Wednesday, August 1, 2018

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

TDM/Toxicology/DAU

B-395

Stability of ethanol in whole blood samples stored in a refrigerator

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Background: Determination of ethanol in whole blood samples is one of the most frequent analytical practices in the toxicology laboratory. This determination has very important legal consequences, such as traffic accidents, labor, maltreatment ... For all these reasons, it is necessary to maintain these samples properly, since they can be required at any time and should not suffer any variation in the concentration of ethanol. In this work, 40 samples of whole blood stored in a refrigerator at 4ºC will be studied and the stability of the ethanol will be checked. **Methods**: Ethanol concentration is analyzed in 40 samples of whole blood stored in the refrigerator, which were already analyzed in their day, each of them with a certain storage time. The results of the two measurements are compared and it is checked if the ethanol concentration is stable. If this is not the case, the possible causes of this variation are determined. For this study, a gas headspace chromatograph (HS-GC) is used, with a flame ionization as a detector (FID). Propanol is used as an internal standard to calculate the concentration of ethanol in the samples. **Results:** The results of the comparison show that:1. Samples that no contain ethanol obtained the same result, that is, no generation of alcohol occurs during storage.2. Samples that contain ethanol suffer a variation in their concentration, they all lose alcohol. This variation is due to several factors, including the storage time and the volume of the air chamber in the sample tubes. This loss is caused by the oxidation of ethanol to acetaldehyde, and later, to acetic acid. This oxidation, dependent on temperature, is catalyzed by oxyhemoglobin (OxHb), which is formed by binding oxygen from the air chamber with hemoglobin (Hb) in the blood. So, if a sample tube has little whole blood volume (or it has a large air chamber), more oxygen can bind to the Hb of the erythrocytes, so more OxHb are formed and more ethanol is oxidized. However, the table shows that this loss of ethanol is not linear with respect to storage time, so it can be deduced that there are more factors that contribute to this loss. These losses could be due to volatilization of ethanol, presence of microorganisms that consume alcohol...3. Loss of ethanol is independent of the initial concentration of alcohol. **Conclusion:** It is clear, that in all samples there is a loss of ethanol. Possible solutions to avoid these losses could be filling the samples tubes up, which it would avoid the air chamber; using urine or serum samples, avoiding the presence of erythrocytes and also the presence of hemoglobin; and, finally, freezing the samples at -20 ° C, with which it prevents the oxidation process.

B-396

High throughput SPE and LC-MS/MS Methods for drugs of abuse in Urine

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Objective

Drugs of abuse are commonly tested in clinical labs by dilute and shoot (D&S) approach, associated with high matrix interference at low concentration and long-term deterioration of the LC-MS system. We evaluated the solid phase extraction with smaller bed weight plate and reduced processing time.

Procedures

Human urine samples were fortified with standards (over 40 drugs of abuse) at different concentrations. For the enzyme hydrolysis recovery test, human urine samples were fortified with codeine-6-β-D-glucuronide, morphine-3-β-D-glucuronide and 6-MAM at ULOQ level (2000 ng/ml of free drug) or with THCA-glucuronide at HQC level (200 ng/ml of free drug). Optimized SPE method for barbiturates and THCA: 0.1 ml of fortified urine was mixed with 0.05 ml of internal standard solution (IS) in 90% methanol, and 0.1 ml of pre-made mixture of 200 mM ammonium acetate pH 6.8 buffer and beta-glucuronidase (BG) solution in 5/2 v/v ratio and incubated at 55ºC for 30 min. A Panthera Deluxe SPE plate (30 mg/well) was pre-conditioned with 1 ml of methanol followed by 1 ml of water. Hydrolyzed urine solution was loaded onto the extraction plate, followed with washing with 1 ml each of water and 20% methanol. The analytes were eluted with 1.5 ml of methanol. Solvent was evaporated under nitrogen at 45ºC and the analytes were reconstituted with 1.5 ml of 30% methanol and analyzed by Gazelle C18 UHPLC column. SPE method for main drug panel: A mixture of 0.4 ml of master mix (ammonium acetate buffer, 25 µl of BG100 glucuronidase and IS) and 0.4 ml of fortified urine was incubated at 68ºC for 30 minutes. Hydrolyzed urine solution was loaded onto the Panthera Deluxe SPE plate (20mg/well) and then washed with 1 ml each of water and 5% methanol. The analytes were eluted with 0.4 ml of 70% acetonitrile, and diluted with 0.6 ml of water, then injected into the Gazelle Biphenyl UHPLC column. An ExionLC-API4500 QQQ MS was operated in negative ion mode for barbiturates and THCA and in positive ion mode for the main drug panel.

Results

Panthera Deluxe SPE gives better reproducibility and recovery than C18 and other types of polymer SPE phases. The extraction recoveries were in the range of 74.2-116.9% for all drugs. Under current hydrolysis conditions, recoveries of all glucuronides were above 89%. Compared to D&S, the Panthera Deluxe procedure gave overall better results demonstrating greater AMR and lower LLOQ. Results for xenobiotic interference/effect testing and matrix induced ion suppression/enhancement were comparable between procedures, while Panthera SPE showed superior results in terms of matrix interference/effect tested at cut-off concentration.

Conclusion

Fast and reliable SPE methods were developed for analysis of over 40 drugs of abuse.. These improved methods demonstrate reduced matrix effects and expand the lower end of the AMR by almost an order of magnitude. The method for main drug panel eliminated evaporation procedure; the processing time per plate is less than 10 minutes maintaining the total cost of sample preparation at a very competitive level.

B-397

Performance Evaluation of the Atellica CH Acet, Dgn, Li, and Sal Assays

J. T. Snyder, K. Estock, C. Tyler, J. Koellhoffer, J. Cheek. *Siemens Healthcare Diagnostics Inc, Newark, DE*

Background: The purpose of the investigation was to evaluate the analytical performance of the Atellica® CH Acetaminophen (Acet), Digoxin (Dgn), Lithium (Li), and Salicylate (Sal) Assays on the Atellica CH Analyzer. Measurement of these assays is useful for assessing overdose and monitoring therapeutic use. The Acet assay uses the enzyme acyl-amidohydrolase and manganese ions to cleave the amide bond of acetaminophen and form a colored compound. The absorbance intensity of the compound is directly proportional to the amount of acetaminophen in the sample. The Dgn assay uses a digoxin-latex complex, with which digoxin in the sample competes for binding sites of an anti-digoxin antibody. The rate of agglutination is inversely proportional the amount of digoxin in the sample. The Li assay is based on the complexation of lithium ions with a lithium-specific chromoionophore, which produces a direct, colorimetric reaction. The Sal assay uses the enzyme salicylate-hydroxylase and NADH to prompt a decrease in absorbance that is proportional to the amount of salicylate in the sample. **Method:** Performance testing included precision and accuracy. Assay precision was evaluated using the Clinical and Laboratory Standards Institute (CLSI) guideline EP05-A3. Each sample was assayed in duplicate twice a day for 20 days. Method comparison studies were conducted according to CLSI EP09-A3, with patient-sample results compared to results from the ADVIA® 1800 Clinical Chemistry System. **Results:**

Assay	Within-Lab Precision Range (%CV)	Sample Type	
Acet	1.5-3.7	Serum/Plasma	
Dgn	2.0-8.2	Serum/Plasma	
Li	1.0-2.1	Serum/Plasma	
Sal	0.5-2.9	Serum/Plasma	
Assay	**Regression Equation**	**r**	**Comparison Assay**
Acet	y = 0.97x - 0.1 mg/dL	0.998	ADVIA 1800 Acet
Dgn	y = 0.97x + 0.18 ng/mL	0.994	ADVIA 1800 Dig
Li	y = 0.98x + 0.02 mmol/L	0.997	ADVIA 1800 LITH
Sal	y = 1.01x - 0.9 mg/dL	0.997	ADVIA 1800 Sal

Conclusions: The Atellica CH Acet, Dgn, Li, and Sal Assays tested on the Atellica CH Analyzer demonstrated acceptable precision. Method comparison results showed acceptable agreement with an on-market comparative analyzer.

B-399

Utilisation of Biochip Array Technology for Detection of Fentanyl and Opioid Novel Psychoactive Substances in Urine

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Background: The use of Novel Psychoactive Substances (NPS) is cause of health concern. Manufactured as synthetic alternatives to traditional drugs, NPS often exhibit similar effects but with heightened potency and legally evasive potential. The increasing number of these drugs represents a challenge in clinical test settings trying to maximise the detection of a large number of these compounds in a sample. Biochip array technology allows the multi-analytical screening of NPS and related analytes from a single sample. By employing simultaneous immunoassays, this technology increases the detection capacity, which is important when facing this opioid epidemic. Rapid development of such assays is also necessary to ensure relevance in a market which is constantly changing. The objective of this study was to evaluate a biochip array, which enables the simultaneous detection of fentanyl and opioid novel psychoactive substances from a single urine sample. Analytes to be detected include: furanyl fentanyl, acetyl fentanyl, carfentanil, ocfentanyl, AH-7921, MT-45, U-47700, W-19, etizolam, clonazepam, mitragynine, buprenorpnine and naloxone.
Methods: Competitive chemiluminescent immunoassays defining discrete test regions on a biochip and applicable to the Evidence series analysers, were employed. The measuring range for each assay were: furanyl fentanyl 0-21.8ng/mL, acetyl fentanyl 0-21.8ng/mL, carfentanil 0-2.5ng/mL, sufentanil 0-5ng/mL, ocfentanyl 0-21.8ng/mL, AH-7921 5ng/mL, MT-45 15ng/mL, U-47700 80ng/mL, W-19 0-40ng/mL, etizolam 0-10ng/mL, clonazepam 0-15ng/mL, naloxone 0-20ng/mL, norbuprenorphine 0-5ng/mL and mitragynine 0-10ng/mL. Recovery at concentrations -50% of cut-off, cut-off and +50% were assessed in human urine to determine inter assay precision (n=18) and validate cut-offs. Intra assay precision was assessed by running precision material replicates. Assay sensitivity was assessed by running negative urine samples (n=20). Results are semi quantitiative
Results: Cut-offs validated for this array were: furanyl fentanyl (1ng/mL), acetyl fentanyl (1ng/mL), carfentanil (0.25ng/mL), sufentanil (1ng/mL), ocfentanyl (2ng/mL), AH-7921 (1ng/mL), ocfentanyl (2ng/mL), AH-7921 (1ng/mL), MT-45 (2ng/mL), U-47700 (10ng/mL), W-19 (2ng/mL), etizolam (2ng/mL), clonazepam (2ng/mL), mitragynine (1ng/mL), naloxone (1ng/mL) and buprenorphine (0.5ng/mL). Recovery (%) was achieved at the tested concentrations within a 70-130% range excepting mitragynine. Mitragynine showed slight over recovery below the cut-off however all replicates spiked at -50% below the cut off reported negative correctly. Inter and intra assay precision were less than 20% for each assay and the limit of detection was less than 50% of the cut-off concentration in each case.
Conclusion: This biochip array, by simultaneously screening fentanyl and opioid NPS from a single urine sample is relevant for the current NPS market, doubling as both a screening method and indication of treatment. It is an anticipated answer for many laboratories facing the crisis of unknown drug combinations and concentration. The array allows rapid and accurate detection of multiple low concentration NPS in a single sample.

B-400

An Evaluation of Analytical Performance of Therapeutic Drugs on the Roche Cobas 8000 Modular Analyser Series

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Background
Therapeutic drug monitoring is a laboratory practice which measures and monitors the concentration of drugs with narrow therapeutic ranges at timed intervals. Determination of serum or plasma drug levels is important to individualise drug dosage to achieve optimum therapeutic efficacy and minimize toxicity. In July 2016, our institution's Department of Laboratory Medicine upgraded the automated chemistry platform to the Roche Cobas 8000 system (Roche Diagnostics, Switzerland) interfaced with the fully automated pre-analytic system Roche Cobas 8100 and the post-analytic system Roche Cobas p501. To minimise laboratory process wastage and overcome manpower shortage, the department intends to move the TDM measurements from our current Roche Cobas Integra 400+ analyser to the c8000 system. We evaluated the analytical performance of nine therapeutic drugs assays, including acetaminophen (ACM) and salicylate (SAL) on the c702 module, and amikacin (AMIK), carbamazepine (CBZ), digoxin (DIG), gentamicin (GENT), phenobarbital (PHNB), theophylline (THEO) and valproic acid (VALP) on the c502 module of the c8000.
Materials and Methods
A correlation study was conducted using human serum or plasma samples (n=40) with concentrations distributed over the analytical measurement range of each therapeutic drug assay. The specimens were analysed on both the I400 and c8000 analysers and results compared. The repeatability and intra-laboratory imprecision of each TDM assay were evaluated using 3 levels of quality control (QC) materials. Each QC level were analysed three times a day (once in the morning, afternoon and night) for five consecutive days. The linearity of the assays was validated using six levels of serum or plasma samples prepared by mixing sera of known high and low concentrations.
Results
The coefficient of variation (CV%) values for repeatability and intra-laboratory imprecision ranges for ACM:1.2-1.9 and 2.61-3.8 (12.07-102.08 µg/mL), SAL:1.5-2.2 and 1.83-2.2 (40.21-466.23 µg/mL), AMIK:1.2-3.6 and 1.3-3.8 (4.79-27.99 µg/mL), CBZ:0.8-2.4 and 1.0-2.5 (3.23-15.9 µg/mL), DIG:1.1-4.8 and 1.5-5.6 (1.02-3.49 ng/mL), GENT:1.9-3.9 and 1.97-4.5 (1.73-6.84 µg/mL), PHNB:2.3-5.5 and 2.5-6.1 (9.67-48.01 µg/mL), THEO:1.2-1.6 and 1.7-2.3 (5.69-30.38 µg/mL), VALP:1.6-2.8 and 2.97-5.6 (34.72-112.34 µg/mL) were within the acceptable range established by manufacturer, except for those of QC level 1 for phenobarbital with CV% of 5.5 and 6.1 (9.67 µg/mL) compared to that of the manufacturer's established CV value of ≤5%. Linear regression between I400 and c8000 for the nine assays are as follows: ACM: y=0.99x-0.72, SAL: y=1.03x+1.15, AMIK: 1.04x-0.23, CBZ: y=0.93x+0.67, DIG: y=0.96x-0.04, GENT: y=0.83x-0.05, PHNB: y=1.03x-0.39, THEO: y=0.99x+0.1 and VALP: y=0.97x+0.17. In the correlation study, I400 and c8000 demonstrated good concurrence with r^2 values of ≥0.98 for all 9 therapeutic drug assays. The percentage of recovery for all the therapeutic drugs was within the allowable linearity limits (≤ ±10%) established by the manufacturer.
Conclusion
Our evaluation data showed that the analytical performance of therapeutic drugs assays on c8000 showed good correlation with the I400. Current reference ranges can also be applied for new assays performed on the new instrument. The incorporation of TDM analysis eliminates the likelihood of human error in pre-analytical phase and increases productivity of the laboratory.

B-401

The incidence of vancomycin-induced nephrotoxicityThe incidence of vancomycin-induced nephrotoxicity in Hong Kong Chinese

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Background: The vancomycin-induced nephrotoxicity (VIN) in Hong Kong was lack of systematic population-wide study until now. This study was to explore the incidence of VIN and identify the characteristics of susceptible patients and the most likely risk factors.
Methods: A retrospective study was conducted using the Hong Kong Hospital Authority Clinical Data Analysis and Reporting System (CDARS). All the data of patients with vancomycin prescription and measurement from 2012 to 2016 in Hong Kong were retrieved from CDARS. With the use of Modified RIFLE criteria, patients with acute kidney injury (AKI) were identified. Patients who had no baseline and follow-up concentration of creatinine, vancomycin treatment <3days or trough concentration not at a steady state were excluded. Results were analyzed by using SPSS version 24. Logistic regression was used to identify the predictors for VIN.

Results: Twenty-three patients were identified as VIN from 140 complete cases in Hong Kong from 2012 to 2016. The cumulative incidence of VIN was 16%. From 2012 to 2016, the incidence was 9%, 23%, 26%, 11% and 13% respectively. There were no significant differences between VIN and non-VIN groups in their demographics. No significant association was found between vancomycin levels and the occurrence of nephrotoxicity. In logistic regression analysis, only length of stay had a significant positive association with VIN (odds ratio 1.020, 95% CI 1.004-1.035). **Conclusion:** The incidence of VIN in Hong Kong is low but shows no decline. Long hospital stay is a risk factor for VIN.

