mass spectrometer (Thermo Fisher Scientific) with a heated electrospray-ionization (HESI) source in positive ionization mode with selected reaction monitoring (SRM). Validation of this method was determined through the characterization of precision (five replicates twice a day over five days), linearity and recovery, and limit of quantitation. Additionally, carryover studies were conducted using high (2000 ng/ml) and low (20 ng/ml) calibrators in randomly alternating runs. All statistical parameters were evaluated with EP Evaluator 8.

Results: Precision was defined as running three levels of docetaxel (35 ng/ml, 350 ng/ml and 1500 ng/ml). The within run precision was evaluated giving a % CV of 16.7%, 13.7% and 13.2% for the aforementioned levels, respectively. Between run and between day precision were resulted with a % CV of 7.3%, 8.4% and 13.4% (between run) and 20.7%, 7.6% and 10.2% (between day) for 35 ng/ml, 350 ng/ml and 1500 ng/ml docetaxel, respectively. The described method was linear from 31.3 ng/ml to 2000 ng/ml (slope of 1.1) and mean recovery ranged from 101.1-108.1% over the linear range. The limit of quantitation (the lowest concentration with a CV <20%) was determined to be 31.3 ng/ml for this method. Carryover studies using high and low docetaxel calibrators resulted in a minimal carryover of 4.2 ng/ml, which is less than three times the SD of the low calibrator (1 SD = 8.8).

Conclusions: This developed and validated LC-MS/MS method allows for the quantitation of docetaxel from direct serum injections. Although this method uses the direct injection of a small quantity of specimen (25 µl) for drug analysis, more studies are being conducted to increase the sensitivity of the assay.

B-23

Development of a Rapid Quantitative/Semi-quantitative LC-MS/MS Method to Monitor Opioids and Glucuronide Metabolites

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Background: Managing chronic pain patients with opioid therapy is a difficult and controversial issue. Random urine drug screens are recommended by the APS and AAPM to help physicians detect aberrant behavior in patients, such as divergence (not taking prescribed drug, and selling or exchanging it for another drug). Several opioids are metabolized primarily to glucuronide metabolites; detecting only the parent compound can lead to false negative results. Regular screening is used to confirm patient compliance, and patients reported as negative are at risk for losing their therapy. Immunoassays are commonly used to screen, but they suffer from a lack of specificity. We have previously developed a quantitative LC-MS/MS method for 14 opioids that offers superior specificity and sensitivity compared with immunoassays. In this work, we aimed to develop a novel LC-MS/MS method to quantify the same 14 opioids and 6 new glucuronide metabolites with minimal sample preparation. While all of the opioids could still be quantified by isotope dilution, internal standards are not universally available for all of the glucuronide metabolites. In addition, several of the glucuronide metabolites are not stable in calibration mixtures. Moreover, it is more important to specifically detect an opioid or its metabolite than it is to accurately quantify the analyte. As a result, we aimed to develop a new semi-quantitative assay by LC-MS/MS using a 50ng/mL cut-off.

Methods: Urine specimens, calibrators, or controls were centrifuged and added in equal volume to 100 µL internal standard solution containing 14 dueterated opioids and 3 dueterated glucuronide metabolites in water. Ten µL were injected for analysis by LC/MS/MS. Separation was performed by UPLC on an Acquity HSS T3 column (2.1 x 50 mm, 3 µm). Mobile phase A contained 2 mM ammonium acetate in water, 0.1% formic acid and mobile phase B contained 0.1% formic acid in acetonitrile. The separation of all compounds was complete in 9 minutes.

Results: The method was evaluated for linearity, precision, and analytical recovery. The assay was linear between 10 and 1000ng/mL. Intra-assay imprecision (150ng/mL) ranged from 1.0 to 8.4% CV. Inter-assay precision ranged from 1.0 to 16%. Recovery was determined by spiking five patient specimens with opioid and glucuronide standards at100ng/mL. The patient specimens contained varying degrees of protein, bilirubin, and pH ranges. Recoveries ranged from 82 to 107% (median 98.9%). The method correlated well with our current quantitative LC-MS/MS assay for opioids. It is very important to point out that during our correlation study we found several patient samples that tested positive for glucuronides that would have tested negative when measuring the parent compound alone.

