Exposure to drugs and toxins is a major cause for the rising number of emergency department visits each year. Immunoassays are commonly used in the emergency department to provide rapid turnaround time for acute care. The purpose of this study was to compare two automated immunoassay chemistry analyzers to determine which platform produced the fewest number of false positive/negative results. Residual patient urine samples were collected for each of the following drugs/drug classes: cocaine (n = 40), opiates (n = 45), and amphetamines (n = 54) and confirmed either positive or negative by mass spectrometry. Sample analyses of these specimens were performed on both the Roche COBAS INTEGRA 400 plus and Ortho Vitros 5,1 FS instruments. The results from the two chemistry analyzers were compared to confirmed results. Both immunoassays were prone to false positive results for cocaine and false negative results for opiates and amphetamines. The Vitros Fusion analyzer generated fewer false positive and false negative results for opiates and amphetamine testing than the Roche Integra, but the platforms performed comparably for cocaine.

Introduction

Substance abuse is a serious issue in the United States and is a major cause for urgent care visits each year (1). Urine drug screens are often ordered by physicians in the Emergency Department (ED) to identify drug use/exposure. Qualitative urine toxicology assays that are of particular interest to an ED include amphetamines, cocaine and opiates. To support the needs of the ED, the National Academy of Clinical Biochemistry recommends Tier I STAT qualitative testing, with a turnaround time of 1 h or less (2). To meet this aggressive time requirement, automated immunoassays, performed with routine chemistry analyzers, are commonly used. Although this approach can meet the turnaround time requirement, immunoassays are limited in sensitivity and specificity when compared to chromatographic (confirmation) assays, which are more definitive. Based on the clinical scenario, it may be important to follow up a positive or negative drug screen result obtained by immunoassay with confirmatory testing.

The objective of this study was to compare confirmed results for amphetamines, opiates and cocaine metabolite (benzylecgonine) with results from two automated immunoassay chemistry analyzers that might be used to support the ED at the University of Utah hospital. The goal of this study was to determine the false positive and negative rates for each platform.

Methods

**Roche COBAS INTEGRA 400 plus**

The Roche Cobas Integra 400 plus (Roche Diagnostics Corp.; Indianapolis, IN) supports competitive turbidimetric immunoassays for drug detection, based on kinetic interaction of microparticles in solution (KIMS), as measured by changes in light transmission. If drug is present in urine, it competes with the drug derivative conjugate for the microparticle-bound antibody that is included in the assay. Turbidimetry is determined by an endpoint reaction at 520 nm. In this study, drug assays were performed according to manufacturer’s instructions, using the following reagent systems: Opiates 300/2000/200T (Cat# 207671580122), Cocaine II 200 (Cat# 03800130 190), Amphetamines (Cat# 20737968 122) (3–5).

**Ortho Vitros 5,1 FS**

The Ortho Vitros Fusion (Ortho Clinical Diagnostics; Rochester, NY) supports competitive immunoassays for drug detection. The assays are based on competition between drug in a patient urine sample and drug labeled with the enzyme glucose-6-phosphate dehydrogenase (G6P-DH), which is included in the assay for antibody binding sites. Enzyme activity decreases upon binding to the antibody and the concentration of the drug in the urine sample is directly proportional to measured enzyme activity. Active enzyme converts oxidized nicotinamide adenine dinucleotide (NAD+) to NADH, resulting in an absorbance increase that is measured spectrophotometrically at 340 nm at two time points (two-point rate reaction). In this study, drug assays were performed according to manufacturer’s instructions, using the following reagent systems: Op (Ver. 5.0 Pub. # J27320 Ref 680 1997), COCM (Ver. 4.0 Pub. # J27318 Ref 680 1995), AMPH (Ver. 4.0 Pub. # J27311 Ref 680 1991) (6–8).