B-402

A rapid ultra-performance LC-MS/MS assay for determination of serum unbound fraction of voriconazole in cancer patients

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Background: Voriconazole (VOR), an antifungal agent, is used in the curative treatment of invasive fungal infections and also the prophylactic treatment of opportunistic fungal infections in immunocompromised patients. In blood, VOR is highly protein-bound and mostly with albumin. Free or unbound VOR is the pharmacologically active form. Increased unbound VOR produces high therapeutic efficacy but also enhances toxicity especially in liver or renal failure patients with decreased albumin levels and drug clearance. Monitoring of VOR is thus, highly recommended. In particular, serum unbound VOR should be measured to provide the most accurate monitoring of VOR therapeutic efficacy and toxicity. Setting the appropriate dose for active VOR is challenging due to its variable unbounded forms. We developed and validated an accurate, simple, fast, and cost-effective test with ultrafiltration and UPLC-MS/MS to measure unbounded VOR in human serum for patient testing in clinical laboratories in addition for the measurement of total serum concentrations of VOR. **Methods:** Agilent ultra-performance liquid chromatography (UPLC) system coupled with a SCIEX QTRAP4000 mass spectrometer was performed with a positive ionization mode. Total analytical run time was 3 min. **Results:** All analyses demonstrated linearity (r^2>0.998) from 0.1 to 10 µg/mL for total VOR, while 0.02 to 2.5 µg/mL for unbounded VOR, acceptable accuracy and precision (%CV<15%), suitable stability under relevant storage conditions. Serum samples from sixty cancer patients were collected and both total and unbounded VOR were measured. The levels of total VOR were correlated well with reference laboratory results. The fraction of unbounded VOR was about 2.55% of the total (1.11-4.69%). The levels of serum total and unbounded VOR were highly correlated (r=0.86, p<0.0001). There was a negative correlation between unbounded VOR fractions and levels of plasma albumin (p<0.05). In cancer patients whose albumin levels were low, the fraction of unbounded VOR was high, suggesting that the unbounded VOR should be measured for TDM, even though their total VOR levels were not high. **Conclusion:** In conclusion, a simple and rapid UPLC-MS/MS method for monitoring unbounded VOR is developed and this assay is suitable for routine therapeutic drug monitoring in clinical laboratories.

B-403

Identification of microRNA-mRNA networks involved in cisplatin-induced renal tubular epithelial cells injury

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Background: Cisplatin is a widely used chemotherapeutic drug that often causes acute kidney injury (AKI) in cancer patients. The mechanism of cisplatin-induced renal damage is not completely understood. Moreover, the contribution of miRNAs to the cisplatin nephrotoxicity remains largely unknown. Here we performed an integrative network analysis of miRNA and mRNA expression profiles to shed light into the underlying mechanism of cisplatin-induced renal tubular epithelial cell injury. **Methods** The human renal tubular epithelial cells HK2 were treated with cisplatin, and then FCM and MTT were used to detect cell apoptosis and viability. In addition, qRT-PCR and western blot were used to detect the mRNA and protein expression levels of the apoptosis related genes. Furthermore, miRNAs and mRNA chip were carried out to identify cisplatin-regulated miRNAs and its' target genes using a cisplatin-induced cell model. Quantitative real-time PCR was applied to validate several differentially expressed miRNAs. Lastly, further bioinformatics analysis including GO/pathway and networks analysis were performed to elucidate the possible biological functions of the differentially expressed miRNAs. **Results:** Microarray analysis identified 47 differentially expressed miRNAs, among them 26 were upregulated and 21 were downregulated. Moreover, integrating dysregulated miRNAs target prediction and altered mRNA expression enabled us to identify 1181 putative target genes for further bioinformatics analysis. Gene ontology (GO) analysis revealed that the putative target genes were involved in apoptosis process and regulation of transcription. Pathway analysis indicated that the top upregulated pathways included MAPK and p53 signaling pathway, while the top downregulated pathways were PI3K-Akt and Wnt signaling pathway. Further network analysis showed that MAPK signaling pathway and apoptosis with the highest degree were identified as core pathways, hsa-miR-9-3p and hsa-miR-371b-5p as the most critical miRNAs, and CASK, ASH1L, CDK6 etc. as hub target genes. **Conclusions:** The integrative analysis combining miRNA and mRNA expression profiles, and the related cellular pathways revealed that miRNAs regulated cell apoptosis and stress response pathways played a crucial role in cisplatin-induced renal tubular epithelial cell injury. Overall, our results provide the molecular basis and potential targets for the treatment of cisplatin-induced AKI.

B-404

Performance Evaluation of the Atellica CH Mycophenolic Acid Assay*

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Background: The purpose of the investigation was to evaluate the analytical performance of the Syva® Emit® 2000 Mycophenolic Acid (MPA) Assay on the Atellica® CH Analyzer. Measurement of the immunosuppressant drug MPA is used to monitor levels and prevent toxic levels in transplant recipients. The assay is a homogeneous-enzyme immunoassay technique. The MPA in a patient sample competes with MPA labelled with glucose-6-phosphate dehydrogenase (G6PDH) enzyme for antibody-binding sites. Enzyme activity increases when the enzyme is not bound to an antibody. The active enzyme converts NAD+ to NADH, and the absorbance is measured spectrophotometrically. **Method:** Performance testing included: precision, linearity, and method comparison. Assay precision, linearity, and method comparison were analyzed using MICROSOFT EXCEL 2010. Precision was tested using 20 replicates run on one day using calibrator and QC samples. Linearity was tested using 5replicates each for a total of nine samples across the assay range. Linearity samples were also tested on the Viva-E® Drug Testing System as a method comparison study. **Results:** The within-run precision ranged from 0.83.8% CV on the Atellica CH Analyzer. The linearity study yielded a regression equation of y = 1.00x - 0.08 µg/mL with r = 1.000 when tested on the Atellica CH Analyzer. The method comparison study yielded a regression equation of y = 1.07x - 0.21 µg/mL with r = 1.000. **Conclusions:** The Atellica CH MPA Assay demonstrated acceptable precision and linearity results when tested on the Atellica CH Analyzer. Method comparison results showed acceptable agreement with an on-market comparative analyzer. *Under development. Not available for commercial sale.

B-405

Performance Evaluation of the Atellica CH Emit Drugs-of-abuse Assays

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Background: The purpose of the investigation was to evaluate the analytical performance of the Atellica® CH Emit® Drugs-of-abuse Assays on the Atellica CH Analyzer. These assays include Amphetamines (Amp), Cocaine Metabolite (Coc), Benzodiazepines (Bnz), Barbiturates (Brb), Propoxyphene (Ppx), Methadone (Mdn), Opiates (Op), Phencyclidine (Pcp), Cannabinoids (Thc), Ecstasy (Xtc), and Methadone Metabolite (EDDP) (MetMtb). Measurement of these assays is used in the determination and semiquantification of illicit drug use. All samples determined to be positive on the Atellica CH Analyzer, relative to a given cutoff value, are confirmed quantitatively by the reference method—gas chromatography/mass spectrometry (GC/MS). The Emit Drugs-of-abuse Assays use a homogeneous enzyme immunoassay technique. The drug in the patient sample competes with drug labelled with glucose-6-phosphate dehydrogenase (G6PDH) enzyme for antibody-binding sites. Enzyme activity increases when the enzyme is not bound to an antibody. The active enzyme converts NAD+ to NADH in the presence of glucose-6-phosphate (G6P), and the absorbance is measured spectrophotometrically. **Method:** Multiple cutoff levels were analyzed for several assays. These include Amp 300 ng/mL (Amp300), Amp 500 ng/mL (Amp500), Amp 1000 ng/mL (Amp1000), Coc 150 ng/mL (Coc150), Coc 300 ng/mL (Coc300), Bnz

200 ng/mL (Bnz200), Bnz 300 ng/mL (Bnz300), Brb 200 ng/mL (Brb200), Brb 300 ng/mL (Brb300), Ppx 300 ng/mL (Ppx), Mdn 150 ng/mL (Mdn150), Mdn 300 ng/mL (Mdn300), Op 300 ng/mL (Op300), Op 2000 ng/mL (Op2000), Pcp 25 ng/mL (Pcp), Thc 20 ng/mL (Thc20), Thc 50 ng/mL (Thc50), Thc 100 ng/mL (Thc100), Xtc 300 ng/mL (Xtc300), Xtc 500 ng/mL (Xtc500), and MetMtb 1000 ng/mL (MetMtb). Performance testing included accuracy. Method comparison studies were evaluated using percent concordance between the Atellica CH Analyzer and Viva-E® Drug Testing System, along with GC/MS for discrepant samples (all Pcp samples were tested using GC/MS), for each cutoff level. **Results:** For the Amp300, Amp500, and Amp1000 assays, the percent concordances were 98.6%, 96.6%, and 97.1%, respectively. For the Coc150 and Coc300 assays, the percent concordances were 98.6% and 97.2%, respectively. For the Bnz200 and Bnz300 assays, the percent concordances were 98.7% and 100%, respectively. For the Brb200 and Brb300 assays, the percent concordances were 91.2% and 100%, respectively. For the Ppx assay, the percent concordance was 96.1%. For the Mdn150 and Mdn300 assays, the percent concordances were 100% and 95%, respectively. For the Op300 and Op2000 assays, the percent concordances were 97.3% and 98.7%, respectively. For the Pcp assay, the percent concordance was 96.4%. For the Thc20, Thc50, and Thc100 assays, the percent concordances were 98.5%, 98.3%, and 98.5%, respectively. For the Xtc300 and Xtc500 assays, the percent concordances were 96.5% and 98.2%, respectively. For the MetMtb assay, the percent concordance was 98.3%. **Conclusions:** Method comparison results showed acceptable agreement with an on-market comparative analyzer.

B-406

Performance Evaluation of the Atellica CH Tacrolimus Assay*

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Background: The purpose of the investigation was to evaluate the analytical performance of the Syva® Emit® 2000 Tacrolimus Assay on the Atellica® CH Analyzer. Measurement of the immunosuppressant drug tacrolimus is used to monitor the therapy of liver and kidney transplant patients. The assay is a homogeneous-enzyme immunoassay technique. The tacrolimus in a patient sample competes with tacrolimus containing glucose-6-phosphate dehydrogenase (G6PDH) enzyme for antibody-binding sites. Enzyme activity increases when the enzyme is not bound to an antibody. The active enzyme converts NAD+ to NADH, and the absorbance is measured spectrophotometrically. Prior to testing, whole-blood samples, calibrators, and controls are pretreated with methanol and a pretreatment reagent. This process lyses the cells, isolates the tacrolimus, and precipitates the majority of blood proteins. The samples are then centrifuged, and the resulting supernatant is used for testing. **Method:** Performance testing included precision, linearity, and method comparison. Assay precision, linearity, and method comparison were analyzed using Microsoft Excel 2010. Precision was tested using 20 replicates run on one day using QC samples. Linearity was tested using two replicates each for a total of nine samples across the assay range. Method comparison was tested on the Atellica CH Analyzer and the Viva-ProE™ Drug Testing System using 18 samples with two replicates each. The first replicate was used in analysis. **Results:** The within-run precision ranged from 3.65.5% CV on the Atellica CH Analyzer. The linearity study yielded a regression equation of y = 0.98x - 0.6 µg/mL with r = 0.997 when tested on the Atellica CH Analyzer. The method comparison study yielded a regression equation of y = 0.98x + 0.2 µg/mL with r = 0.990. **Conclusions:** The Atellica CH Tacrolimus Assay demonstrated acceptable precision and linearity results when tested on the Atellica CH Analyzer. Method comparison results showed acceptable agreement versus an on-market comparative analyzer. **Under development and not available for commercial sale*

B-407

An Evaluation of the New ARK Technologies Methotrexate Immunoassay Method onTwo Automated Chemistry Analyzers

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Background: Methotrexate (MTX) therapy has been approved for a number of malignancies and autoimmune diseases. Unfortunately, MTX toxicities can occur at any dose and require timely rescue using eitherLleucovorin and Gucarpidase to prevent irreversible toxicity. However, accurate and frequent monitoring of MTX plasma concentrations can maximize therapeutic efficacy, improve patient safety, and prevent unnecessary adverse outcomes. In anticipation of future unavailability of Abbott TDx Methotrexate II assay, we evaluated the new ARK Technology methotrexate assay run to two different chemistry analyzers: the Beckman DxC 700 AU and Siemens ADVIA 1800. **Objectives:** Determine the precision and accuracy the new MTX immunoassay run on both the Beckman DxC 700 AU and Siemens ADVIA 1800 automated chemistry analyzers using our traditional fluorescent polarization method on the Abbott TDx as a reference method. **Methods:** Analytical precision was determined using known patient plasma samples of low (n = 3), intermediate (n = 5), and high (n =3) MTX concentrations over a period of 5 days. Analytical accuracy was assessed using the ARK Technology methotrexate immunoassay deployed on both Beckman DxC 700 AU and Siemens ADVIA 1800 chemistry analyzers compared to reference method (Abbott TDx Methotrexate II). **Results:** The ARK MTX Immunoassay method on both the Siemens ADVIA 1800 and Beckman DxC 700 AU showed good-to-excellent correlation when compared to our reference TDx MTX method (Siemens Advia 1800; n=86, y = 1.038x + 0.020; R^2 = 0.971; Beckman DxC, n=86, y = 1.081x + 0.0160; R^2 = 0.952). However, for higher MTX values, the Beckman DxC method showed a consistent negative bias. While both methods showed acceptable accuracy at low concentrations, accuracy was unacceptable for samples with MTX levels below the manufacturer's suggested reportable range. Precision with the ARK MTX Immunoassay using the Siemens ADVIA 1800 platform showed an average coefficient of variance of 3.93% (ranging from 1.10% to 8.89%). **Conclusion:** While the ARK Technology methotrexate assay using either the Beckman DxC 700 AU or Siemens ADVIA 1800 showed good-to-excellent correlation with our reference TDx method, higher MTX levels showed a consistent negative bias on the Beckman DxC. Based on the comparable reportable range and the good-to-excellent precision and accuracy compared to our reference method, we conclude that ARK MTX Immunoassay is an acceptable alternative method on our Siemens Advia 1800 to our previously validated MTX assay on the Abbott TDx.

B-408

Hydrolysis Efficiency Study of Selected Beta-glucuronidase Enzymes for Opioid Measurement in Urine

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Background: β-glucuronidase enzymes are used for hydrolysis of opioid glucuronides in urine for consistent measurements. Originally we used β-glucuronidase from *patella vulgate* (limpets), 1-3.2M U/g in acetate buffer with an 18 hour incubation at 60°C. The aim of this study was to investigate whether other commercially available glucuronidase enzymes may offer better hydrolysis efficiency with a much shorter incubation time. **Method:** Six different studies were performed. In each study two levels of prepared glucuronide urine samples (300 ng/mL and 1000 ng/mL) were extracted with one of the enzymes and incubated at 3 time points (30 minutes, 1 hour and 3 hours). The enzymes studied were β-glucuronidase from E.Coli recombinant from overexpressing BL21, >20M U/g in phosphate buffer incubated at 37°C, IMCSzyme, a genetically modified β-glucuronidase , >50K/mL in phosphate buffer incubated at 55°C, β-glucuronidase from *Haliots refuescens* (red abalone) 1–3.2M U/g incubated at 60°C, and β-glucuronidase from *patella vulgate* (limpets),1-3.2M U/g in acetate buffer incubated at 60°C. One extraction had no enzyme using only phosphate buffer incubated at 55°C. The extraction was completed following the incubation and the aliquots were analyzed by an established HPLC-MS/MS method. **Results:** Codeine showed the lowest degree of hydrolysis across all enzymes and time points. The extractions with only buffer showed no sign of hydrolysis. *Haliots refuescens* had the lowest degree of hydrolysis of all enzymes across all time points. E.Coli had the highest degree of hydrolysis at the 3 hour time point. IMCSzymne had the highest degree of hydrolysis at 30 minutes. **Conclusion:** Both E.Coli and IMCSzyme showed improved hydrolysis efficiency over the limpets enzyme. Codeine glucuronide had the lowest hydrolysis efficiency of all the glucuronides tested. IMCSzyme provided the highest hydrolysis efficiency of codeine at the shortest time point (30 minutes) offering the overall best results.

