cutoff. Since the number of samples available was low, we used computational techniques to determine whether a pantoprazole metabolite shares any structural or electrostatic similarities with THC-COOH. Pantoprazole sulfate, the major metabolite, and THC-COOH were built in Maestro and tautomeric states determined using Epik in water at a pH of 7 (± 2). A 25K step mixed torsional/low mode conformational search was performed and electrostatic potential energy was calculated in an OPLS3 forcefield. Comparison of structures shows very little overall similarity in shape or electrostatics between the two molecules. **Conclusion:** We find no supporting evidence of pantoprazole or its metabolites as the cause of false positives in the EMIT II Plus Cannabinoid assay and caution the use of PPIs as a possible explanation for THC immunoassay false positives.

B-332

Trends in Heroin Related Deaths and 6-MAM Concentrations from the Hennepin County Medical Examiner's Office Between 2004 and 2015

S. A. Love¹, J. Lelinski², J. Kloss¹, O. Middleton³, F. S. Apple¹. ¹Hennepin County Medical Center, Minneapolis, MN, ²Milwaukee County Medical Examiner's Office, Milwaukee, WI, ³Hennepin County Medical Examiner's Office, Minneapolis, MN

Background: Over the past decade, prescription opiate use and widespread abuse have become major concerns driving changes to opiate prescribing practices and prescription monitoring programs. These changes have accompanied increases in both the use of heroin and in heroin related deaths. This study evaluated the trends in heroin

related deaths and associated concentrations of the heroin metabolite 6-monoacetyl morphine (6-MAM) in urine, blood, and vitreous humor. **Methods:** We retrospectively reviewed the cause of death (COD) for cases from the Hennepin County Medical Examiner's Office between 2004 and 2015 where heroin was suspected as a potential contributor to COD either based on investigative information or as identified by blood or urine drug screen. **Results:** We identified 287 heroin related deaths. From 2004 to 2006 there were an average of 12 heroin related deaths per year. This significantly increased ($p=0.009$) to a mean of 57 heroin related deaths per year from 2012 to 2014. 79% of the decedents were male and the mean (SD) decedent age was 36 (12) years. Mean (SD) 6-MAM concentrations were significantly different between sample types: urine 0.540 (0.439) mg/L; blood (various sources) 0.025 (0.027) mg/L; vitreous humor 0.064 (0.063) mg/L. Mixed drug toxicity was the COD in 46% ($n=130$) of cases. Ethanol was also present in 104 (36.2%) cases, with a mean (SD) concentration of 0.113 (0.124) g/dL. Mean (SD) blood concentrations of morphine were: total 0.899 (1.08) mg/L and free 0.233 (0.278) mg/L. In addition to opiates, cocaine ($n=81$) and benzodiazepines ($n=61$) were the most frequently identified drug classes on screening. Accident was the manner of death (MOD) in 94% ($n=265$) of the identified cases, with the remaining cases' MOD evenly split between natural, homicide, and suicide. **Conclusion:** We found the number of heroin related deaths increased nearly 5-fold between 2004 and 2015. Postmortem urine, blood, and vitreous humor specimens are all useful in identifying 6-MAM to confirm heroin related deaths.

B-333

Evaluation of a New ELISA Based Test for the Determination of Pregabalin in Biological Samples

M. Welsh, V. Anderson, J. Darragh, P. Lowry, S. Lindsay, E. Benchikh, R. McConnell, S. FitzGerald. Randox Laboratories Ltd, Crumlin, United Kingdom

Background: Pregabalin is a gamma-aminobutyric acid analog used for the treatment of neuropathic pain and partial seizures, as well as generalised anxiety disorder. It is also used to treat fibromyalgia in the USA and is currently listed as one of the top 30 prescribed medications. Pregabalin is eliminated from the systemic circulation primarily by renal excretion as unchanged drug with a mean elimination half-life of 6.3 hours in subjects with normal renal function. In recent years, pregabalin abuse has increased, especially among heroin addicts and prison inmates.

As the demand for a method suitable for high-volume screening of pregabalin is increasing, this study reports a new Enzyme-Linked Immunosorbent Assay (ELISA) for the detection of pregabalin in urine and whole blood. This is relevant for compliance monitoring of pain patients and for clinical toxicology applications.

Methods: Pregabalin was derivatised and conjugated to immunogenic carrier material (Keyhole Limpet Haemocyanin). The resulting immunogen was administered, via intramuscular injection, to adult sheep, to initiate polyclonal antiserum production. The antiserum generated was employed in the development of a competitive colorimetric immunoassay. Ig fraction derived from the antiserum was immobilized and stabilized on a 96-well micro-titre plate. The analyte, if present in the sample, competed with horseradish peroxidase labelled pregabalin conjugate for antibody binding sites on the microtitre plate. Absorbances were read at 450nm.