Conclusion: We have developed a quantitative/semi-quantitative method to simultaneously monitor 14 opioids and 6 of their glucuronide metabolites with minimal sample preparation. The assay can be used to identify non-compliance and diversion with high specificity in the chronic pain population.

B-24

A Highly Specific 6-Acetylmorphine Immunoassay for Detecting Heroin Use

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Background: Immunoassays directed against the unique metabolite of heroin, 6-acetylmorphine (6-AM), are increasingly used to screen for heroin use. These assays are more specific for assessing heroin use than opiate immunoassays which target morphine. However, in our population of patients treated for chronic pain, we observed a high false positive rate with the CEDIA 6-AM immunoassay, which was suspected to be due to interference from other structurally-related opioids.

Objective: To evaluate whether a recently approved automated urine 6-AM immunoassay is more specific than our current CEDIA 6-AM assay for detection of 6-AM.

Methods: A total of 214 urine samples sent to our laboratory from patients treated for chronic pain (192 consecutive, 22 previously screened positive for 6-AM by CEDIA) were analyzed by the Microgenics CEDIA Heroin Metabolite (6-AM) assay (Thermo Fisher Scientific, Inc.) and the 6-AM enzyme immunoassay (EIA) (Lin-Zhi International, Sunnyvale, CA) on the Olympus AU480 analyzer (Beckman Coulter, Inc.) using the manufacturers' recommended 10 ng/mL cutoffs. All positives by CEDIA and/or EIA were tested for 6-AM by GC/MS (5 ng/mL reporting limit). Samples were also tested for the presence of morphine (free and conjugated forms) and other opioids by LC-MS/MS (100 ng/mL reporting limit). Water spiked with free morphine concentrations up to 50,000 ng/mL was tested to determine the morphine cross-reactivity of both assays.

Results: CEDIA and EIA demonstrated equivalent sensitivity for 6-AM detection, both correctly identifying the eight 6-AM positive specimens. However, the EIA 6-AM assay provided improved specificity for 6-AM (100%) compared to CEDIA (91%). The CEDIA false positive rate was 69%. Upon investigation all but one of the eighteen CEDIA false positive samples were found to contain high levels (>30,000 ng/mL) of morphine. Subsequent spiking experiments revealed that spiked morphine concentrations as low as 12,500 ng/mL generated positive CEDIA results, whereas EIA showed no morphine cross-reactivity up to 50,000 ng/mL.

Conclusion: The EIA assay offers sensitive and specific 6-AM detection without the significant morphine cross-reactivity of the CEDIA assay. Implementation of the EIA 6-AM screening assay should facilitate accurate detection of recent heroin use in clinical laboratories that do not perform confirmatory testing.

B-25

Performance Charectersitcs Of Ark Diagnostics Quantitative Immunoassay For Levetiracetam On The Beckman Random Access Unicel® Dxc System & Comparsion To Lc-Ms-Ms Batch Analysis

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Background: Levetiracetam [(-)-(S)-α-ethyl-2-oxo-1-pyrrolidine acetamide] is a second generation anticonvulsant medication indicated as adjunctive therapy in the treatment of certain types of seizures in people with epilepsy. It is marketed under the trade name Keppra®. Levetiracetam is a single enantiomer and the precise mechanism(s) by which levetiracetam exerts its antiepileptic effect is unknown. However, high doses of levetiracetam can induce adverse effects, including dizziness, somnolence, asthenia, headache, behavioral problems, depression, and psychosis (Kanner et al., 2004). The therapeutic drug monitoring of levetiracetam concentrations plays an important role as an aid in management of patients treated with levetiracetam for toxicity issues.

Objective: To evaluate the performance characteristics of the new ARK Diagnostics Levetiracetam Assay on the Beckman Random Access UniCel DxC system for routine clinical laboratory use.

Methods: The ARK Levetiracetam Assay is a homogeneous immunoassay used in the quantitative determination of levetiracetam in human serum or plasma. When sample and reagents are mixed, drug in the sample competes with drug labeled by the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for antibody binding sites. Enzyme activity decreases upon binding to the antibody so that the drug concentration in the sample can be measured in terms of enzyme activity. Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that is measured spectrophotometrically. The NADH absorbance is directly proportional to drug concentration in the sample. Endogenous serum G6PDH does