B-409

Performance Evaluation of Representative Clinical Chemistry Assays from the Therapeutic Drug Monitoring Panel on the Alinity c System from Abbott Laboratories

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Background: The Abbott Therapeutic Drug Monitoring Panel, or TDM, determines drug concentration measurements in body fluids as an aid to the management of drug therapy for the cure, alleviation, or prevention of disease. Routine monitoring ensures that therapeutic drug concentrations are in the right therapeutic range for optimal patient care. Abbott TDM assays are carefully designed to measure drug concentrations of analytes that require strict dosage control under STAT or routine testing conditions. The Alinity ci system is part of a unified family of systems that are engineered

for flexibility and efficiency. The design is based on insights from customers, resulting in a number of benefits including a smaller footprint, improved workflow, and greater throughput with up to 1350 tests per hour. The Alinity ci system has an increased reagent load capacity, holding up to 70 Clinical Chemistry reagents, onboard QC and calibrators, clot and bubble detection ability, and smartwash technology to provide consistent and reliable results. **Objective:** To demonstrate the analytical performance of representative assays from the Therapeutic Drug Monitoring Panel of the Alinity c system, which consists of assays that utilize photometric technology for the quantitative determination of analytes in human serum or plasma. **Methods**: Key performance testing including precision, limit of quantitation (LoQ), linearity and method comparison were assessed per Clinical and Laboratory Standards Institute protocols. The assay measuring interval was defined by the range for which acceptable performance for bias, imprecision, and linearity was met. **Results:** The observed results for precision, LoQ, method comparison, and defined measuring intervals for representative assays in the Therapeutic Drug Monitoring Panel are shown in the table below.

Assay	Total %CV	LoQ	Method Comparison to ARCHITECT (Slope/Correlation)	Measuring Interval
Amikacin	≤5.3	2.3 µg/mL	1.02/1.00	2.3 to 50.0 µg/mL
Theophylline	≤3.7	1.3 µg/mL	0.94/1.00	2.0 to 40.0 µg/mL
Tobramycin	≤3.1	0.3 µg/mL	1.01/1.00	0.3 to 10.0 µg/mL
Valproic Acid	≤2.5	5.1 µg/mL	1.00/1.00	12.5 to 150.0 µg/mL
Phenobarbital	≤5.8	1.9 µg/mL	0.98/1.00	2.0 to 80.0 µg/mL

Conclusion: Representative clinical chemistry assays utilizing photometric technology on the Alinity c system demonstrated acceptable performance for precision, sensitivity, and linearity. Method comparison data showed excellent agreement with on-market ARCHITECT clinical chemistry assays.

B-410

Biomonitoring of Chromium (Cr) and Cobalt (Co) for Joint Implant Failures by ICP/MS

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Background: Artificial joint implants are intended to restore mobility and relieve pain. The composition of these implants vary, but the metal-on-metal style carries some additional risks. During movement, metal can be released from the implant into the circulatory system. Adverse effects associated with toxicity of these metals include vision and hearing loss, dermatitis, and myocardial failure. Evaluation of patient risk includes clinical history, physical examination, imaging tests, and laboratory analysis. Because both chromium (Cr) and cobalt (Co) are essential elements, it is suggested that baseline levels are obtained prior to surgery. The objective of this work is to present a validated method for the determination of Cr and Co concentrations in blood, serum/plasma, urine, and fluid specimens. **Methods:** Quantitative determinations of Cr and Co concentrations in blood, serum/plasma, urine, and fluid were performed using an octopole collision cell to reduce matrix interferences from polyatomic species. Internal standards (Sc, Ge and Rh) were added to calibration standards, controls, and samples, and then introduced into the instrument. Analyte concentrations were calculated from the measured ion signal and the internal standard isotope signals using aqueous-based calibration curves. The analytical measurement ranges for Cr and Co are 1.0 mcg/L - 1,250 mcg/L and 0.5 mcg/L - 625 mcg/L, respectively, in blood, serum/plasma, and urine; and 1.0 mcg/L - 625 mcg/L in fluid for both elements. For reference purposes, normal Cr and Co concentrations are typically less than 1.0 mcg/L in blood and serum/plasma specimens. In urine, normal Cr and Co concentrations are typically less than 1.0 mcg/mL and 2.0 mcg/L, respectively. **Results:** Retrospective data analysis of samples submitted for both Cr and Co analyses from July 2014 to February 2018 was completed. The data set consisted of 280 blood samples, 640 serum/plasma samples, 6 urine samples, and 258 fluid samples. In blood, positive Cr (n=171) and Co concentrations (n=198) averaged 4.89 mcg/L (median: 4.80 mcg/L; range: 1.0 - 51 mcg/L) for Cr and averaged 4.89 mcg/L (median: 5.00 mcg/L; range: 0.51 - 66 mcg/L) for Co. In serum or plasma, positive Cr (n=359) and Co concentrations (n=475) averaged 4.97 mcg/L (median: 2.00 mcg/L; range: 1.0 - 120 mcg/L) for Cr and averaged 6.87 mcg/L (median: 3.80 mcg/L; range: 0.50 - 160 mcg/L) for Co. In urine, positive Cr and Co concentrations (n=5) averaged 146 mcg/L (median: 35 mcg/L; range: 3.0 - 390 mcg/L) for Cr and averaged 226 mcg/L (median: 89 mcg/L; range: 9.9 - 790 mcg/L) for Co. In fluid, positive Cr (n=119) and Co concentrations (n=140) averaged 15,301 mcg/L (median: 95 mcg/L; range: 2.5-390,000 mcg/L) for Cr and averaged 3,002 mcg/L (median: 145 mcg/L; range: 1-29,000 mcg/L) for Co. **Conclusion:** Potential health risks are associated with metal-on-metal implants as a consequence of elevated metal levels. Patients with evidence of excessive device wear or a localized adverse tissue reaction should be assessed for systemic effects of exposure to metal ions. The presented method can be used to monitor Cr and Co exposure in patients before and after joint implant surgery in several matrices.

B-411

Development and validation of an UPLC-MS/MS analytical method to quantify voriconazole in human plasma

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Background: Voriconazole is essential to the treatment and prophylaxis of invasive fungal infections. Significant pharmacokinetic variability combined with positive exposure-response relationship has increased demand for therapeutic drug monitoring of voriconazole. Here, we develop and validate a fast and simple ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method to measure voriconazole in human plasma, which is suitable for the needs of clinic. **Methods:** Samples were purified via liquid-liquid extraction using methyl tert-butyl ether. Cyproheptadine was used as the internal standard (IS). The optimal chromatographic behavior was achieved on a BEH C18 column (1.7 µm, 2.1 mm× 50 mm) using a mixture of 0.3% formic acid in 0.02 mol/L ammonium acetate and acetonitrile (60:40, v/v) as the mobile phase. Mass spectrometric detection was conducted with a triple quadrupole detector equipped with electrospray ionization in the positive-mode using multiple reaction monitoring (MRM). **Results:** The total analytical run time was within 3 min. Our method provided consistent recovery of more than 86.56% for both voriconazole and IS. The calibration curve was prepared, which provided an excellent linearity from 0.5 to 1000 ug/L with a correlation coefficient (r^2) >0.99. The limit of detection (LOD) was determined to be 0.125 µg/L. The intra- and inter-day presicion were 1.81% and 3.57% on average. The overall accuracy were −0.85 ~ 5.14% and 2.20 ~ 4.23% for intra- and inter-day values, respectively. Selectively, dilution integrity and stability were also validated. The method was successfully used to evaluate voriconazole clinical practice in the "real-world" setting. **Conclusions:** A rapid, sensitive and robust UPLC-MS/MS method for quantifying voriconazole levels in human plasma was validated. It was helpful in improving voriconazole related personalized medicine strategies.

B-412

Sample stability and method comparison of a urine benzodiazepine quantitation method by LC-MS/MS

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Background: Benzodiazepines (BZD) are one of the mostly prescribed classes of drug worldwide. They are central nerve system depressant used as sedatives, anticonvulsants, anxiolytics, and muscle relaxants. BZD are frequently been identified in illicit use, mostly for recreational purposes but also been used in certain crimes. BZD is relatively safe in overdose if taken alone; however, there is an increasing number of BZD abuse with opioids among pain management patients and the co-administration will cause excessive suppression to the central nervous system and sometimes leading to death. We have previously reported an LC-MS/MS quantitation method for measuring seven BZD and metabolites (7-aminoclonazepam, α-hydroxyalprazolam, α-hydroxytriazolam, oxazepam, lorazepam, nordiazepam and temazepam). Here, we present study results for sample stability and method comparison. **Methods:** The seven BZD were spiked into negative urine samples to three concentration levels at 100, 1000 and 5000 ng/mL. The spiked samples were then stored at ambient, 4 °C, and -20 °C. Samples were removed and stored at -70 °C when each storage time was reached, and analyzed in triplicate as one batch at the end of the study. The sample storage condition was acceptable if the calculated mean values were within 20 % of the initial values. We compared our LC-MS/MS method to a GC-MS method offered at an independent laboratory using both patient urine samples and spiked urine samples. To further evaluate the accuracy of this method, another comparison was performed using commercial BZD urine toxicology controls (UTAK control) at levels of 100 ng/mL and 400 ng/mL. Percent difference from the UTAK target value and reference value (result from an undisclosed laboratory measurement using LC-MS/MS methodology) were calculated. **Results:** We found 7-aminoclonazepam was less stable with 12 h at both

ambient and 4 °C, 7 days at -20 °C storage. All other six BZD were stable for 24 h at ambient, 14 days at 4 °C, and 3 month at -20 °C. For the method comparison, all negative urine samples tested by our LC-MS/MS method were also been tested negative by the GC-MS method. For the positive urine samples, Deming regression showed correlation coefficient (R) > 0.96 for all analytes. Six of the seven analytes showed positive bias with 7-aminoclonazepam being the highest at 24.5 %, which was most likely due to its instability during shipping and transportation to the other laboratory. Whereas α-hydroxyalprazolam showed a negative bias at -15.4 % and the bias was mainly from the high concentration samples (> 1700 ng/mL by our method). All bias for all the remaining analytes were within 13 %. For the commercial control materials, our LC-MS/MS method showed -15. 8 % to 17.3 % difference from the UTAK target values and -2.7 % to 37.9 % difference from their reference values. **Conclusion:** Additional validation data support the use of this LC-MS/MS method for clinical use.

B-413

Cigarette Smoke And Oxidative Stress-Induced Hypertension

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Background: Cigarette smokers are exposed to significant quantities of reactive oxygen species (ROS) which play a major pathophysiological role in oxidative stress-induced hypertension through the process of lipid peroxidation. The mechanisms underlying oxidative stress-induced hypertension in cigarette smokers are not completely understood. Here, we evaluated the possible effects of cigarette smoke on malondialdehyde (MDA), total antioxidant status (TAS), systolic blood pressure (SBP), diastolic blood pressure (DBP) and body mass index (BMI). **Methods:** A total of 90 male subjects were recruited and grouped into control group (nonsmokers: n=30) and test group (active smokers: n=60). The plasma levels of MDA and TAS were determined; SBP and DBP were measured while BMI was calculated using standard formula. **Results**: A significant increase ($p< 0.01$) was observed when the means of SBP, DBP and MDA in smokers were compared with corresponding control group, while a significant decrease ($p<0.01$) was observed when TAS levels in smokers were compared with corresponding nonsmokers**.** Although there was a subtle increase in the BMI of smokers when compare with nonsmokers, no statistically significant difference was observe. **Conclusion:** The increase in SBP, DBP and MDA concentration coupled with decrease in TAS observed in cigarette smokers indicate that cigarette smokers are at high risk of developing oxidative stress-induced hypertension due to increase lipid peroxidation. **Key words:** Smokers; Oxidative stress; Hypertension.

B-414

Genotyping of selected alleles involved in tramadol metabolism provide evidence for additional factors beyond CYP2D6-inffered phonotype that may contribute to observed metabolite patterns.

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Background: Tramadol is a centrally acting opioid analgesic used to treat moderate to severe nociceptive pain. Structurally, it bears similarities to codeine and morphine, and exhibits similar analgesic potency. The parent prodrug inhibits neurotransmitter reuptake and its bioactivation by CYP2D6 to *O*-desmethyl tramadol (M1) stimulates the μ-opioid receptors, enhancing its analgesic properties. *N*-demethylation by CYP2B6 and CYP3A4/5 renders tramadol inactive by generating *N*-desmethyl tramadol (M2). Interpatient variability in response to tramadol treatment ascribed to polymorphisms in the genes that code for proteins involved in the pharmacokinetics and pharmacodynamics of tramadol. The Clinical Pharmacogenetics Implementation Consortium provides guidelines for CYP2D6-guided therapy for tramadol; however the guideline does not account for additional factors that may contribute to opioid analgesic outcomes including multigene variants and drug-drug interactions. In this study, we have analyzed metabolic patterns for tramadol in serum/plasma and urine for results reported by a national reference laboratory and, for a subset of patients, we determined their genotypes and interrogated the correlation between CYP2D6-inffered metabolizer phenotype and observed metabolic patterns. **Methods**: Concentrations of tramadol, M1 and M2 for 1,321 serum/plasma and 21,686 urine samples were retrospectively analyzed. Testing was performed by LC-MS/MS and 50ng/mL, 100ng/mL and 100ng/mL were used as positive cutoffs for tramadol, M1 and M2, respectively. Positivity for parent drug and M1 or M2 and ratios of M1/M2 were assessed to identify the metabolic patterns. DNA was extracted and selected pharmacogenes involved in tramadol pharmacology (ABCB1, COMT, CYP2B6, CYP2D6, CYP3A4, CYP3A5, and OPRM1) were evaluated using a custom OpenArray genotyping panel assay on the Quant Studio 12K instrument (Thermo-Fisher) for twelve patients taking tramadol. A copy number variation assay was also performed for each patient to identify large deletions or duplications of CYP2D6. For CYP2D6, linear regressions were carried out to compare metabolite concentrations with the predicted phenotype or activity score. Pearson coefficient was determined to evaluate the degree of correlation. **Results:** Presence of parent drug and both metabolites was a common finding for urine samples (90.5%) compared to serum/plasma (13.8%). Parent drug, in the absence of any metabolites was detected for 57.9% of serum/plasma specimens compared to 2.8% in urine. Highest frequency of results was observed at 1.0-1.2 ratio of M2/ M1 with median concentrations of 208 and 178ng/mL (S/P) and 4953 and 5371ng/mL (urine). CYP2D6 genotyping for twelve patients identified nine distinct genotypes with predicted CYP2D6 activity scores ranging from 0.5 to >2. Comparison between predicted phenotypes based on CYP2D6 activity revealed a moderate correlation with M1 (r=0.52), a low correlation with M2 (r=0.33) and no significant correlation with M1/M2 (r=0.14). **Conclusions:** Differences between the CYP2D6-inffered phenotype and tramadol metabolic phenotypes suggest involvement of other factors that contribute to the patient's response to tramadol treatment. This study was limited by lack of clinical information including co-medications and tramadol dosing, but patterns described here showed relatively poor correlations with CYP2D6 genotypes alone. The data highlight the importance of monitoring clinical response, drug-drug interactions and polymorphisms in several genes that may impact tramadol pharmacology.