Results: Specificity was determined by calculating percentage cross-reactivity with the major metabolite N-methylpregabalin and a range of therapeutic compounds. The assay exhibited specificity for pregabalin and < 1% cross-reactivity with the other compounds tested. The analytical range of the assay was 0 - 50 $\mu\text{g/mL}$ pregabalin with Limits of Detection (LOD) of 0.05 $\mu\text{g/mL}$ for urine and 0.159 $\mu\text{g/mL}$ for whole blood. Recovery and inter-assay precision were assessed using spiked negative urine and whole blood at the cut-off and +/- 50% cut-off (1, 2 and 3 $\mu\text{g/mL}$). Three replicates of each level were assessed over 5 separate runs. For each level the recovery was within the target range of 100 +/- 20%, while inter-assay precision expressed as CV(%) was ≤ 10 . Additionally, 20 authentic urine samples, 10 positive for pregabalin and 10 for gabapentin, were assessed and correlated with the concentration obtained via liquid chromatography mass spectrometry (LC-MS). Specificity for pregabalin was confirmed and the assay exhibited 100% agreement with the LC-MS method.

Conclusion: This evaluation indicates applicability of this new ELISA to the detection of pregabalin in blood and urine. The LODs for both matrices were below the cut-off concentration of 2 $\mu\text{g/mL}$. The assessment of samples showed optimal concordance whilst maintaining specificity to pregabalin with minimal detection of gabapentin. Thus, the ELISA represents a highly specific and useful analytical tool, which could be applied within a clinical setting to compliance monitoring of pain patients and toxicology applications.

B-335**Long-term Storage Stability of Opioid Drugs in Urine, Dependence on Sample pH**W. R. Adams, D. N. Schiller. *NMS Labs, Willow Grove, PA*

Background: Urine analysis of opioids is employed for a variety of purposes, including pain management, employment eligibility, and diagnosis of drug intoxication. There is often a need for long-term storage of urine samples prior to analysis. We observed that most opioids were stable in frozen (-20 C) urine, with two notable exceptions: buprenorphine and 6-monoacetylmorphine (6MAM) fell below acceptable limits prior to 30 days of storage. The recovery of other analytes suggested this was an analyte-specific phenomenon. We hypothesized that the pH of the sample was outside of the normal range of 6-8, leading to premature degradation of sensitive analytes.

Methodology: Discarded urine samples containing buprenorphine-glucuronide or 6MAM were pooled and the pH was verified between 6 and 8. Samples were stored frozen in preservative free plastic cups. Buprenorphine-glucuronide samples were prepared by enzymatic hydrolysis and liquid-liquid extraction, then quantitated by liquid chromatography tandem mass spectrometry (LC-MS/MS) using a method that performs within a 9.8% CV for between run replicates. 6MAM samples were prepared by solid phase extraction, then quantitated by LC-MS/MS using a method that performs within a 6.0% CV for between run replicates.

Results: Concentrations (ng/mL ± standard deviation) of sensitive analytes in routine and pH controlled stability experiments. *Indicates result below acceptable limit (80% of Day 0 mean). Data from the lower of two target concentrations is shown.

Day	Spiked Buprenorphine	Patient Buprenorphine, pH 6 to 8	Day	Spiked 6MAM	Patient 6MAM, pH 6 to 8
0 (n=5)	14.8 ± 0.6	15.8 ± 0.7	0 (n=5)	10.5 ± 0.1	20.6 ± 0.7
1 (n=3)	10.0 ± 0.3*	13.4 ± 0.4	1 (n=3)	10.4 ± 0.2	
2 (n=3)	13.7 ± 1.1	12.8 ± 0.1	7 (n=3)	9.4 ± 0.1	
7 (n=3)	13.1 ± 0.5	13.0 ± 0.2	14 (n=3)	7.2 ± 0.2*	18.5 ± 0.3
14 (n=3)	8.5 ± 0.7*	13.0 ± 0.3	30 (n=3)	7.6 ± 0.4*	18.3 ± 0.4
30 (n=3)	10.4 ± 1.2*	16.5 ± 0.4	60 (n=3)		19.8 ± 0.4

Conclusions: Free buprenorphine exhibits variable recovery when spiked into urine. By utilizing positive patient samples and maintaining pH between 6 to 8, the standard deviation of replicates was reduced, giving a more accurate determination of stability. Selecting samples with normal pH was also beneficial for determining the stability of 6MAM in urine. Clients are now advised that published stability data is applicable to urine samples with pH of 6 to 8 for tests that include sensitive analytes.