B-415

Multidisciplinary Approach for Standardized Care and Control of Opioid Administration for Pain Management Patients

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Objective: A multidisciplinary team (Anesthesiology, Behavior Health, Family Practice, Internal Medicine, Pathology, Psychiatry) developed a patient-centered approach to manage chronic noncancer patients in order to minimize the risk of opioid abuse, diversion, and addiction. A clear and concise guide was created for ordering and interpreting pain management drugs to help providers better manage cases and serve patients safely and effectively. **Relevance:** Abuse and addiction to opioid analgesics has become a major patient safety risk in the United States and has worsened over the past few years. According to the Michigan Department of Health and Human Services, in 1999 only 22%of the state's drug overdose deaths were attributed to opioids and heroin abuse. Recently, it was reported as up to 67 percent. **Methodology**: We developed a primary care policy to ensure providers and patients consider the safest and most effective treatment for non-cancer, non-palliative, chronic pain patients. Incorporated into the standard work of medical assistants was a screen for opioid risk of abuse, diversion, and overdose every 12 months. Patients that violate the medication management agreement are flagged with a banner on the medical information system splash page. A chronic pain registry was developed to track patients on the chronic pain syndrome problem list. Patients on the registry trigger health maintenance components, drug screens every 12 months, an automated prescription systems program every 12 months, pain and wellness score every 6 months, and pain treatment contract renewal. A dashboard was created to track providers' use of the pain registry and individual providers are coached for use of the registry. Pathology implemented a new directed chronic pain panel, where a negative opiates screen (and negative cocaine screen) will reflex to a confirmatory opiates (GCMS) order. A drug screen ordering tip sheet was developed to guide screen (qualitative) and confirmation (quantitative) ordering practices. **Validation:** In a survey of primary care providers, 92% requested a streamlined process for prescribing opioids for patients who actually need them. The extent to which providers enrolled their patients into the pain registry was monitored. To examine the effectiveness of the physician education component of our program, we assessed whether narcotics ordering volumes changed. Provider ordering practices were examined before and after focused education regarding proper drug screening and confirmatory strategies. **Results and Conclusions**: Of the 5927 patients being cared for chronic pain during the study period, enrollment by the provider to the registry increased from an average of 22% (median 16%) to 36% (33%). Patient enrollment varied widely by clinic site, from 0% to 73% of their patients. The program resulted in more appropriate test ordering by providers, with 56.9% before program initiation and nearly complete compliance afterward. Correct screening panel ordering increased from 75.7 to 237.9 per month and opiates confirmation testing increased from 11.4 to 19.8 per month. Patients managed by pain clinics has remained relatively constant, while prescriptions for narcotics decreased 4.3% year over year. A standardized approach has been instituted to better manage chronic noncancer patients on opioid drugs.

B-416

Evaluation of the measurement of serum TPMT concentration as a novel method to monitor thiopurine therapy

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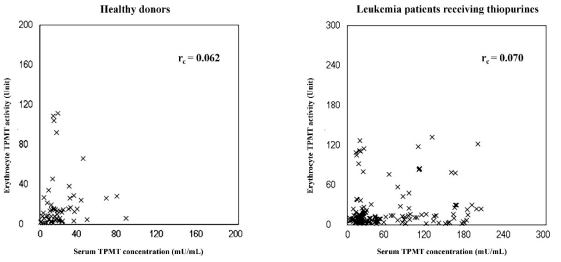
Background:
Thiopurine S-methyltransferase (TPMT) metabolizes thiopurine drugs which are used in various disciplines as immunosupressors and anti-cancer drugs. Decreased activity of the enzyme poses a high risk of severe adverse drug reactions which can be prevented by prescribing decreased thiopurine doses if enzyme status is identified by a prior testing. Currently available methods of TPMT status evaluation are the measurement of the enzyme activity in red blood cells (RBC) and molecular analysis of *TPMT* mutations. *TPMT* genotyping is rather labor-intensive and time-consuming method, while measurement of the enzyme activity is dependent on the RBC condition of the patient which is a matter of concern especially in regions with higher prevalence of hemoglobinopathies. Therefore, considering all the drawbacks of current methods we set up a new study to evaluate the measurement of serum TPMT concentration as a new method.
Methods:
400 healthy blood donors (319 Male/81 Female) and 143 leukemia patients receiving thiopurine therapy (91 Male/52 Female) were included in the study. Measurement of TPMT enzyme activity was chosen as a control method. Serum TPMT concentration (MyBioSource, MBS938845) and enzyme activity (BIOMERICA, REF7019) were both measured by ELISA. Correlation between enzyme activity and serum concentration was analysed via Lin's concordance correlation, and Student's *t*-test was used to analyze between-group differences.
Results:
TPMT serum concentration was higher in female (40.7±10.5) comparing to male (34.5±9.5) donors ($p<0.0001$), and there was a significant increase with the age ($p<0.0001$). Correlation of two methods was $r_c=0.062$ in healthy donors, and $r_c=0.070$ in leukemia patients.
Conclusion:
Measurement of serum TPMT concentration would be a fast and less labour-intensive comparing to *TPMT* genotyping, and it is not dependent on quality and lifespan of erythrocytes and is not influenced by transfusions like the enzyme activity testing. However, the correlation data was not significant enough to support further evaluations of the method.



B-417

BISPHENOL-A EXPOSURE: IMPACT ON HUMAN HELTH

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Introduction: Bisphenol-A, a constituent of plastic and an endocrine disrupting chemical has been implicated to several negative effect on human health.This study was aimed at evaluating some arthropometric measures, oxidative stress indicators and reproductive hormones in males occupationally exposed to Bisphenol-A in a plastic industry in Ibadan, Nigeria. **Methodology:** 80 apparently healthy males aged 18-62 years with normal renal function indices were enrolled into this cross-sectional study. They were forty male employees of a plastic industry (PIW) age matched with forty males who were non-employees of any plastic industry (NPIW, Control). Sexual history, blood pressure (BP), socio-demographic and anthropometric indices were obtained by standard methods. Blood (10mls) was obtained from participants for sex hormones analysis by enzyme linked immunosorbent assay while nitric oxide and superoxide dismutase activities were estimated spectrophotometrically. Bisphenol-A was estimated in spot urine samples using high performance liquid chromatography-tandem mass spectrometry. Data analysed statistically were significant at $p<0.05$. **Result:** Bisphenol- A was detected in both groups but was significantly raised in NPIW compared with PIW ($p<0.003$). NPIW also showed significantly raised diastolic BP and adiposity indices but lower nitric oxide compared with PIW ($p<0.05$). In NPIW, bisphenol-A had a direct relationship with systolic BP and waist circumference but indirect relationship with diastolic BP and waist height ratio ($p<0.05$).No associations were observed between bisphenol-A and physical sexual function indices. However, bisphenol-A had a direct relationship with oestradiol in PIW ($p<0.010$). **Conclusion:** Bisphenol-A was present in both exposed and unexposed groups but was not associated with sexual dysfunction. However, its endocrine disrupting capacity especially in the exposed group is suggested. It's lipophilic nature was shown in the presentations of arthropometric measures and oxidative stress markers in this study.
Keywords: Bisphenol-A, Exposure, Industrialisation

B-418

Ethylene Glycol Elimination Kinetics in an Infant

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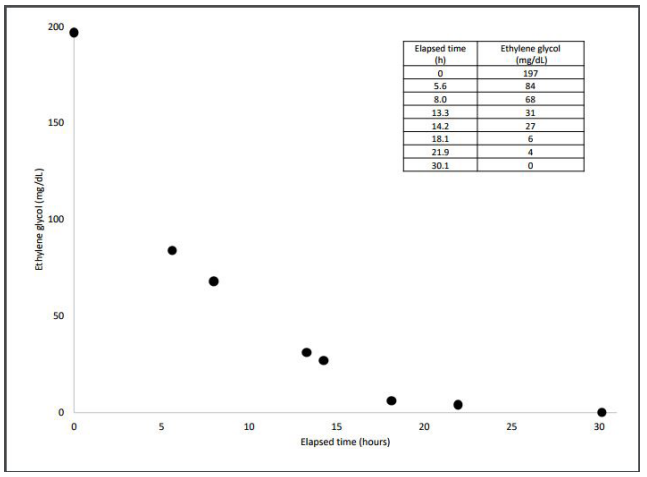
Background: Ethylene glycol (EG) is an ingredient in many industrial products, most commonly found in antifreeze. When consumed, EG is metabolized to toxic metabolites that may result in an elevated anion gap metabolic acidosis (AGMA), renal failure, and potentially death without appropriate therapy. EG toxicity has been previously well described; however, the pharmacokinetics of EG metabolism in infants prior to receiving antidotal therapy has not. The objective of our case study was to determine the pharmacokinetics of EG in an infant. **Methods:** A four-month old infant was brought to the Emergency Department (ED) after one day of altered mental status and vomiting. The patient was noted to be lethargic, tachypneic, tachycardic, and afebrile. Initial laboratory studies were remarkable for a lactate 1.9 mmol/L (17 mg/dL), creatinine 1.93 mg/dL, a $CO2 < 5$mmol/L, and an incalculable anion gap. No infectious source was identified, and the patient was transferred to the pediatric intensive care unit (PICU) at a local pediatric tertiary care hospital. Testing for inborn errors of metabolism resulted on hospital day (HD)2, concerning for EG toxicity. A specimen tested the same day was negative for EG, as determined by gas chromatography with flame ionization detection (Agilent). With a persistent AGMA and worsening AKI, the patient was treated with fomepizole, thiamine, and pyridoxine and continuous renal replacement therapy (CRRT). **Results:** Retrospective analysis of serial specimens from initial presentation showed EG with an elimination half-life of 4.5 hours (Figure). In a single ingestion, an estimated volume of 18 ml of 50% EG containing antifreeze would be required. No other volatiles were detected. The patient subsequently recovered with normal renal function. **Conclusions:** We have uniquely demonstrated the pharmacokinetics of EG in an infant, prior to receiving antidotal therapy to block alcohol dehydrogenase (ADH) activity or hemodialysis, which appears similar to that of adults.



Elapsed time (h)	Ethylene glycol (mg/dL)
0	197
5.6	84
8.0	68
13.3	31
14.2	27
18.1	6
21.9	4
30.1	0

B-419

Therapeutic Drug Monitoring of Lipophilic Immunosuppressive Drugs during Hyperlipidemia

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Background: Therapeutic drug monitoring (TDM) is an integral component for management of transplant patients prescribed immunosuppressive drugs such as cyclosporine, tacrolimus and sirolimus. Due to the lipophilic nature of these drugs, lipemia may interfere with accurate analysis. This investigation was prompted by a cyclosporine order on a grossly lipemic whole blood specimen (>50 mmol triglyceride/L) in a 10 year post-allograft-bone-marrow-transplant patient with polymyositis. Ultracentrifugation is frequently used to overcome lipemia interferences; however this is not appropriate for whole blood specimens and the lipophilic nature of cyclosporine made ultracentrifugation questionable. Furthermore, there is limited data surrounding lipemia interference with our liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay. **Objectives:** To investigate whether hyperlipidemia affects LC-MS/MS quantification and the whole blood distribution of cyclosporine, tacrolimus, and sirolimus. **Methods:**
Non-lipemic patient TDM specimens for cyclosporine, tacrolimus, and sirolimus were spiked with a 20% soybean lipid emulsion (Intralipid®) followed by extraction with water, zinc sulfate, and internal standards ([d^{12}]cyclosporine; ascomycin in methanol). Extracts were centrifuged for varying times at 12,000 rpm and by ultracentrifugation. The linearity of hypertriglyceridemic specimens were assessed by dilution with non-lipemic TDM specimens. The *in vitro* distribution of immunosuppressive drug in blood was investigated mixing fresh RBCs with drug-negative lipemic or non-lipemic plasma. Briefly, pooled TDM blood was centrifuged to quantify drug distribution in plasma and washed RBC fractions. Grossly lipemic plasma or non-lipemic plasma were added to washed RBCs (i.e. **lipemic RBCs** and **non-lipemic RBCs**, respectively) and rocked for 24h at 37C. Separately, fresh, non-TDM blood specimens were pooled, and plasma fraction was replaced with equal volumes of either lipemic or non-lipemic plasma. QC material containing cyclosporine, tacrolimus, and sirolimus was added to lipemic (**lipemic+QC**) and non-lipemic (**non-lipemic+QC**) samples prior to 24h incubation. *In vitro* drug distribution was quantified in plasma and RBC fractions after incubation. **Results:** Lipemia did not interfere with LC-MS/MS quantification of cyclosporine, tacrolimus and sirolimus. Ion suppression or chromatographic shifts were not observed; and ultracentrifugation of extracted lipemic specimen in the presence of internal standard did not affect results. Linearity of grossly lipemic blood was acceptable for cyclosporine (R^2=0.99), tacrolimus (R^2=0.98), and sirolimus (R^2=0.99). Whole blood levels of cyclosporine (58 ng/mL), tacrolimus (5.5 ng/mL), and sirolimus (3.8 ng/mL) were largely in RBCs (cyclosporine: 13628 ± 307 ng/10^{12}RBC; tacrolimus: 1477 ± 37 ng/10^{12}RBC; sirolimus: 985 ± 55 ng/10^{12}RBC) versus plasma fraction (cyclosporine: 2.8 ng/mL; tacrolimus: 4.8 ng/mL; sirolimus: 0.01 ng/mL). After 24h incubation, **lipemic RBCs** recovered 36, 66, and 71% of cyclosporine, tacrolimus, sirolimus. In contrast, tacrolimus and sirolimus recovery in **non-lipemic RBCs** was ~105% after incubation, although RBC cyclosporine recovery was 80% in **non-lipemic RBCs**. Lastly, the RBC fraction of **lipemic+QC** contained 64, 80, and 73% of total cyclosporine, tacrolimus, and sirolimus, respectively. Comparatively, cyclosporine, tacrolimus, and sirolimus levels in RBC fraction of **non-lipemic+QC** sample were 70, 94, and 87% of total, respectively. **Conclusion:** Lipemia interferences were minimal in whole blood for cyclosporine, tacrolimus, and sirolimus measurement. However, TDM interpretation of lipophilic drugs should be with caution during hyperlipidemia where drug levels may correlate with lower concentration in RBCs.

B-420

Urine Drug Testing Ranges for Oxycodone, Oxymorphone, and Noroxycodone

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Background: Oxycodone is a semi-synthetic opioid synthesized from naturally occurring thebaine. This strong opioid is commonly used as an alternative to morphine. However, concern about oxycodone is increasing given that oxycodone has similar abuse potential to morphine. Therefore, Urine drug testing (UDT) is often used to help establish whether the patient is indeed adherent to the prescribed drug or is diverting the drug to other purposes. As such, it is important for physicians to recognize drug levels that are outside expected ranges. **Methods:** We present the results of testing a large number of patient urine samples for oxycodone and its metabolites, oxymorphone, and noroxycodone. The data were collected from quantitative liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis. A data set of 25,259 oxycodone positive urine specimens (50 ng/mL cutoff) collected over a 6 months period (July 2017- December 2017) is used for this study. We separated the data according to the prescribed daily dosages ranging from 5 mg to above 400 mg. We performed Kruskal-Wallis non-parametric test for multiple comparisons. Furthermore, we generated box-whisker plots for the drug concentrations at different daily dosages. **Results:** Kruskal-Wallis test suggested that there are no differences in urine oxycodone concentrations among different groups with daily dosages above 100 mg. When daily doses smaller than 100 mg, the urine oxycodone concentration increased as the dosage increased. Similar trends were also observed for oxymorphone and noroxycodone. **Conclusion:** Drug monitoring results by LC-MS/MS for patients prescribed with oxycodone were used to generated box-whisker plots, giving an expected range for different dosages. The hope is that the data presented herein can aid physicians in determining whether patients are adherent or need additional counseling.