B-336**Evaluation of the ARK Diagnostics Lamotrigine Immunoassay on Beckman AU 400 for Therapeutic Drug Monitoring**C. Abou-Diwan¹, M. Torrey², E. J. Kaleta³. ¹Banner - University Medical Center Phoenix, Phoenix, AZ, ²Laboratory Sciences of Arizona, Tempe, AZ, ³Sonora Quest Laboratories, Tempe, AZ

Background: Lamotrigine is an anti-epileptic drug used as an adjunct therapy for partial seizures, and has since gained indications as monotherapy for partial seizures and also as treatment for bipolar disorder. Lamotrigine is commonly used in pregnant women with epilepsy due to its solid safety record in pregnancy. Objective: Validate the Ark Diagnostics homogenous immunoassay for the quantitative determination of Lamotrigine levels in plasma, and to compare the immunoassay to a liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay. Method: ARK Lamotrigine Assay is a homogeneous immunoassay based on competition between the unlabeled lamotrigine in the patient sample and lamotrigine labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for binding to the antibody reagent. Active enzyme converts the coenzyme nicotinamide adenine dinucleotide (NAD) to NADH that is measured spectrophotometrically as a rate of change in absorbance, by using the Beckman AU 400 automated chemistry analyzers. Validation of this method was performed against a Hitachi 917 chemistry analyzer and an LC-MS/MS method. Performance evaluation included accuracy, linearity, analytical sensitivity, imprecision, carryover, and comparison studies. Results: The assay showed excellent correlation to the Hitachi 917 chemistry analyzer ($y = 0.989x - 0.32$; $r^2 = 0.9956$; $N = 20$, with an analytical measurement range of 1.0-40 µg/dL. The analytical sensitivity was 2.0 µg/dL. Both within-run (3.5%-3.9%) and between-run (4.8%-5.6%) imprecision were within acceptable limits, and the assay exhibited no carryover (<1%). Conclusions: The ARK Lamotrigine immunoassay is suitable for

therapeutic drug monitoring of the novel antiepileptic drug lamotrigine. The method provides rapid turn-around-time (2hours), compared to 2-3 days as a send out test.

B-337**Analytical evaluation of cyclosporine and tacrolimus on the Roche automated electrochemiluminescence immunoassay platform**M. J. Knauer, I. M. Blasutig, D. Colantonio, V. Kulasingam. *University of Toronto, Toronto, ON, Canada*

Objectives: Cyclosporine (CSA) and tacrolimus (TAC) are immunosuppressant medications that require careful therapeutic drug monitoring following organ transplantation. This study evaluated the analytical performance of the semi-automated Roche Elecsys immunoassays for CSA and TAC on the Roche e411 platform. **Design and Methods:** Precision was evaluated using manufacturer and BioRad Whole Blood Immunosuppressant controls. Linearity across the measuring range was assessed using CAP Linearity Survey material. Method comparison studies comparing Roche e411 with Abbott Architect and LC-MS/MS were performed using patient samples spanning the analytical measuring range. Analytical sensitivity and lot-to-lot assessment were also assessed. **Results:** Precision ranged from 3.4 to 8.0% for CSA and 4.1 to 9.9% for TAC. Linearity was verified from 48.0 µg/L to 960.9 µg/L for CSA and from 1.4 µg/L to 27.1 µg/L for TAC. The functional sensitivity met the manufacturer's claim and was determined to be 44 µg/L for CSA and 0.7 µg/L for TAC (CV ≤ 20%). Deming regression analysis of the Abbott Architect method comparison (n = 102) yielded slopes of 0.92 (95%CI: 0.89-0.95) for CSA and 0.92 (95%CI: 0.88-0.97) for TAC. Deming regression analysis of the LC-MS/MS method comparison (n = 20) yielded slopes of 1.33 (95%CI: 1.17-1.50) for CSA and 0.92 (95%CI: 0.84-1.01) for TAC. Lot-to-Lot comparison (n = 20) yielded slopes of 0.998 (95%CI: 0.97-1.03) for CSA and 0.97 (95%CI: 0.94-1.01) for TAC. **Conclusions:** The Roche Elecsys CSA and TAC assays have acceptable precision, linearity, and functional sensitivity and are comparable to Abbott Architect and LC-MS/MS methods and is fit for purpose for the therapeutic drug monitoring of CSA and TAC.

B-339**An Investigation of Illicit Fentanyl Substances in a Clinical Setting**A. Darragh, E. L. Ryan, J. Bekemeyer, L. Contella, M. L. Snyder. *LabSource, LLC, Greenville, SC***Background**

Acetyl-fentanyl is a powerful opioid analgesic that is an increasing concern in the treatment of patients in the pain management and addiction settings. More information is coming to light regarding the introduction of fentanyl and acetyl-fentanyl into adulterated pills and street drugs. The DEA has confirmed 52 acetyl-fentanyl associated deaths between 2013 and 2015. Heroin related overdose and death has reached epidemic proportions in the United States. Mainstream media frequently associates this increase in overdoses with the possibility of fentanyl and/or acetyl-fentanyl laced heroin but little scientific research has been published investigating these theories. Acetyl-fentanyl laced cannabis is also a concern with the changing legalities surrounding recreational and medicinal cannabis, the suspicion of laced cannabis may be falsely decreased. Illicit sources of the drug will not have the stringent testing requirements that most surely will blossom in the changing cannabis landscape. LC-MS/MS confirmation must be performed to identify acetyl-fentanyl in urine as immunoassays cannot differentiate it from fentanyl.

Objectives

To determine the prevalence of acetyl-fentanyl, fentanyl, and norfentanyl in urine of patients confirmed positive for 6-acetylmorphine and THC.

Methods

A total of 365 urine specimens were analyzed. The sample group was comprised of all 6-acetylmorphine confirmed specimens (positive cut off 5.0 ng/mL) from a 90 day period and all THC samples confirmed > 500 ng/mL over a 30 day period. All samples were tested by LC-MS/MS (Agilent, Santa Clara CA) for the presence of free and conjugated forms of acetyl-fentanyl, fentanyl, and norfentanyl, employing IMCSzyme recombinant beta-glucuronidase pretreatment, with a reporting concentration limit of 2.5 ng/mL for all compounds.

Results

Of the 156 6-acetylmorphine urine specimens tested, 3 (1.9%) confirmed positive for acetyl-fentanyl, 19 (12.2%) confirmed positive for fentanyl, and 20 (12.8%) confirmed positive for norfentanyl. Fentanyl prescriptions were not disclosed in any of the 6-acetylmorphine cases. None of the 209 THC urine specimens tested confirmed

positive for acetyl-fentanyl however, 2 (1.0%) confirmed positive for fentanyl, and 3 (1.4%) confirmed positive for norfentanyl. Fentanyl prescriptions had been disclosed in 3 (1.4%) of the THC positive cases.

Conclusions

Due to the lack of disclosure of illicit substance use the source of acetyl-fentanyl in our study cannot be determined. In all positive cases acetyl-fentanyl was present in addition to fentanyl and/or norfentanyl. Adulterated or mislabeled illicit pills are an increasing presence in the street drug market. Our experiment filtered samples for positive heroin use in an attempt to investigate low level norfentanyl positive, negative fentanyl results seen in tandem with 6-acetylmorphine in our patient population. The results obtained however cannot rule out the additional use of illicit fentanyl pills. Although acetyl-fentanyl was identified in 6-acetylmorphine samples, the lack of acetyl-fentanyl in THC positive samples indicates the acetyl-fentanyl lacing of cannabis may not be as high of a concern as with heroin. While acetyl-fentanyl is present in specimens our lab received, the low prevalence does not warrant the addition of this assay to our current testing panel at this time.

B-340

Evaluation of a point of care test for urine fentanyl screening

J. M. Boyd, A. Klavins, H. Sadrzadeh. *Calgary Laboratory Services, Calgary, AB, Canada*

Introduction: Fentanyl is a synthetic opioid that is estimated to be 100X more potent than morphine and 40X more potent than heroin. Deaths due to fentanyl have dramatically increased in Canada in the past 2 years, especially in Alberta where in 2015, over 250 deaths were associated with fentanyl. The fentanyl crisis has impressed the need for a screening method. There are few fentanyl immunoassay kits available that can be used to screen for the drug. Mass spectrometry will be the choice for confirmation, if needed. Here we present the evaluation of a point of care fentanyl strip for use in urine drug screening.

Methods: The point of care strip (Innovacon) is a qualitative lateral flow immunoassay calibrated against norfentanyl, a major metabolite of fentanyl. The analysis time is 15 minutes after specimen application. The cutoff is 20 ng/mL. Results are read manually with the appearance of two lines indicating a negative and one line indicating a positive. The strip performance was compared to our quantitative opioid confirmation assay which consists of dilute and shoot followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis that detects fentanyl and norfentanyl with a lower limit of quantitation of 10 ng/mL for each.

Results: 50 samples (2 drug free urine blanks, 4 QC, 44 patients) were compared using the fentanyl strip and LC-MS/MS. The fentanyl and norfentanyl concentrations in the patient specimens ranged from <10 to 4584 ng/mL and <10 to 11197 ng/mL, respectively, as determined by LC-MS/MS. Overall, results generated by the point of care (POC) kit agreed well with those generated by LC-MS/MS analysis (both positive and negative), with agreement for 45/50 samples (90%). Four of the remaining 5 specimens with fentanyl or norfentanyl concentrations of 12 ng/mL or lower (by LC-MS/MS) were also positive by POC kit; however, the 5th specimen with norfentanyl concentration of 25 ng/mL and fentanyl concentration <10 ng/mL by LC-MS/MS was a false negative by the POC kit.

Conclusion: The point of care fentanyl strip compares well with our LC-MS/MS opioid confirmation assay and may be used for qualitative analysis of fentanyl in the lab.