B-421

ARK METHOTREXATE ASSAY ON SIEMENS ATELLICA CH ANALYzER

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BACKGROUND: Methotrexate (MTX), a classical antifolate, can be safely administered over a wide dose range as maintenance chemotherapy for acute lymphoblastic leukemia and treatment of nononcologic diseases including rheumatoid arthritis or psoriasis. When combined with leucovorin (LV) rescue, high-dose MTX (HDMTX; doses of 1,000-33,000 mg/m²) is usually administered as a prolonged i.v. infusion for a variety of cancers, including acute lymphoblastic leukemia, lymphoma, osteosarcoma, breast cancer, and head and neck cancer. HDMTX can be safely administered to patients with normal renal function by vigorously hydrating and alkalinizing the patient to enhance the solubility of MTX in urine. Serum levels may reach 1000 µmol/L or more. Pharmacokinetically guided LV rescue by monitoring MTX serum levels is required to prevent potentially lethal MTX toxicity. Ability to measure MTX accurately at 0.05 µmol/L enables clinical determination of non-toxic status. **OBJECTIVE:** Evaluate the analytical performance of the ARK™ Methotrexate Assay on the Siemens® Atellica CH Analyzer. Compare performance of the assay on the Atellica analyzer to the predicate analyzer (Roche/Hitachi 917). **METHODS:** The ARK™ Methotrexate Assay is a homogenous enzyme immunoassay for quantifying the quantitative determination of MTX in human serum or plasma on automated clinical chemistry analyzers. The assay was evaluated on the Siemens® Atellica CH Analyzer. Increasing reaction rate correlates to increasing MTX concentration for a six point calibration curve (0 to 1.20 µmol/L). Tri-level (0.07, 0.40, and 0.080 µmol/L) quality controls were run. Performance of the assay was determined by assessing precision, limit of quantitation, linearity, analytical recovery, high sample dilution, on board auto dilution, and method comparison. Method comparison samples above the measurement range were serially diluted to within range. **RESULTS:** Total Precision (%CV) for controls was 8.8% (0.07 µmol/L), 3.5% (0.42 µmol/L), and 3.8% (0.81 µmol/L). Limit of Detection (LOD) and Quantitation (LOQ) were comparable to that on the Roche/Hitachi 917: LOD ≤ was 0.02 µmol/L and LOQ was 0.04 µmol/L (0.003 RMSSD, Mean 0.042 µmol/L). Analytical recovery

was within 10% for nominal values >0.10 µmol/L and ±0.01 µmol/L for values ≤0.10 µmol/L. The ARK Methotrexate Aassay was linear from 0.03 to 1.20 µmol/L. High sample dilution was evaluated by performing serial dilutions up to 1:10,000 and resulted in recoveries between 91.7% to 105.0%. Parameters for 1:10 on board automatic dilution with DI water were evaluated and compared to manual dilution using ARK Methotrexate Dilution Buffer. On board dilution resulted in -7.0% to 2.4-5.6% difference from manual dilution. For method comparison, Passing Bablok regression analysis was used. There were 142 samples within measurement range (samples ranged from 0.04 to 1.16 µmol/L): Atellica = 0.95*Hitachi917 + 0.01 (r^2 = 0.97) and 152 samples total including those above the measurement range (samples ranged from 0.04 to 1030 µmol/L): Atellica = 0.95*Hitachi917 + 0.01 (r^2 = 0.99). **CONCLUSION**: The ARK™ Methotrexate Assay performance on the Siemens® Atellica CH Analyzer is substantially equivalent to the Roche/Hitachi 917 analyzer. The ARK™ Methotrexate Assay system on the Atellica analyzer was shown to be safe and effective for its intended use based on performance studies.

B-422

Multiple Drug Classes and Metabolites: Qualitative Analysis in Serum/Plasma by LC-MS/MS

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INTRODUCTION: In emergency department, patients often present with unknown medical conditions and some may present with signs and symptoms of adverse drug reactions. Emergency department laboratory testing may utilize immunoassay or other point of care devices to identify the unknown drugs [1, 2]. However, the numbers of available drugs on immunoassay platforms and POC devices are limited, which may require additional identification and confirmation by GC-MS or LC-MS/MS [2]. **OBJECTIVE:** The purpose of this study was to develop a qualitative method to identify160 drugs/metabolites in serum/plasma by LC-MS/MS to help support testing for adverse drug reactions. This qualitative multi-analyte drug panel consists of different classes of compounds: antiarrhythmic, anticoagulant, anticonvulsant, antidepressant, antihistamine, anti-inflammatory, antipsychotic, barbiturates, benzodiazepines, cannabinoids, decongestant, hallucinogens, hypoglycemic, muscle relaxants, opioids, sedative-hypnotics, stimulants, and tricyclic antidepressants. **METHODS:** PLD+ columns (Biotage) were placed on a 96-well deep plate for extraction. 750 µl of crash solvent that consisted of acetonitrile and a mix of internal standards were aliquoted into each PLD+ column. 250 µl of control, calibrators, or patient sample was added to the appropriate columns, which is an improvement over the previous method requirement of 6 ml of sample. The supernatant was then pushed through the column at $4.1x10^4$-$8.3x10^4$ pascal and collected. The eluted supernatant was then dried, reconstituted, and analyzed on an ABSciex 5500 LC-MS/MS. The run time for the positive mode was 11.5 minutes, and the negative mode was 5.5 minutes. The new method was compared to the current method which utilized a 30 minute GC-MS run, in addition to testing by LC-MS/MS, immunoassay and GC-FID to accommodate a broad spectrum drug screen. **RESULTS:** The cutoffs determined in this assay are analyte specific and ranged from 1 to 100 ng/ml. The presence of a particular analyte above or below the analyte cutoff determines the "present" or "not detected" results of the test. Samples spiked at 50% (n = 4 over 5 days, n = 20 total) of the cutoff screened negative, while samples spiked at 150% (n = 20) of the cutoff screened positive. The LC-MS/MS method had a 70.9% agreement rate with the old extraction and GC-MS method. Further, the new method was able to detect 42.1% more analytes than the old GC-MS method. **CONCLUSIONS:** The new method incorporated an extraction procedure that included a protein crash followed by phospholipid removal. The new LC-MS/MS method enabled the detection of 160 compounds on a single platform, using both positive and negative ionization modes, and reduced the analytical run-time for analysis.

B-423

Therapeutic teicoplanin monitoring reduce the duration of hospitalized days of patients with methicillin-resistant Staphylococcus aureus infections

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Background: Teicoplanin, a glycopeptides antibiotic, has been reported to be comparable to vancomycin in efficacy, but has fewer adverse effects than vancomycin for the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) infection. Therapeutic drug monitoring (TDM) of teicoplanin and appropriate loading doses of teicoplanin are required because of potential treatment failure due to suboptimal concentrations of circulating teicoplanin. However, monitoring of teicoplanin concentration in patients is currently not performed routinely in clinical practice in Taiwan. In this study, we conducted a TDM of teicoplanin from patients with MRSA infections. The lengths of patient stay in hospital (LOS) of patients were collected and analyzed. The importance of TDM of teicoplanin in LOS determination was determined. **Methods:** Between July 2016 and July 2017, we prospectively collected 30 blood samples from 12 patients with MRSA infections after five days prescription with three teicoplanin loading doses (12 mg/kg/12 h) followed by maintenance doses of 6 mg/kg/24 h. The teicoplanin concentrations in blood samples were measured by using an immunoassay kit (QMS teicoplanin, Thermo Fisher Scientific). Three other important renal biomarkers, including blood urea nitrogen, creatinine, and cystatin C were also determined in this study. Medical charts of ten patients with multiple teicoplanin results were reviewed and LOS were collected and analyzed. The importance of TDM of teicoplanin in LOS determination from MRSA-infected patients was analyzed by using a multivariate linear regression model. The difference between predictive LOS and true LOS was determined in patients with and without TDM of teicoplanin. **Results:** Among the 12 patients, the teicoplanin concentration ranged from 1.7 to 41.4 mg/L and the average was 19.4 mg/L. Optimal therapeutic concentration (10-15 mg/L) was determined in nine patients and three patients were suboptimal (less than 10 mg/L) during the study period. The LOS of the 10 patients ranged from 9 to 32 days and the average was 21 days. To analyze the importance of TDM of teicoplanin in LOS determination, a multivariate linear regression model was used. The difference between predictive and true LOS was lower than three days if TDM of teicoplanin was applied, and more than five days differences were determined if TDM of teicoplanin was absent. Moreover, the LOS was extended when the teicoplanin concentration was suboptimal. **Conclusion:** Therapeutic teicoplanin monitoring plays an important role in MRSA patient treatment. Maintenance of optimal dosing in the patient during teicoplanin therapy can reduce the duration of hospitalized days.

B-424

Comparing different alcohol markers in routine laboratory testing

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Background: Phosphatidylethanol (PETH), detected in whole blood samples, is like ethylglucuronid (ETG) a metabolite of ethanol and a direct biomarker for alcohol consumption. The verifiability of PETH is longer than the ETG one, but the sensitivity is the same. ETG is detectable in urine as well as in serum. Gamma-glutamyltransferase (γ-GT) and Carbohydrate deficient transferrin (CDT) are indirect biomarker and useful to detect chronical alcohol abuse. A combination of the CDT and γ-GT is the Antilla-Index (AI=0.8 ln(γ-GT)+1.3 ln(%CDT)) with a higher sensitivity and specify as the analytes alone. Aim of the study was to detect and compare results for alcohol consumption with direct and indirect biomarkers in whole blood samples of patients with laboratory request for CDT and γ-GT. **Method**: 111 whole blood samples were analyzed for PETH, ETG, CDT and γ-GT. For positive results the laboratory intern cut offs were used, PETH > 20 ng/ml, ETG >3.2 ng/ml, CDT >2.0%, γ-GT >55 U/L (man) and >38 U/L woman and AI >4.11 (man) and 3.81 (woman). **Results:** Over 50% of the samples have positive results for PETH, but only 30% for ETG, 20% for AI and 10% for CDT. All samples that where positive for ETG, AI and CDT were also positive for PETH. **Conclusion:** The study has shown that determining PETH concentrations in whole blood samples helps to identify alcohol consumption more often than other direct and indirect biomarkers. The problem of all alcohol biomarker is, that it is not possible to find out when the alcohol consumption happened and how high the amount of alcohol consumed was. However a combination of all biomarkers could be a possible way to bring us closer to an answer respectively to frequency and addiction of drinking alcohol.

B-425

Analytical Performance of MyCare Psychiatry Assays for the Detection of Antipsychotic Medications: Risperidone, Clozapine, Arpiprazole, Olanzapine, Quetiapine and Paliperidone

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Background: Adherence to antipsychotic medication is critical to treatment outcomes for patients with mental illness. Measurement of drug blood levels can provide clinicians with objective evidence they need to avoid treatment failures. LC/MS-MS is currently used, however, the time to reported result is days. Optimally clinicians

want to have a reported result sooner. Saladax Biomedical developed rapid immunoassays for the most common antipsychotic drugs to run on random access clinical chemistry analyzers. All measuring ranges are based on recent literature and on established AGNP consensus and Maudsley prescribing guidelines. The objective of this study was to validate assays for the detection of antipsychotic medications in serum. **Methods:** Automated homogeneous immunoassays were developed for risperidone, clozapine, aripiprazole, olanzapine, quetiapine and paliperidone. All assays utilize the same multianalyte calibrators and controls. Analytical performance of each assay was evaluated according to CLSI guidelines using three reagent and three calibrator lots, on two Beckman Coulter® AU480 analyzers. Repeatability and within-laboratory precision were evaluated over 20 days with controls, spiked pooled serum samples, and pooled patient serums. Recovery and Limit of Quantitation were assessed with spiked serum from individual donors. Over 140 prescription and OTC drugs were tested for cross-reactivity. Comparison to a validated LC-MS/MS method was performed with over 400 patient samples. **Results:** The LoQ, linear range, precision, and the Deming regression statistics from the method comparison for each assay are shown in the table below. Co-administered and common prescription medicines and supplements caused less than a 10% bias in the assays' results. All method comparisons between analyzers and lots resulted in slopes of 1 ± 8%.

		Median Imprecision of Serum Pools (CV)		Method Comparison				
Analyte	Measuring Range	Repeat-ability	Within-Labor-atory	R	N	Slope	Inter-cept	Range (ng/mL)
Risperidone	16 - 120 ng/mL	2.4%	4.0%	0.96	146	0.98	1.2	16 - 118
Clozapine	75 - 1423 ng/mL	2.2%	4.8%	0.92	120	0.97	-2.7	80 - 1317
Aripiprazole	25 - 1,000 ng/mL	7.0%	10%	0.99	50	0.94	9.3	29 - 802
Olanzapine	5 - 125 ng/mL	1.5%	2.6%	0.98	82	1.00	2.7	4 - 111
Quetiapine	15 - 700 ng/mL	1.3%	3.2%	0.91	86	1.04	-4.9	15 - 688
Paliperidone	16 - 120 ng/mL	2.8%	4.1%	0.95	119	0.99	2.8	16-120

Conclusion: The MyCare Psychiatry Assays demonstrated robust performance, allowing for rapid, precise, sensitive, specific, and automated measurement of antipsychotic drugs in human serum in clinical chemistry laboratories.

B-426

Can opiate addiction treatment with naltrexone be monitored using saliva?

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Objective. Naltrexone is a potent and safe opiate receptor antagonist used in treatment of opiate addiction. However, patients must be monitored to ensure that therapeutic levels, >2 ng/ml, are maintained. Blood sampling is the preferred method but recovery of a blood sample is challenging in this patient population. We examined the feasibility of using saliva for the purpose of monitoring naltrexone levels. Methods. Healthy subjects (n=7) providing informed written consent were administered a single dose of naltrexone as oral tablet at dose of 25 mg (n=3) or 50 mg (n=4). Blood (serum) and saliva samples were obtained prior to dosing and 1, 3, 5 and 7 hours later. Saliva was obtained using Salivette (Sarstedt). Samples were evaluated by liquid chromatography using tandem mass spectrometer (LC-MS/MS) for detection. Naltrexone assays were evaluated for linearity, matrix effect, stability and interferences. and showed linear response from 0.2 to 1,000 ng/ml. Total protein in saliva was also determined using the bicinchoninic acid assay (BCA assay) method and is linear between 0.5 to 100 ng/ml. Results. One subject was immediately removed from study because nursing staff was unable to obtain a pre-dose blood sample. Serum levels of naltrexone declined in 5 of 6 subjects in a log-linear manner from 1 to 7 hours. One subject showed a steadily increasing naltrexone serum concentration due to slow gastric emptying. Serum concentration of naltrexone (NTX_{Blood}) was shown to be linearly related to saliva concentration (NTX_{Saliva}) by the following equation: $NTX_{Blood} = 0.358 + 0.106 * NTX_{Saliva}$ $R^2 = 0.856$. Saliva concentration of many molecules has been shown to depend on relative hydration state of the individual at the time that a saliva was taken. Total protein, a concentration that changes in response to hydration status, ranged from 1.46 to 4.01 mg/ml showing that hydration status varied significantly. NTX_{Saliva} was divided by total protein to normalize for hydration dependent variation. Normalized saliva naltrexone ($NTX_{Saliva, Norm}$) was regressed against NTX_{Blood}, resulting in the following relationship: $NTX_{Blood} = 1.071 + 0.138 * NTX_{Saliva, Norm}$, $R^2 = 0.892$. A clinical decision rule for increasing naltrexone dose based on $NTX_{Saliva, Norm}$ was explored using Receiver Operator Characteristic (ROC). A clinically important event is when $NTX_{Blood} < 2$ng/ml. ROC AUC = 0.992 for $NTX_{Saliva, Norm}$. Values of $NTX_{Saliva, Norm} < 10$ are associated with false positive and false negative decisions <5%. Conclusion. Obtaining blood from patients undergoing treatment for addiction can be problematic because of poor health and absence of usable peripheral veins. In addition, patients in treatment are often very sensitive to painful stimulus and needles often evoke painful conflicting emotions. Results demonstrate that saliva can be used to monitor therapy with naltrexone. More research is required to assess how naltrexone levels in saliva are related to blood levels in patients undergoing treatment with sustained release naltrexone dosage forms.

B-427

Novel serum biomarker candidates for gastric and/or duodenal ulcers in humans

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Background:
Gastric and/or duodenal mucosal ulceration and hemorrhage are frequently observed side effects in clinical settings. These findings are currently only detectable by endoscopy, and non-invasive diagnostic methods, including biomarkers, have not been identified. Therefore, identifying sensitive and non-invasive biomarkers could facilitate gastric ulcer diagnosis. We previously conducted metabolic profiling of gastric ulcerations induced by aspirin, ibuprofen, ethanol, and stress, and metabolomic analysis of the effects of omeprazole and famotidine on aspirin-induced gastric injury in rats. Based on these studies, we hypothesized that serum hydroxyproline could be a potential biomarker for gastric ulceration. However, we were unable to clarify the usefulness of this biomarker in humans. Here, we confirmed the usefulness of hydroxyproline as a diagnostic biomarker and explored for additional biomarkers through metabolic analysis of sera from gastric and/or duodenal ulcer patients.
Methods:
Twenty-nine patients who suffered gastric and/or duodenal ulcers and were treated with proton pump inhibitors participated in this study. Serum samples were collected four times from each patient during hospitalization or at clinic visits. A total of 509 metabolites in serum were identified and semi-quantified by capillary electrophoresis−time of flight−mass spectrometry (CE−TOF-MS).
Results:
Serum concentrations of metabolites in each sample were categorized into three groups based on the number of days after starting treatment (Group 1: upto3 days, Group 2: between 4 and 14 days, Group 3: from 15 or more days). Metabolite levels were compared among the groups using analysis of variance and Dunnett's test. Statistical analysis showed lower levels of hydroxyproline and higher levels of 2-hydroxybutyrate, 3-hydroxybutyrate, and 2-aminobutyrate in Group 1 compared to Group 3. Therefore, hydroxyproline increased while 2-hydroxybutyrate, 3-hydroxybutyrate, and 2-aminobutyrate decreased with treatment.
Conclusion:
We identified hydroxyproline, 2-hydroxybutyrate, 3-hydroxybutyrate, and 2-aminobutyrate in human serum as potential biomarker candidates for the diagnosis of gastric ulceration. Changes in hydroxyproline in human serum were similar to those observed in a gastric ulceration rat model. Although larger scale studies are needed to confirm the usefulness of these biomarkers, our findings suggest that these new noninvasive biomarker candidates may be useful for gastric injury diagnosis in clinical settings.