B-341

Comparison of cobalt and chromium results from two Canadian laboratories

J. M. Boyd¹, J. Powell², H. Sadrzadeh¹. ¹*Calgary Laboratory Services, Calgary, AB, Canada*, ²*University of Calgary, Calgary, AB, Canada*

Introduction: Metal on metal (MoM) prosthetics for hip replacement have proved popular as they exhibit less wear than their metal on polyethylene counterparts. Following MoM joint replacement, it is common for the levels of prosthetic metals (cobalt and chromium) to become elevated in the blood and are used by the surgeon as indicators of wear and tear and/or to assess for possible toxicity. At present, there is little consensus on the use of blood cobalt and chromium measurements, especially with respect to the specimen of choice (whole blood vs serum). Our lab recently switched referral labs for trace metals testing from Lab A, who performed cobalt and chromium in whole blood, to Lab B, who measures cobalt in whole blood and chromium in serum. Here we present the results of a study comparing the cobalt

(whole blood vs. whole blood) and chromium (serum vs whole blood) results from these two labs.

Materials & Methods: Both Lab A and B use inductively coupled plasma-mass spectrometry (ICP-MS) for trace element analysis.

Patients with existing MoM hip replacement being monitored by orthopedics were included in the study. Following their regular blood draw for whole blood cobalt and serum chromium which was sent to Lab B as per normal laboratory protocol. An aliquot of the whole blood tube was taken and sent to Lab A for whole blood chromium and cobalt analysis. Results were collated and analyzed using our in house patient comparison spreadsheet. So far, results have been obtained from 68 patients with a MoM joint replacement.

Results: Preliminary results showed that comparison between Lab A and B had an R2 value of 0.9969 ($y=0.79x + 2.163$) and displayed a bias towards Lab A. For whole blood cobalt concentrations below 100 mmol/L, the percent bias ranged from +10% to -30%. Above 100 ng/mL, the percent bias was consistent at -20%. This was of interest as we expected better agreement between the two labs when using the same specimen type and technology.

The comparison of whole blood chromium (Lab A) to serum chromium (Lab B) showed a clear positive bias towards Lab B ($R2 = 0.9944$; $y=1.41x-5.05$). The majority of chromium values obtained were below 100 ng/mL and exhibited a % bias ranging from -30% to +60%. This is consistent with previous studies which have shown poor correlation between serum and whole blood chromium.

Conclusion: Cobalt and chromium measurements can differ substantially between labs, even when using the same specimen type and analytical method.

B-342

Optimization and validation of qualitative testing for prescribed medications in urine using liquid chromatography-mass spectrometry

S. Y. Wu, D. Silverstern, R. Houda, D. Turner, F. Hassouna, S. Kone-Coulibaly. *Confirmatrix Clinical Laboratory, Lawrenceville,, GA*

Background: Urine qualitative testing for prescribed medications by liquid chromatography-mass spectrometry (LC-MS) provides scientific data that a clinician can use to assess patient medication management. In this study, we statistically evaluated the performance accuracy of qualitative testing methods by LC-MS. By optimizing the cutoff values and instrument data processing, we further lowered cutoffs, enhanced test sensitivity, and reduced the false negative rate.

Methodology: Patient data, along with LC-MS results and instrument integration threshold parameters, were collected and statistically evaluated with Receiver Operating Characteristics (ROC) analysis. The data from 50 to 80 patients with positive and negative results for each drug were collected. The documented information on patient prescribed medications, combined with higher resolution and sensitivity high performance liquid chromatography-tandem mass spectrometry, were used as gold standards to confirm result accuracy. R statistical programming was used to construct ROC curves and curves of accuracy across the range of possible cutoffs for multiple drugs. Area under the curve (AUC) for each ROC, sensitivity and specificity for each possible cutoffs, positive and negative predictive values (PPV and NPV), and accuracy were calculated by running the written R scripts.

Results: The AUCs of ROC curves for most of drugs in the test panel ranged from 0.96-0.98. At optimal cutoff, the sensitivity and specificity were 97-98% and 94-95%, respectively.

Conclusion: The setup for optimal cutoffs and instrument parameters for qualitative LC-MS drug testing significantly enhances the test accuracy and sensitivity, and reduced the false negative rate.

B-343

LC-MS/MS quantification of 11-nor-9-carboxy-Δ9-tetrahydrocannabinol and 11-nor-9-carboxy-Δ9-tetrahydrocannabinol-glucuronide in urine

J. D. Buse, J. Boyd, S. M. H. Sadrzadeh. *Calgary Laboratory Services, Calgary, AB, Canada*

Introduction: Cannabis is the most commonly used illegal substance worldwide, with 7.5% of American's older than 12 years of age and 10.2% of Canadians older than 15 years of age projected to have used cannabis in the past year. Such high prevalence of usage and its association with the initiation of illicit drug use has required the detection of cannabis and its metabolites by clinical toxicology laboratories engaged with addiction screening and drug treatment programs. The quantification of the

main psychoactive cannabinoid, Δ^9 -tetrahydrocannabinol (THC), as well as its metabolites, 11-hydroxy-THC (THCCOH) and 11-nor-9-carboxy-THC (THCCOOH) predominately rely upon either GC-MS or LC-MS/MS analysis, with these methods requiring hydrolysis of 11-Nor- Δ^9 -THC-9-carboxylic acid glucuronide (THCCOOH-glucuronide) to measure total THC.

Objective: Development of a LC-MS/MS quantitative method for the quantitative analysis of THCCOOH and THCCOOH-glucuronide in patient urine, including the investigation of the source of THCCOOH and THCCOOH-glucuronide interferences.

Material and Methods: A low volume and sensitive LC-MS/MS method has been developed for the quantification of THCCOOH and THCCOOH-glucuronide in 200 μ L of urine. Quantitative analysis for both THCCOOH and THCCOOH-glucuronide analytes is simultaneously achieved on an Agilent 1290/6460 LC-MS/MS without the need for hydrolysis. Chromatographic separation utilized an acetonitrile/water gradient on a Restek Ultra Biphenyl II (2.1 x 100 mm, 5 μ m) column, with a total run time of 4.5 minutes. Detection of each analyte and isotopically labeled internal standard relied upon multiple reaction monitoring (MRM); THCCOOH (343.2 \diamond 299.1/245.1), THCCOOH-D3 (346.2 \diamond 302.1, 248.1) THCCOOH-glucuronide (519.2 \diamond 343.2/299.2) THCCOOH-glucuronide-D3 (522.2 \diamond 346.2/302.1). Quantification relied upon the ratio between the cps intensity of the MRM quantifier transitions of the analyte and internal standard, while analyte confirmation relied upon the ratio between the MRM quantifier and qualifier transitions.

Results: Preliminary results show that both THCCOOH and THCCOOH-glucuronide analyte were chromatographically separated and displayed excellent peak resolution. The lower limit of quantification (LLOQ) was assessed to be 10 ng/mL for THCCOOH using both calibrators prepared in blank urine and patient samples analyzed using GC-MS, while the LLOQ of THCCOOH-glucuronide was assessed as 10 ng/mL using calibrators prepared in blank urine. Linearity for both analytes was from 10 to 2000 ng/mL. During analysis of patient samples an unidentified compound was found to interfere with the ratio of the MRM quantifier and qualifier for THCCOOH-glucuronide; we are investigating this finding.

Conclusion: We developed an accurate and cost effective LC-MS/MS method to quantify both THCCOOH and THCCOOH-glucuronide in patient urine with a linearity of 10-2000 ng/mL; work is in progress to fully evaluate this assay and will be presented at the meeting.

B-344

Comparison of a Test Strip Versus an Automated Assay for Urine Specimen Validity Testing

A. Ridgway, D. R. Bunch, S. Wang. *Cleveland Clinic, Cleveland, OH*

BACKGROUND: Ensuring urine specimen validity is essential to proper urine toxicology testing. Products and information are available online to help a person subvert a urine drug testing. To counteract these products and techniques, laboratories utilize testing to confirm the absence or presence of common adulterants such as bleach and vinegar, as well as techniques such as excessive hydration. This study compares Intect® 7, a urine adulteration test strip to a Roche SVT assays for adulteration testing on Cobas 311. **METHOD:** The Intect 7 method is a colorimetric test strip which allows for subjective judgment while the SVT assays give quantitative values. Individual patient urines (n=30) without preservative were collected and assayed on the C311 for specific gravity (SVTSG) and pH (SVTpH). A second group of patient urine (n=24) was used for the quantitative creatinine assay. A pool of patient urine was spiked with 1:10 v/v bleach:urine then serially diluted to 1:20, 1:40, 1:80, and 1:160. The resulting samples were assayed by the SVTOx assay which detects bleach and other oxidants. Meanwhile, all samples were tested using the Intect 7 strips. **RESULT:** The specific gravity ranged from 0.999 to 1.023 on the C311 and from 1.000 to >1.020 by the Intect 7 method. The reference interval for urine specific gravity is 1.005-1.030. The average difference between the two assays was -0.0003 without the >1.020 values and 0.0003 if the >1.020 were set at 1.020. For samples <1.005 (low abnormal) by Intect 7, 5 out of 10 agreed. Both assays determined the pH of the urine samples to be between 5-10. The creatinine values ranged from 10.89 to 346.47 mg/dL by C311 with 19 of 24 samples matching the Intect 7 ranges. Of the 5 discrepant samples, four were higher on the C311. Intect 7 creatinine classification ranges were <10, 10-20, 20-50, and >50 mg/dL. Intect 7 was only able to detect bleach to the 1:80 dilution while the C311 detected bleach at all dilution levels. While the comparisons were acceptable, there is an element of subjectivity and color discrimination that may make the cut-offs softer than a calibrated quantitative assay. **CONCLUSION:** The C311 had an acceptable comparison to Intect 7 for the detection of specific gravity, pH, and creatinine, and had higher sensitivity for bleach than the Intect 7 assay.