B-428

Effect of middleware implementation on LC-MS/MS workflow for immunosuppressant testing

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Background: Middleware is a powerful tool to automatically transfer information from analyzers to laboratory information systems. Although this is routinely in use for main chemistry analyzers, middleware for liquid chromatography-tandem mass spectrometry (LC-MS/MS) instruments has is less available. In the past few years, as demand of LC-MS/MS testing has increased, so has interest in interfacing these instruments. Interfacing has many advantagnes including reduced technologist time to enter results, reduced risk of transcription error and improved turnaround time. Here we describe our experience in implementing middleware on our routine immunosuppressants bench and evaluate the benefits gained in time savings and improved workflow. **Methods:** Middleware from Data Innovations was implemented on the LC-MS/MS bench (Sciex API3000) performing daily immunosuppressant testing (tacrolimus, sirolimus and cyclosporine). An LIS report file (.csv format) was generated from LC-MS/MS software (Analyst) and loaded into the middleware equipped with a Sciex instrument specific driver. The middleware then converted the file into a form acceptable by the laboratory information system (Cerner Millennium). Prior to middleware implementation, the workflow consisted of a manual extraction, manual input of patient accession numbers into the MS software, analysis of data, manual typing of data into the LIS and batch verification of results. Following middlware implementation, accession numbers were scanned into the MS software to make the analytical worklist and all patient results were automatically transferred from the MS software to the LIS through the middleware. **Results:** Prior to middleware implementation, the immunosuppressant bench was struggling with completing the workload each day within a 7.75 hour shift, partially due to the 1-1.5 hours spent per day entering results into the LIS. The extra time gained from middleware implementation has significantly helped in managing the bench workload allowing the technologist to focus on extracting samples and running the LC-MS/MS. Implementation has also had several other positive effects on workflow in the laboratory. For example, as results are no longer manually entered, a second technologist does not need to double check manual transcription into the LIS saving around 20 minutes per day. In addition, the amount of paper printed for record keeping has been significantly reduced by an estimated 3000 sheets per month. And finally, turnaround times have improved on average by 30-40 minutes, which allows for better patient care. **Conclusion:** Middleware implementation has had significant positive impact on the LC-MS/MS workflow in our laboratory

B-429

Multiplexing LC-MS/MS: Addition of Lacosamide to an Anti-Epileptic Panel

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Background Lacosamide is an anti-epileptic used for the treatment of partial seizures. It enhances the slow inactivation of sodium channels, resulting in stabilization of neuronal membranes and inhibition of repetitive neuronal firing. Both LC-MS/MS and immunoassay-based methods are available for measurement of lacosamide. A major technical advantage of LC-MS/MS is the ability to multiplex reactions. In our laboratory, the current anti-epileptic panel consists of 6 compounds: lamotrigine, levetiracetam, topiramate, zonisamide, 10-hydroxycarbamazepine, and rufinamide. In this study, we successfully added lacosamide to the existing LC-MS/MS panel and assessed the performance characteristics of the assay. **Methods** Assay precision was assessed by replicate measurement (n = 12) of lacosamide in patient plasma pools across the concentration range, 4.0 to 20.0 mg/L. The lower limit of quantification (LOQ) was determined by means of the functional sensitivity, assessed by replicate analysis (n = 5) of commercial product pool across the concentration range, 0.5 to 2.0 mg/L. A direct comparison of methods study between our method (Multiplex LC-MS/MS) and a reference method (Reference LC-MS/MS) was performed by measurement of patient specimens (n = 21) across the concentration range, 2.0 to 20.0 mg/L and assessed by Deming-regression analysis. An interference study was performed by testing commercial multi-level therapeutic drug monitoring (TDM) material. **Results** Assay precision was assessed at 2 lacosamide concentrations (mean: 4.8 mg/L and 19.0 mg/L). The mean coefficient of variation (% CV) was 5.3% and 5.7% at 4.8 mg/L and 19.0 mg/L lacosamide respectively. The functional sensitivity of the assay was determined at 1.0 mg/L. Comparison of methods by Deming-regression produced the following values for slope and intercept: Reference LC-MS/MS = 0.037*Multiplex LC-MS/MS + 0.996. **Conclusion** The multiplexing LC-MS/MS method for lacosamide performed with acceptable precision and was comparable to a reference LC-MS/MS method across the therapeutic concentration range. The LOQ value was also acceptable for routine therapeutic drug monitoring. This study has demonstrated that lacosamide can be easily added to an existing LC-MS/MS panel for anti-epileptic drugs.

B-430

Inter-Laboratory Performance of QMSTM Omecamtiv Mecarbil Immunoassay in a Large Global Clinical Study

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Background: Heart failure affects approximately 23 million people worldwide, including more than 5 million in the United States. Omecamtiv mecarbil (OM), a novel selective cardiac myosin activator, is being studied as a potential treatment for heart failure with reduced ejection fraction. Phase 2 clinical study results indicated that a pharmacokinetic (PK)-based dose titration strategy was useful to identify the optimal dose of OM for heart failure patients, a strategy that has been carried into Phase 3 studies. The QMS Omecamtiv Mecarbil Immunoassay was developed for the rapid (time to first result ~ 10 minutes), quantitative determination of OM concentration. The assay is being implemented in central laboratories in the US, EU and Asia to support the OM clinical development program.**Methods:** The QMS Omecamtiv Mecarbil Immunoassay is a homogeneous particle-enhanced turbidimetric inhibition immunoassay. It is based on competition for anti-OM antibody binding sites between omecamtiv mecarbil in the sample and omecamtiv mecarbil coated onto microparticles. A concentration-dependent classic agglutination inhibition curve can be obtained to determine the OM concentration in the sample. The system has two reagent components, six calibrators that span 0 to 1200 ng/mL, as confirmed by a validated LC-MS/MS reference method, and multi-level controls. **Results:** The lower limit of quantification, precision, accuracy, cross-reactivity, potential for interference by endogenous and exogenous substances, method comparison, carry-over and kit stability were found to meet design specifications. The manufacturer's laboratory (TFS) and three central laboratories in US, EU and Asia demonstrated over a twenty (20)-day period lab-to-lab reproducibility less than 3% CV and less than 10% bias versus the validated LC-MS/MS reference method, among other performance characteristics. The results from this study were used to establish quality control limits in a quality control concept that features Westgard Rules and other measures to ensure precision and accuracy. **Conclusion:** QMS Omecamtiv Mecarbil Immunoassay turn-around-time, precision, accuracy and other performance characteristics support its use. CAUTION: As of July 2017, omecamtiv mecarbil (OM), and the QMS immunoassay for omecamtiv mecarbil (OM), are for investigational use only. Limited by United States law to investigational use.

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Retrospective Untargeted and Targeted Urine Drug Screen Trends in Suspected Drug-facilitated Sexual Assault Cases

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Background & Objectives: Drug facilitated sexual assault (DFSA) is the use of drugs to compromise an individual's ability to consent to sexual activity. Drugs commonly identified as used in DFSA include alcohol, flunitrazepam, ketamine and gammahydroxybutyrate. Use of pharmacological agents for DFSA vary significantly, and depend on local availability, ease of use, and the pharmacokinetic properties of drug. Indeed, many cases of DFSA are not confirmed due to a short detection window of certain drugs, or the victim was consuming the drug in moderation (e.g. alcohol). The objective of this study was to investigate retrospective trends in urine drug screen results in suspected sexual assault cases received by our laboratory. **Methods:** Data was obtained from our laboratory information system (Cerner Millennium) for all cases from 2010-2017 under institutional data policies. The dataset included urine drug screen results performed on all patient specimens by an initial immunoassay screen (i.e. amphetamines, benzodiazapines, barbiturates, cannabinoids, cocaine metabolite, ethanol, methadone, methadone metabolite, opiates, oxycodone) followed by untargeted screening and confirmation by gas chromatography-mass spectrometry (GC-MS). Data was analyzed using a custom C++ program. **Results**: 334 suspected sexual assault cases were identified between 2010 and 2017. The number of cases increased from 7 in 2011 to 61 in 2016. 95% of results were from female patients with a median age of 23. Immunoassay screens were positive for ethanol (31%), cannabinoids (22%), cocaine metabolite (19%), amphetamines (16%), benzodiazapines (11%), opi-

ates (7%), and oxycodone (1%). Methadone, methadone metabolite, and barbiturates were present in <1% of cases. GC-MS confirmed the immunoassay screen results for 98% of amphetamines, 30% of benzodiazapines, 68% of cannabinoids, 100% of cocaine, 25% of ethanol, 100% of oxycodone, and 88% of opiate results by immunoassay. Other specific drugs identified by GC-MS including methamphetamine, diphenhydramine and ketamine in 11, 7, and 2% of cases, respectively. Interestingly, 88% of all opioid positive urine specimens were reported since 2014, and 44% of all diphenhydramine positive specimens occurred in 2016. Our results are consistent with previous reports that ethanol remains a commonly detected drug in suspected sexual assault cases. However, flunitrazipam and gammahydroxybutyrate were not detected in this patient population with untargeted GC-MS. **Conclusion**: Real-time monitoring of urine drug screen positivity among suspected DFSA cases may allow the laboratory to target specific drug classes that emerge within specific patient populations. **:**

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UPLC-MS/MS determination of voriconazole in human plasma and its application

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Background: Voriconazole(VCZ), a triazole antifungal agent, was approved for the treatment of invasive fungal infection with a broad spectrum, including Aspergillus Cryptococcus and Candida species. However, a high incidence of adverse reactions may occur during the treatment, such as liver dysfunction and neurological toxicity. Because of the above findings, it has suggested that the blood concentration of VCZ should be maintained between 1.5 and 5.5μg/mL and the measurement of blood levels could assist with decisions about dose adjustment. So we develop a sensitive and rapid ultra performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method to determine VCZ concentration in human plasma. **Methods:** We build a simple UPLC-MS-MS method for quantifying VCZ concentration in human plasma, using Cyproheptadine as an internal standard (IS). VCZ and IS were extracted from plasma samples by liquid-liquid extraction with 1 ml of Methyl Tertiary Butyl Ether. Chromatographic separation was carried out on an Acquity UPLC BEH C18 column (2.1*50mm, 1.7um) using an isocratic mobile phase system composed of acetonitrile and 0.02mol/L NH_4Ac containing 0.1% formic acid (40:60, v/v) at a low rate of 0.30 mL/min. Mass spectrometric analysis was performed using a TQ-S mass spectrometer coupled with an electrospray ionization source in the positiveion mode. The multiple reaction monitoring (MRM) mode was used, and the transitions selected for quantification were m/z 350.4→m/z 127.2 and m/z 288.4→ m/z 96.2for VCZ and IS, respectively. **Results:** Good linearity (R^2= 0.9991) was observed throughout the range of 0.0005-10ug/ml in 0.1 ml plasma. The overall accuracy of this method was 99.2-109.5%, and the lower limit of detection was 0.25ng/ml. The intra- and inter-day variations were lower than 3.84% and 6.72%, respectively. Plasma concentrations of VCZ in 793 patients were determined, the blood concentration level of VCZ were between 0.01 and 55.74ug/ml, there are 44.24% VCZ concentration at1.5-5.5ug/ml, 37.45% VCZ concentration below 1.5ug/ml and 18.31% VCZ concentration above 5.5ug/ml. **Conclusion:** A UPLC-MS/MS method for the determination of VCZ in human plasma was developed and validated, This method was rapid, sensitive, specific, selective, reproducible, and successfully applied in therapeutic drug monitoring of VCZ.

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Performance of the ARK Diagnostics, Inc., ARKTM Methotrexate Assay on the VITROS® 4600 Chemistry System and the VITROS® 5600 Integrated System.

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Introduction: The ARK Methotrexate Assay quantitatively determines the concentration of methotrexate in human serum or plasma on automated clinical chemistry analyzers. Methotrexate is an antimetabolite used in the treatment of certain neoplastic diseases, severe psoriasis, and adult rheumatoid arthritis. Methotrexate monitoring helps ensure appropriate therapeutic levels of less than 0.05 – 0.1 μmol/L, and avoid possible toxic effects of the treatment. **Method:** The ARK Methotrexate Assay is a homogeneous immunoassay based on competition between methotrexate present in the specimen and methotrexate labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for binding to the antibody reagent. Antibody binding to G6PDH decreases enzyme activity, while binding of methotrexate from the specimen to the antibody reduces antibody bound to G6PDH, thereby increasing enzyme activity. Active enzyme converts the coenzyme nicotinamide adenine dinucleotide (NAD) to NADH that is measured spectrophotometrically as a rate of change in absorbance. The assay was conducted using 8.5 µL of patient sample and the two ARK Methotrexate Assay reagents. Two-point rate measured at 340nm is converted to concentration using a Logit/Log 4 calibration model. Enzyme activity (rate) is directly related to the concentration of Methotrexate in the patient specimen. **Results:** The performance of the ARK Methotrexate assay was assessed on the VITROS 4600 Chemistry System and the VITROS 5600 Integrated System. The stated reportable range is 0.04 – 1.2 µmol/L. Linearity testing using an 11 level admixture series resulted in an observed linear range of 0.029 – 1.122 µmol/L. We evaluated accuracy with 50 serum samples (0.09 – 1.04 µmol/L) on the VITROS 4600 and VITROS 5600 Systems compared to the Beckman Coulter AU680 Clinical Chemistry Analyzer. The results show: VITROS 4600 System = 1.052 * Beckman - 0.01388; (r^2) = 0.98. VITROS 5600 System = 1.032 * Beckman - 0.01754; (r^2) = 0.982. A 5-day precision study was conducted on the VITROS 4600 and VITROS 5600 Systems using control fluids at mean methotrexate concentrations of 0.076, 0.418, and 0.852 µmol/L. These resulted in within–laboratory standard deviation (SD) of 0.009 for the low fluid, and within-laboratory percent coefficient of variation (%CV) of 3.36% and 4.25% respectively for the mid and high fluids for the VITROS 4600 System, and 0.007 (SD) for the low fluid and 3.11% and 4.55% respectively for the mid and high fluids for the VITROS 5600 System. The Limit of Quantitation (LoQ) for the VITROS 4600 and VITROS 5600 Systems is 0.04 µmol/L based on 40 determinations per system with 3 samples at 0.03, 0.04, and 0.05 µmol/L methotrexate. The Limit of Detection (LoD) for the VITROS 4600 and VITROS 5600 Systems is 0.023 µmol/L based on 60 determinations per system with 1 low-level sample. The Limit of Blank (LoB) for the VITROS 4600 and VITROS 5600 Systems is 0.005 µmol/L based on 60 determinations per system with 1 blank sample. **Conclusions:** The Methotrexate Assay run on the VITROS 4600 and VITROS 5600 Systems exhibited excellent correlation with the Beckman Coulter AU680 Clinical Chemistry Analyzer, optimal precision and low end sensitivity. RD0082

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A Liquid Chromatography Tandem Mass Spectrometry method for the Simultaneous Screening and Quantification of 10 Analgesics and Narcotics from Micro Plasma Collection Card

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Abstract:
Background: Addiction and abuse of analgesics and narcotics are epidemic worldwide. It is essential to quickly identify and accurately determine those drugs when drug poisoning is suspected. Here we present an application of micro plasma collection card for simultaneous screening and quantification of 10 typical drugs of analgesics and narcotics in plasma by liquid chromatography tandem mass spectrometry method. These drugs include Meperidine, Fentanyl, Morphine, Oxycodone, Tramadol, Acetaminophen, Heroin, Ketamine, Nimetazepam and Methamphetamine. **Methods:** One drop of blood (10-20 microliter) was collected by a micro plasma collection card, and then Dried Plasma Spot (DPS) was extracted before the sample was analyzed by liquid chromatography coupled to quadrupole-time-of-flight mass spectrometry (LC-QTOF) and liquid chromatography coupled to quadrupole mass spectrometry (LC-MS/MS). The drugs were identified based on retention time and exact mass acquired from molecular ions and fragment ions. After a positive identification by LC-QTOF, the sample was once again quantified by a LC-MS/MS method. Plasma volume factor of the 10 drugs was aquired by calculating the ratio of drug concentrations between DPS and wet plasma from a same blood sample. Hematocrit were evaluated the impact on plasma volume factor. **Results:** All the drugs were well extracted from DPS with recoveries higher than 70%. For LC-QTOF screening method, the limit of detection was 10-50 ng/mL. For the LC-MS/MS quantification method, the accuracy was between 88-113% and precision was less than 10% with linearity curve ranged from 10-1000 ng/mL. Plasma volume factor of each drug was a constant value (from 0.0301 to 0.0597) when hematocrit was between 30-50% or 30-60%. The concentration conversion formula was: Wet plasma (ng·mL-1) =DPS (ng·mL-1) / Volume factor. **Conclusions:** DPS card was a useful tool for convenient and stable biological matrix aimed for screening and quantifying the 10 analgesics and narcotics in human plasma.