B-345

A Case of Mitragynine Adverse Effect

L. Liu, S. Giannoutsos, J. A. Rymer, R. Venkataramanan, K. Tamama. *University of Pittsburgh Medical Center, Pittsburgh, PA*

Background: Mitragynine is the primary psychoactive alkaloid of the plant kratom indigenous to South East Asia. Being illegal in some countries, mitragynine has started to emerge in the U.S. as a legal psychoactive product on numerous websites. Pharmacologic studies have shown that mitragynine produces stimulant effects at low dose, but sedative narcotic effects at high dose by acting as a selective and full agonist of the μ -subtype opioid receptor. Animal studies and user experiences indicate an addictive potential with cognitive impairments, which suggest its classification as a harmful drug. Although restricted in some states in the U.S., mitragynine is not scheduled under the Controlled Substances Act at the federal level. As of now, there is no immunoassay available for mitragynine detection, and our clinical experience and knowledge of mitragynine abuse in U.S. are very limited.

Case Report: A 26-year-old male with a prior history of polysubstance abuse presented to the emergency department with severe constipation and urine retention. His symptom started approximately 3 weeks before when he had difficulty in having bowel movements. Over-the-counter laxative was not helpful. One week later, he started to have difficulty in urinating and subsequently developed abdominal and lower back pain. At the time of presentation, he can only dribble urine. He denied saddle anesthesia, fever, or vomiting. His abdomen was moderately distended. CT scan of abdomen showed severe constipation with fecal impaction. Foley catheter placement returned two liter of urine. Aggressive bowel regimen including enema was employed. A comprehensive urinary untargeted drug screening by gas chromatography-mass spectrometry (GC-MS) revealed the presence of mitragynine in urine.

Conclusion: This is the first detection of mitragynine by GC-MS based untargeted drug screening at our institution. The severe constipation and urine retention in this individual is likely caused by mitragynine-induced activation of opioid receptors in the gastrointestinal and urinary systems. As mitragynine gains popularity in the U.S. in the recent years, it has caused more serious clinical manifestations, including psychosis. The mass spectrometry-based mitragynine assay plays a key role in the diagnosis and detection of mitragynine abuse. Its usage monitoring by national drug abuse surveys might also be warranted.

B-346

Fully automated analysis platform for the routine determination of immunosuppressant drug compounds in whole blood

H. Shibata. *Shimadzu, Columbia, MD*

Background: LC-MS/MS shows higher sensitivity and superior specificity compared to immunoassay-based approaches for the analysis of immunosuppressant medications. Nevertheless, LC-MS/MS approaches lack in standardization and the necessary throughput for the application in routine analysis. We report a fully automated, high-throughput platform for the quantitation of four major immunosuppressant (cyclosporine A, tacrolimus, sirolimus and everolimus) in whole blood sample. **Method:** The analysis of immunosuppressant was performed using a fully automated LCMS preparation Unit (CLAM-2000, Shimadzu) online with HPLC-LCMS (NexeraX2-LCMS8050, Shimadzu) starting from Standard Blood Collection Tubes using "MassTox®" kit (Chromsystems). Samples (EDTA whole blood), calibrators, Extraction Buffer, Precipitation Reagent, and Internal Standards (Chromsystems) were loaded into the CLAM-2000. The fully automated preparation/analysis procedure consisted of: I) 25 μ L of sample was dispensed in filtration-collection vial; II) 50 μ L of Extraction buffer was added (liquid-liquid extraction); III) 12.5 μ L of internal standards (IS) were added; IV) stirring (10 sec) and incubation (2 min); V) 125 μ L of precipitation reagent were added; VI) stirring (1 min) and Filtration (deproteinization); VII) injection by HPLC autosampler (5 μ L) and quantitation following MRM for drugs and IS. **Preliminary results:** Usually LC-MS/MS analysis of whole blood samples require some manual preparation steps for extraction and protein precipitation before the injection. With the aim to reduce the operator involvement, to increase the throughput and the data quality, we completely eliminated the manual sample preparation procedure by the use of a novel automatic preparation unit (CLAM-2000, Shimadzu). First, we tested if the LOQ and linearity of our method were compatible with suggested therapeutic intervals for immunosuppressant (100-350 μ g/L Cyclosporin A, 3-8 μ g/L everolimus, 3-20 μ g/L sirolimus, tacrolimus 4-20 μ g/L). The LOQ for all the compounds resulted <0.5 μ g/L (Cyclosporin A 0.45 μ g/L, tacrolimus 0.15 μ g/L, everolimus 0.5 μ g/L and sirolimus 0.25 μ g/L, with S/N>10). We