Compounds	LOD (ng/mL)	Standard Curve (ng/mL)	Accuracy (%)	Precision (%)	Recovery (%)	Volume Factor	Hematocrit (%)
Meperidine	20	10-1000	88.60-111.3	2.46	81.43	0.0465	30~60
Fentanyl	10	10-1000	90.62-108.7	7.86	84.68	0.0301	30~60
Morphine	50	10-1000	95.20-111.3	6.34	86.39	0.0482	30~60
Oxycodone	10	10-1000	95.40-105.8	4.72	85.91	0.0589	30~60
Tramadol	10	10-1000	97.38-112.3	5.42	81.57	0.0365	30~60
Acetaminophen	50	10-1000	93.00-103.2	9.66	78.84	0.0343	30~50
Heroin	20	10-1000	94.52-105.0	3.48	71.59	0.0401	30~50
Ketamine	15	10-1000	100.2-105.6	5.88	74.29	0.0476	30~50
Nimetazepam	20	10-1000	95.39-104.6	6.09	78.63	0.0329	30~60
Methamphetamine	50	10-1000	97.42-102.5	6.58	80.09	0.0597	30~60

LOD: limit of detection.

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General Unknown Screening of Urine Samples with LC-MS/MS

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Background: Urine drug screening is generally performed in a targeted manner, where analysis is limited to the detection of predefined drugs and metabolites. Untargeted methods for the detection and identification of exogenous molecules, in which analysis is performed in an unbiased manner, have the potential to detect new drugs and unexpected additives. Such a test would be useful for public health purposes, to identify new local trends and aid in investigations of interferents. Here we explore the use of an untargeted LC-MS/MS method combined with database searching to identify exogenous compounds in urine from pain management patients, assessing the identification of drugs previously found by targeted methods and the identification of additional exogenous drugs not previously assessed by available methods. **Methods:** Urine specimens were mixed with acetonitrile, centrifuged to remove particulate matter, then mixed with 0.1% formic acid in water. Liquid chromatography of specimens was performed with a 250 mm pentafluorylphenyl column, using 0.1% formic acid in methanol and 0.1% formic acid in water as the mobile phase with a 30 min gradient. Samples were analyzed in positive mode on a Thermo Q Exactive™ mass spectrometer collecting data-dependent fragmentation spectra, without the use of inclusion or exclusion lists. Data was analyzed using Compound Discoverer™ 2.1 (Thermo) following the forensics unknown workflow, which searches the mzCloud™ database of >7,000 compounds for precursor ion and product ion pattern matches. Commercial drug-free urine was used as a negative control. A mixture of 43 recreational and prescription drugs at 200 ng/mL served as a positive control. De-identified remnant patient samples, obtained from urine toxicology testing, were analyzed. **Results:** Analysis of the positive control with this method detected and accurately identified 38 out of 43 (88%) compounds. An estimate of the limit of detection was obtained by analysis of serial dilutions of this control sample, with most compounds detectable at 20 ng/mL and approximately half detectable at 2 ng/mL. Due to the data-dependent nature the analysis, limits of detection will necessarily vary with the complexity of the sample. Analysis of pain management samples revealed a 72% concordance of the untargeted method with identifications from a validated targeted method, with lack of agreement occurring for analytes present at <100 ng/mL. Additionally, this method was able to identify prescription drugs (e.g. Zyrtec, Nexium, gabapentin) and non-prescription drugs (e.g. psychoactive beetle nut compound arecoline, designer stimulant 4-methoxy-alpha-pyrrolidinobutiophenone) that are not included in the targeted analysis. These results are qualitatively compared to prescription records and patient surveys containing self-reports of drug usage. **Conclusion:** This untargeted LC-MS/MS method, coupled with data analysis with Compound Discoverer™, is able to identify a large variety of compounds, including recreational and therapeutic drugs. We anticipate that this methodology could be used for public health surveillance to aid in the identification of new recreational drug trends, including additives and excipients, and for investigations of potential interferents in targeted assays.

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Performance Evaluation of the New Emit II Plus Oxycodone Assay on the Viva-E System

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Background: Oxycodone is a semisynthetic opioid analgesic prescribed for the relief of moderate to severe pain. Oxycodone structurally resembles codeine and morphine, with similar analgesic properties and potential for addiction and abuse. A new Emit® II Plus Oxycodone Assay for human urine screening has been developed by Siemens Healthineers. The Emit II Plus Oxycodone Assay has cutoffs of 100 and 300 ng/mL. The assay consists of ready-to-use liquid reagents that provide qualitative and semiquantitative results. The data presented in this study was generated on the Viva-E® Drug Testing System. **Methods:** Precision was evaluated at the cutoffs, ±25% controls, and other levels according to CLSI EP5-A2. Analytical recovery was studied by spiking oxycodone into human urine at levels that span the assay range (50-1000 ng/mL). Specimens (100 per cutoff) were analyzed and the results compared to those of LC-MS/MS. Cross-reactivity with structurally related drugs was assessed at different cross-reactant concentrations. The effect of common interferences was assessed by spiking the interferents into human urine in the presence of oxycodone at levels of ±25% of the cutoffs. On-instrument stability was assessed by testing the assay controls over time. **Results:** Evaluation of precision demonstrated qualitative repeatability CVs (rate) for all levels that ranged from 0.18 to 0.36%, and within-lab CVs ranged from 0.41 to 1.32%. Semiquantitative repeatability CVs (ng/mL) ranged from 0.68 to 2.54%, and within-lab CVs ranged from 2.49 to 4.87%. Semiquantitatively, the assay quantified oxycodone-spiked samples between 50 and 400 ng/mL for the 100 cutoff curve and 100-1000 ng/mL for the 300 cutoff curve within ±20% of nominal values. The percent agreement of specimens between the assay run on the Viva-E Drug Testing System and LC-MS/MS was 96% at the 100 ng/mL cutoff and 98% at the 300 ng/mL cutoff. The assay demonstrated 84% detection of oxymorphone in urine relative to oxycodone at the 100 ng/mL cutoff. The assay demonstrated minimal cross-reactivity to structurally related opioids. Potentially interfering substances gave acceptable results relative to the 100 and 300 ng/mL cutoffs. The reagents were stable onboard the Viva-E system for a minimum of 4 weeks. **Conclusion:** The Emit II Plus Oxycodone Assay on the Viva-E Drug Testing System is a suitable screening method for urine specimens at the cutoff levels of 100 ng/mL and 300 ng/mL for both qualitative and semiquantitative analysis of oxycodone.

B-437

Prevalence and Trends in Drug Use: Urine Drug Screening Positivity Rates for Community-based Patients in Ontario, Canada from 2014 to 2017

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Background: Comprehensive multi-year reports detailing the prevalence and annual trends in drug use within a specific patient cohort are often not widely available or current. Urine drug screening positivity rates derived from qualitative liquid chromatography tandem mass spectrometry-based (LC-MS/MS) patient testing may be used to obtain this information. This approach to identifying recent drug use trends in community-based patients in Ontario, Canada has not yet been published. ***Objective:*** Identify multi-year trends in drug use by examining qualitative LC-MS/MS urine drug screening positivity rates. ***Methods:*** All LC-MS/MS urine drug screening results from 2014 (N=136,864), 2015 (N=153,329), 2016 (N=106,687) and 2017 (N=75,774) were retrospectively reviewed. Following enzymatic hydrolysis and protein precipitation, all urine specimens received targeted LC-MS/MS screening which identified the presence of drugs within the following drug classes: anesthetic; anticonvulsant; antidepressant; benzodiazepine; cannabinoid; opioid; stimulant; and illicit. Relevant drug metabolites and related compounds were also included in this test. A total of N=63 different compounds were screened using their respective positive/negative cut-off concentrations. The positivity rates for all analytes were tabulated and partitioned by month of testing. ***Results:*** Over the examined four-year testing period, urine drug screening positivity rates ranged from 76.6% (cotinine) to <0.01% (7-aminoflunitrazepam, desalkylflurazepam, diazepam, flunitrazepam, flurazepam, JWH018, JWH200, MDEA, MDPV, mephedrone and phenazepam). From 2014 to 2017, annual significant ($p \leq 0.05$) increases in urine drug screening positivity rates were observed for: 6-acetylmorphine (0.6% to 1.4%); amphetamine (3.4% to 6.8%); gabapentin (5.4% to 7.6%); and methamphetamine (2.9% to 5.7%). From 2015 to 2017, annual sig-

nificant positivity rate increases were observed for: benzoylecgonine (9.7% to 13.3%); cocaine (2.8% to 4.2%); fentanyl (3.5% to 4.2%); and norcocaine (0.8% to 1.5%). From 2016 to 2017, significant positivity rate changes were observed for: buprenorphine (8.7% to 9.7%); levamisole (8.0 to 5.9%); meperidine (0.08% to 0.04%); methylphenidate (2.0% to 1.7%); naloxone (8.5% to 9.7%); norbuprenorphine (10.0% to 11.1%); norhydrocodone (1.7% to 2.0%); normeperidine (0.1% to 0.07%); and ritalinic acid (3.3% to 2.8%). Relative to the 2017 observed positivity rates, all other analytes included in the LC-MS/MS screening panel did not show significant annual trends or differences within the tested patient population. The 2017 positivity rates for methadone and EDDP were 37.0% and 37.6%. THCA positive rates in 2014, 2015, 2016 and 2017 were 29.6%, 28.9%, 29.5% and 28.6%. ***Conclusions:*** This retrospective review of qualitative LC-MS/MS urine drug screening positivity rates from 2014 to 2017 identified several significant annual changes in licit drug use and provided evidence of an increasing prevalence of illicit drug consumption within the community-based patient cohort. Use of amphetamine, cocaine, fentanyl, heroin and methamphetamine significantly increased but cannabinoid-use was consistent. Use of Suboxone significantly increased in 2017 but evidence of methadone-based opioid antagonist therapy was more common. Laboratories can provide detailed information on drug use trends within a specific patient population by tabulating, interpreting and communicating urine drug screening positivity rates to their clinical communities.

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A novel activity-based concept to screen biological matrices for the presence of (synthetic) opioids

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Background: Highly potent synthetic opioids, which mimic the effects of heroin and morphine, are a growing health threat. Detection of these novel opioids remains challenging as new compounds continue to enter the market. The objective of this study was to set up a novel concept for screening biological matrices for the presence of opiates and (synthetic) opioids, not relying on antibody-based or mass spectrometry-based recognition of the structure of these compounds, but based on their opioid activity. **Methods:** The μ opioid receptor (MOR) belongs to the class of G-protein-coupled receptors (GPCRs). Activation of these receptors results in recruitment of a signaling molecule, β-arrestin 2 (βarr2). We used this principle to set up a bioassay in which we expressed MOR and βarr2, each fused to one part of nanoluciferase, in HEK293T cells. Upon GPCR activation, βarr2 is recruited, which brings both parts of nanoluciferase into close proximity, resulting in its functional complementation, which, after application of a substrate, can easily be monitored via luminescence. In the optimized set-up, applied in 96-well format, HEK293T cells are used that are transiently transfected with plasmids (ratio 4:4:1) encoding respectively: i) MOR, C-terminally fused to the large part of nanoluciferase; ii) βarr2, N-terminally fused to the small part of nanoluciferase; and iii) G-protein coupled receptor kinase 2 (GRK2). Following washing the cells with serumfree medium, 90 µl of medium (Opti-MEM® I) and 25 µl of 20-fold diluted Nano-Glo Live Cell Reagent is added and luminescence is monitored until stabilization. Subsequently, 20 µl of biological extract (reconstituted in medium) is added and luminescence is monitored for 2 hours. Scoring is always done blind-coded. **Results:** Sensitivity and specificity were evaluated using 107 authentic postmortem blood samples with known presence or absence of the synthetic opioids U-47700 or furanylfentanyl, as determined by LC-MS/MS and QTOF analysis. A first finding was that in 8 synthetic opioid positive samples no positive signal was obtained. In these samples, Q-TOF analysis revealed the MOR antagonist naloxone, which can obviously also prevent receptor activation *in vitro*. Hence, evaluation was further based on non-naloxone containing samples. For U-47700 and furanylfentanyl positives, sensitivity was 100% (8/8), respectively 95% (21/22). The missed furanylfentanyl positive sample could not be retested for the presence of naloxone. Of the 59 opioid negative samples, 55 samples were correctly scored negative, yielding a specificity of 93% (55/59). An additional 5 samples (found to contain opioids codeine, (nor) buprenorphine or loperamide) was correctly scored positive. In 5 negatively scored samples, Q-TOF analysis revealed presence of alfentanil (1) or sufentanil (1) (both < 1 ng/ml) or dextromethorphan/levomethorphan (2) or dextrorphan/levorphanol (1) (for the latter, non-detection could be explained by presence of inactive form). **Conclusion:** The MOR reporter assay allows rapid identification of opioid activity in blood samples. Although the co-occurrence of opioid antagonists is currently a (solvable) limitation, the high sensitivity, selectivity and the untargeted nature of the technique may render it a useful first-line screening tool to investigate potential opioid intoxications in clinical and forensic settings, complementing conventional analytical methods which are currently used.

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The Detection and Analytical Confirmation of Synthetic Fentanyl Analogues in Human Urine & Serum using an Ultivo LC/TQ

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Background: During this research study, a sensitive, robust and relatively fast targeted analytical method was developed for the quantitation of 12x synthetic fentanyl opioids, 4-ANPP the synthetic precursor molecule and a similar powerful opioid-like synthetic known as W-18. Simple sample preparation routines were employed to make samples ready for analysis using an Ultivo triple quadrupole mass spectrometer LC/MS (LC/TQ) from both human serum and urine matrices. Several separate batches were prepared and analyzed to obtain statistically valid analytical performance results. The lower limits of quantitation, chromatographic precision, calibration linearity, range and accuracy for each synthetic opioid will be presented herein. A comparison of the analytical performance of each analyte for both urine and serum matrices will also be outlined. **Methods:** LC/MS analysis was performed using an Agilent 1290 UHPLC/Ultivo LC/TQ with electrospray ionization (ESI) in positive mode. The chromatographic column used was a Poroshell EC-C18 column (2.1x50mm, 2.7 µm). The UHPLC mobile phases used, A and B respectively, were 0.01% formic acid and 5mM ammonium formate in water and 0.01% formic acid in methanol. Two MRM transitions were monitored for the analytes and a single transition for the deuterated or C^{13} internal standard, during a 7-minute analysis. Human serum samples (250µL) were spiked with calibrators at various concentration levels, cold acetonitrile (500µL) containing the deuterated internal standard was added to affect protein precipitation and centrifuged at 5000rpm. The supernatant was further diluted (1:2) with a 10:90 methanol:water solvent mixture prior to instrument injection. Negative urine was spiked with internal standards and specified calibration levels, centrifuged at 5000rpm (4ºC) for 10 minutes, then 100µL of the supernatant was made up to 1mL in the sample vial by the addition of 900µL de-ionized water. **Results:** Excellent linearity and reproducibility were obtained for human serum extracts typically within an actual concentration range from 10 or 50pg/ml to 500ng/ml (50/250fg to 2500pg on-column) for each synthetic opioid analyte with a linearity coefficient of >0.997 for three batches. Precision data observed over the three batches resulted with a %RSD variation of <7% across all calibration levels in this research study. Typical results for the diluted urine samples yielded an actual concentration range from 50 or 100pg/ml to 500ng/ml (250/500fg to 2500pg on-column) for each synthetic opioid analyte with a linearity coefficient of >0.996 for three batches. Precision data observed over the three batches resulted with a %RSD variation of <9% across all calibration levels in this research study. In general, the LLOQ sensitivity for each serum-spiked synthetic fentanyl opioid analyte measured in this research exercise was approximately 2x of that obtained from the urine-spiked matrix. **Conclusion:** This research project demonstrates that the performance of theUltivo LC/TQ with the analytical methodology described herein generated excellent linearity, precision and sensitivity across the range of 10 or 50pg/ml through 500ng/ml for each respective synthetic opioid in human serum and sensitivity across the range of 50 or 100pg/ml through 500ng/ml for the respective synthetic opioid in human urine. For Research Use Only. Not for use in diagnostic procedures.