used a wide range of quantification (6 levels) for all the compounds (up to 930 µg/l for Cyclosporin A, and 45 µg/l for everolimus, sirolimus, tacrolimus). Also, we obtained good linearity over the entire range ($r^2 > 0.997$). Since the difference in concentration between the highest calibration level and the LOQ was remarkable we tested the carry-over effect, and it was negligible. We tested the intra-day precision (repeatability) of the method, by analyzing 4 reference samples (spanning from low concentration to high concentration levels). Interestingly, the automated sample preparation resulted in providing low CV% values for the compounds (<2.5% Cyclosporin, <6% sirolimus, <8% Everolimus, <3.5% tacrolimus). Also the inter-day precision (reproducibility) was in accordance with CLSI guidelines, with CV% <12% over all 4 levels of concentration. The method showed good correlation and accuracy to the reference material for all compounds (Bias% <5% for everolimus, tacrolimus, and sirolimus, <10% for cyclosporine A). Finally, results on whole blood samples showed a strong agreement with immunoassay results.

Conclusion: The completely automated quantification method for Immunosuppressant drug compounds allows routine analysis with high data quality/precision, reduced time, increased throughput and safety.

B-347

Quantitation of Urine Fentanyl and Norfentanyl by UPLC-MS/MS

B. Wright, D. Giacherio, H. Ketha. *University of Michigan, Ann Arbor, MI*

Background: Fentanyl, a powerful synthetic opiate analgesic more potent than morphine has a high abuse potential on its own or in combination with other prescription and street drugs. The abuse of fentanyl at the present time is high in the state of Michigan and has risen in recent years. Available immunoassays show poor cross reactivity towards fentanyl leading to false negative results.

Relevance: Measuring urine concentrations of fentanyl and its metabolite norfentanyl is of clinical value, for diagnosing acute overdoses, monitoring compliance and forensic investigations.

Objective: The first objective was to develop and validate a liquid chromatography tandem mass spectrometry (UPLC-MS/MS) assay for measuring urine fentanyl and norfentanyl concentrations. The second objective was to monitor urine concentrations of patients taking fentanyl.

Method and Validation: An UPLC-MS/MS assay for measuring urine fentanyl and norfentanyl was developed. Briefly, 200 µl of urine was added to 100 µl of methanol containing Fentanyl-D₅ (100 ng/ml) and Norfentanyl-D₅ (1,000 ng/ml), plus 750 µl ammonium acetate (3M, pH 5.5). The solution was passed through a conditioned cation exchange SPE column (Supelco Inc, PA, USA), washed and eluted with 5% ammonium hydroxide in methanol. The eluate was neutralized with 0.4M Acetic Acid, and 10 µl injected onto a Acquity UPLC BEH C-18 50mm x 2.1 mm column (Waters), and separated using an acetonitrile/0.1% formic acid and water/0.1% formic acid gradient over 3.0 minutes at 50 °C and analyzed on a mass spectrometer (Xevo TQ-S, Waters Corp., Milford, MA) using multiple reaction monitoring mode (MRM). Fentanyl transition used were (m/z 337.45>188.3) and norfentanyl transition used were (m/z 233.35>84.2). Fentanyl and Norfentanyl standards (10, 25, 100, and 1000 ng/ml for each) and quality control samples were prepared in fentanyl free urine. 50 samples from the Mayo Clinic were also analyzed.

Results: The UPLC-MS/MS assay was linear across an analytical measurement range of 5-1000 ng/ml with ($r^2 = 0.999$). The intra assay imprecision for fentanyl was 2.0 % at 12 ng/ml, 2.0% at 30 ng/ml, 1.7% at 50 ng/ml (n=5) and 1.6% at 750 ng/ml. The intra assay imprecision for norfentanyl was 3.1% at 12 ng/ml, 2.0% at 30 ng/ml, 1.9% at 50 ng/ml, and 0.5% at 750 ng/ml (N=5 replicates each). The inter assay imprecision for fentanyl ranged from 2.7-2.8%. The inter assay imprecision for norfentanyl ranged from 5.0-7.1%. The limit of quantitation was set at 10 ng/ml for both fentanyl and norfentanyl. Our method compared well with the Mayo Clinic LC-MS/MS assay Fentanyl: $UM_{UPLC/MS} = 1.11 * MAYO_{LC-MS/MS} + 1.67$ ($r^2 = 0.97$); Norfentanyl: $UM_{UPLC/MS} = 0.83 * MAYO_{LC-MS/MS} + 7.62$ ($r^2 = 0.99$).

Conclusions: We have developed an UPLC-MS/MS assay for measuring Fentanyl and Norfentanyl concentrations in urine. In our initial cross sectional study, fentanyl and norfentanyl could be reliably quantitated in samples from patients taking fentanyl. In the future, studying the fentanyl/norfentanyl ratio in urine of patients is an attractive option in for compliance monitoring.