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Activity-based detection of cannabinoid activity in serum and plasma samples

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Background: Synthetic cannabinoids continue to be the largest group of new psychoactive substances monitored by the European Monitoring Center of Drugs and Drug Addiction. The rapid proliferation of novel analogues makes the detection of these new derivatives challenging and has initiated considerable interest in the development of so-called 'untargeted' screening strategies to detect these compounds. Starting from an existing proof-of-concept that worked in urine samples, the objective of this study was to set up an improved activity-based screening assay for the detection of cannabinoid activity in plasma and serum. **Methods:** We previously developed cell-based cannabinoid reporter bioassays for the detection of synthetic cannabinoids and their metabolites, capable of demonstrating cannabinoid activity in authentic urine samples. The principle of these bioassays is activity-based, where activation of the cannabinoid receptors CB1 or CB2 leads to β-arrestin 2 (βarr2) recruitment, which results in functional complementation of a split NanoLuc luciferase, thereby restoring luciferase activity. In the presence of the substrate furimazine, this results in a biolu-

minescent signal, which can be read out with a standard luminometer. Based upon this successful proof-of-concept, we have developed new stable cell lines, in which a truncated rather than a full-length βarr2 molecule was used, with the aim to further improve the assay's performance. This new bioassay was evaluated using extracts of authentic serum (n = 45) and plasma (n = 73) samples. For sample preparation, 500 µl of matrix was subjected to a simple liquid-liquid extraction using hexane:ethyl acetate (99:1 v/v). Following evaporation and reconstitution in 100 µl of Opti-MEM® I/methanol (50/50 v/v), 10 µl of these extracts was analyzed in the bioassays, which were performed in HEK293T cells that stably expressed an optimized combination of either CB1 or CB2, along with a modified βarr2. Scoring was performed blind-coded. **Results:** Truncation of βarr2 significantly (P = 0.0034 and 0.0427 for CB1 and CB2, respectively, unpaired student's t-test) improved the analytical sensitivity over the previously published bioassays, applied on urine samples. For CB1, the best result was obtained when fusing CB1 to the large part of NanoLuc and combining this in the cell system with βarr2, truncated at residue 366 and fused N-terminally to the small part of NanoLuc. For CB2, the best result was obtained when fusing CB2 to the small part of NanoLuc and combining this in the cell system with βarr2, truncated at residue 382 and fused N-terminally to the large part of NanoLuc. These new CB1 and CB2 bioassays detected cannabinoid receptor activation by authentic serum or plasma extracts, in which synthetic cannabinoids (such as MDMB-CHMICA, AB-CHMINACA, 5F-PB-22, 5F-ADB, 5F-APINACA, EG-018, PB-22 and/or ADB-FUBINACA) were present at low- or sub-ng/ml level or in which Δ^9-tetrahydrocannabinol was present at concentrations above 12 ng/ml. For synthetic cannabinoid detection, analytical sensitivity was 82%, with an analytical specificity of 100%. **Conclusion:** The new CB1 and CB2 bioassays have the potential to serve as a first-line screening tool for (synthetic) cannabinoid activity in serum or plasma and may complement conventional analytical assays and/or precede analytical (mass spectrometry based) confirmation.

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Therapeutic Drug Monitoring of Monoclonal Antibody in Inflammatory Bowel Diseases: Laboratory Evidence to Predict Patient Responses

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Background: Monoclonal antibody (mAb) biologic drugs that target inflammatory mediators hold promise in the treatment of inflammatory bowel disease (IBD). Therapeutic drug monitoring (TDM) of both drugs and anti-drug antibodies (ADAbs) is a valuable tool that can guide a personalized treatment plan. This study aims to provide laboratory evidence to predict patient responses to these therapies in IBD by reviewing TDM testing results of 6 biologics; adalimumab (ADA), certolizumab (CER), golimumab (GOL), infliximab (INF), ustekinumab (UST), and vedolizumab (VED). **Methods:** A total of 18,837 sera samples collected at trough levels from adult and pediatric IBD patients receiving mAb treatments were analyzed using the InformTx™ assay (Inform Diagnostics, Inc.), which employs an ELISA-based method to measure concentrations of drugs and free ADAbs. Patient responses were predicted on the basis of drug and ADAbs status. The needs for potential drug optimization were assessed by comparing drug and ADAbs concentrations with regard to the recommended therapeutic drug levels (ADA: 5.0-12.0 µg/mL, CER: >27.5 µg/mL, GOL: >1.4 µg/mL, INF: 5.0-1.0 µg/mL, UST: >4 µg/mL, and VED: >10 µg/mL) and laboratory-defined higher ADAbs levels (A-ADA: >25 ng/mL, A-CER: >25 AU/mL, A-GOL: 10.0 ng/mL, A-INF: >25 ng/mL, A-UST: >20 AU/mL, A-VED: 100 ng/mL). **Results:** 64.1%, 30.2%, 83.9%, 60.4%, 25.2%, and 69.1% of the patients treated with ADA, CER, GOL, INF, UST, and VED, respectively, had drug level equal to or greater than the recommended therapeutic level and undetectable ADAbs. 4.5%-33% patients had a drug concentration above the recommended therapeutic level. In contrast, patients (31.0% in ADA, 57.0% in CER, 12.1% in GOL, 32.5% in INF, 74.4% in UST, and 30.6% in VED) had undetectable or suboptimal levels of drugs and undetectable or lower levels of ADAbs. The overall ADAbs positive ratio for ADA, CER, GOL, INF, UST, and VED was 5.3%, 15.1%, 5.6%, 8.0%, 0.6%, and 0.4%, respectively. **Conclusion:** This study provides laboratory evidence to dictate the patient responses to mAb treatments in IBD patients. Undetectable or suboptimal drug levels may portend loss of response or unsatisfactory response to mAb therapies. Additional ADAb measurements are useful in distinguishing patients with low/undetectable levels of ADAbs (12.1% - 74.4%) who may benefit from dose escalation or shortening of dose interval from those with higher levels of ADAbs (0.3% - 12.6%) who need to be switched to different drugs. In patients with drug concentrations above the recommended therapeutic level (4.5% - 33%), de-escalation of therapy might reduce associated risks and costs. Moreover, the immunoresponse to mAbs varies among drugs with the lowest in UST and VED and is consistent with the degree of humanization of mAbs. Not surprisingly, the overall ADAbs positive ratios reported in this study were significantly lower than what have been previously described using methodologies that detect both free and drug-bounded ADAbs. Given that only the free ADAbs have the capacity to neutralize drugs in future infusions, quantitation of free ADAbs may represent a more clinically informative measurement. Additional clinical validation is imperative to confirm potential of TDM to improve efficacy, safety, and cost-effectiveness of these biologic therapies.

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Stability of Oxidants in Urine Specimens Used for Specimen Validity Testing

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Background: The analysis of a urine specimen to determine if it is "consistent with normal human urine" is referred to as specimen validity testing (SVT). The measured indices normally include pH, specific gravity, nitrites, chromate, and oxidants. These indices are used to determine if a urine specimen has been diluted, adulterated, or substituted. The purpose of this study was to determine the stability of a urine specimen that has been adulterated using sodium hypochlorite (NaOCl) as an oxidant. **Method:** Two pools were created by combining patient urine specimens that had previously been tested negative for sample adulteration. Pools were spiked with NaOCl in order to achieve an initial oxidant concentration that would reflect results of "normal" (<200mg/L) and "adulterated" (≥200mg/L) as defined in the *Mandatory Guidelines for Federal Workplace Drug Testing.* The "normal" pool (8.0mL) was spiked with 10 µl of NaOCl and the "adulterated" pool (8.0mL) was spiked with 17 µl of NaOCl. Each pool was then tested for oxidants immediately after the spiking (time 0). The two pools were then aliquoted and stored at 4°C and -20°C for the following time periods: 0, 5, 10, 24, and 48 hours. Samples at each time point for each storage condition were assayed in triplicate by the Roche ONLINE DAT Specimen Validity Test Oxidant assay on the cobas c501 clinical chemistry analyzer. The mean results for each specific storage condition and time point were then compared to their respective time 0 mean. **Results:** The percent differences measured at each time interval for both pools were >20% in comparison to time 0 means and continuously decrease over the study course. **Conclusion:** Patient urine samples that are tested for the presence of adulterants by the SVT oxidant assay are not stable when NaOCl is used as the adulterant. This indicates that false negative results for oxidants may occur if urine samples cannot be tested immediately post collection.

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Evaluation of Roche ONLINE TDM Acetaminophen Gen.2 assay and its robustness for analysis of hemolyzed samples

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Background and objectives: Sunnybrook Health Sciences Centre is a regional trauma centre with the largest Trauma, Emergency and Critical Care Program in Canada. Unfortunately, the currently used acetaminophen method (Roche Acetaminophen assay on MODULAR® P analyzer, Gen.1) is susceptible to hemolysis interference, resulting in high rates of sample rejection and turn-around-time delay in our patient population. This is especially challenging in our critical patients where samples can be more difficult to obtain. The objective of this study was to evaluate the performance of the newer generation Roche ONLINE TDM Acetaminophen Gen.2 assay (ACET2, on the Roche COBAS® c502 analyzer, Gen.2) and to confirm its robustness in the presence of hemolysis. **Methods:** The Gen.1 assay is an enzymatic method based on the hydrolysis of acetaminophen to p-aminophenol and acetate via arylacylamidase, with subsequent conversion of p-aminophenol to a chromogenic indophenol in the presence of o-cresol and sodium periodate. The Gen.2 assay is a homogeneous enzyme immunoassay based on competition between endogenous drug and G6PDH-labeled drug for anti-acetaminophen antibody binding sites. The Gen.2 assay was evaluated for its precision, linearity and accuracy based on CLSI guidelines. The hemolysis interference study was performed by spiking serum samples with a concentrated hemolysate stock solution. **Results:** Precision was assessed using two levels of Bio-Rad Liquichek IA Plus™ quality control material. The overall %CVs were 2.5% and 3.0% for the low (mean = 214 µmol/L) and high (mean = 657 µmol/L) quality controls, respectively, across 37 runs. Linearity was assessed using both TDM1 ACTM linearity material (Maine Standards) and patient samples. Across a concentration range of 38 to 3452 µmol/L (extended range with dilution), the assay demonstrated linearity with <10% difference from targets. Correlation between the Gen.1 and Gen.2 was assessed using patient samples with acetaminophen concentrations ranging from 36 to 687 µmol/L. Overall, the Gen.2 assay displayed an average negative bias of 21.7%

(n = 31) comparing with Gen.1. Due to this observed negative bias, accuracy of the Gen.2 assay was further assessed using external quality assurance (EQA) materials. The Gen.2 assay results were well within acceptable ranges when compared to the all methods mean. Finally, interference of hemolysis was assessed by spiking various levels of hemolysate into acetaminophen-naïve samples and samples with approximately 200 µmol/L of acetaminophen. Across a range of hemolysis levels (hemoglobin concentration 0 to 10 g/L), there was negligible interference in the Gen.2 assay for both acetaminophen-naïve and acetaminophen of 200 µmol/L samples (difference <6.2%). However, the Gen.1 assay gave various levels of false positive results in acetaminophen-naïve samples as well as falsely elevated results in the samples with acetaminophen. **Conclusions:** Evaluation of the Roche Gen.2 acetaminophen assay has shown that this immuno-based assay has an acceptable analytical performance and is less susceptible to hemolysis interference compared to the Gen.1 enzymatic method. Implementing the Gen.2 assay on our COBAS® c502 analyzer will thus allow for a lower sample rejection rate due to hemolysis and improve the quality of care, especially for our trauma, emergency and critical care patients.

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Urine buprenorphine and metabolite patterns in a large cohort of patients

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Background: Products co-formulated with buprenorphine (BUP) and naloxone (NX), such as Suboxone and Zubsolv, are frequently prescribed as therapy for opioid use disorder. An integral part of therapy is monitoring for adherence. Understanding the typical patterns of BUP and metabolites in urine would inform interpretation of results. BUP metabolites include norbuprenorphine (NOR) and the glucuronide forms (B3G and N3G). Additionally, NX is measurable in urine. Our lab previously reported that 91% (n=1,946) had three (NOR/B3G/N3G) or four (BUP/NOR/B3G/N3G) metabolites present and that free BUP and NX concentrations >100 ng/mL were suggestive of adulteration. Our objective was to evaluate urine BUP and metabolites and NX in a recent, large cohort of samples and to assess the ratio of NOR+N3G to BUP+B3G (N:B) as a potential indicator of consistent therapy. **Methods:** Data for all quantitative urine BUP and metabolite tests performed by LC-MS/MS from February 2011 to January 2018 were retrieved. Cutoff concentrations were 2 ng/mL for free BUP and NOR, 5 ng/mL for B3G and N3G, and 100 ng/mL for NX. Results were analyzed for: 1) the occurrence of BUP and metabolites; 2) the distribution of concentrations for NX, BUP and metabolites and; 3) the ratio (%) of N:B in patients with at least 50 separate test requests for comparison. **Results:** Results for 128,709 tests requests were obtained from 44,299 different patients. The median age was 34 years (interquartile range (IQR) 27-44 years). Similar to previous, 93.5% of patients had three or four metabolites present (NOR/B3G/N3G=40.6%; BUP/ NOR/B3G/N3G=52.9%). Of the total cohort, 24.9% of samples were negative for all compounds. There were 3,342 (2.6%) samples with NX, of which 2,030 (60.7%) had both NX and BUP concentrations ≥1000 ng/mL suggestive of sample adulteration. There were 912 patients with BUP >100 ng/mL but <1000 ng/mL. Within those 912 patients, 162 had no quantifiable metabolites (including 62 with NX <100 ng/mL), while 631 patients had quantifiable metabolites and NX <100 ng/mL, suggestive of possible adulteration with a BUP product not co-formulated with NX. Among 58 patients with at least 50 incidences of monitoring, the ratios of N:B varied significantly inter-individually ($p<0.0001$) and intra-individually (mean CV%=55.2±17.3%), likely indicating variation in urine collection time since last dose, changes in dose, or adulteration. However, the N:B ratio was significantly different among three groups representative of samples from patients who had not recently taken the medication (late metabolism), had recently taken the medication (mid-metabolism), and those with ≥1 analyte ≥1000 ng/mL (early metabolism/high-dose therapy/adulterated sample) (median (IQR): 343 (243-543) ng/mL vs. 271 (159-452) ng/mL vs. 169 (129-251) ng/mL, respectively, $p<0.0001$). **Conclusion:** More than 90% of patients taking a BUP formulation will have three or more metabolites present in their urine. Which metabolites are present and the N:B ratio may aid in assessing the stage of metabolism or if adulteration has occurred. Consistency with respect to the time of dose, time of urine collection, and consideration of urine dilution may improve the utility of metabolite ratios to evaluate whether a patient is being adherent with their therapy.