Sample preparation for cocaine analysis

This assay was designed to determine concentrations of benzylecgonine over an analytical measurement range of 50–5,000 ng/mL. The sample preparation procedure was adopted from a method published by Taylor and Jain in 1987 (9). Briefly, 100 μL of deuterated internal standard for benzylecgonine-d8 (Cerilliant; Round Rock, TX) was added to 1 mL of urine specimen. To adjust the pH of the specimens, 2 mL of 0.1 M sodium phosphate buffer (pH 6.0) was added for optimal adsorption of drug onto the PSCX solid-phase extraction (SPE) column (SPEware; Baldwin Park, CA). The samples were vortexed, centrifuged at 3,500 rpm at 0°C for 5 min, and then...
loaded onto the column by adjusting the vacuum pressure to 1 drop per 4 s. The SPE column was then washed with 1 mL of 0.1 M sodium bicarbonate buffer, 1 mL nanopure water, 1 mL of 0.1 M hydrochloric acid (made in-house), 1 mL of methanol (HPLC grade; VWR International; Radnor, PA) and 1 mL of ethyl acetate (VWR International). The SPE column was then dried for 2–5 min at a pressure of 25 psi. Cocaine and metabolites were eluted from the SPE column with 1 mL of ethyl acetate–isopropanol–ammonium hydroxide (50:45:5) (VWR International), by adjusting the vacuum pressure to 1 drop per 4 s into a clean vial. The samples were dried to completion at 40°C at 25 psi for 20–30 min. The samples were derivatized by adding 25 μL of acetonitrile (HPLC grade, VWR International) and 25 μL of N-methyl-trimethylsilyl-trifluoroacetamide (MSTFA; United Chemical Technologies, Inc; Bristol, PA). The samples were capped and vortexed. Calibrators (purchased from Cerilliant) and controls (made in-house) were prepared in a similar manner as the patient samples.

Gas chromatography with mass spectrometric detection

The derivatized solution was injected onto a capillary column (part #21512-141, VWR International), with ultra-high purity helium at a column temperature of 140°C, followed by fragmentation by electron impact. Selected ions were monitored in selected ion monitoring (SIM) mode on a gas chromatography–mass spectrometry (GC–MS) 6890N/5973 instrument (Agilent Technologies; Santa Clara, CA). The results were quantified by comparison to a calibration curve using the instrument’s microprocessor.

Sample preparation for opiate analysis

This clinical assay was designed to determine concentrations for six unconjugated opioids (morphine, codeine, 6-monoacetylmorphine, hydrocodone, hydromorphone and dihydromorphone). Twenty-five microliters of an opiate internal standard solution, which consisted of deuterated analogs (codeine-d6, dihydrocodeine-d6, hydrocodone-d3, morphine-d6, hydromorphone-d3, oxycodeone-d6, oxymorphone-d3 and 6-acetylmorphine-d6) were pipetted into a 2-mL polypropylene 96-well PSCX SPE column (SPEware). Two hundred fifty microliters of urine specimen was transferred into the SPE column. To adjust the pH of the urine specimen, 750 μL of 0.1 M sodium phosphate buffer (pH 6.0) was added to the well for optimal adsorption of drug onto the column. The samples were loaded onto the column by adjusting the vacuum pressure to 1 drop per 4 s. The SPE column was then washed with 1 mL of nanopure water, 1 mL of 0.1 M acetic acid (made in-house) and 1 mL of methanol (HPLC grade; VWR International). The SPE column was then dried for 5 min at a pressure of 60 psi. Amphetamines were eluted off of the SPE column by gravity flow with 1 mL of methanol–ammonium hydroxide (95:5) (VWR International) into a clean 96-well plate. The plate was covered for direct injection onto the mass spectrometer. Calibrators (purchased from Cerilliant) and controls (prepared in-house) were prepared in the same manner as patient samples.

Liquid chromatography with tandem mass spectrometric detection

Ten microliters of eluted samples were injected onto an Acquity HSS C18 ultra-performance liquid chromatography (UPLC) analytical column, 1.8 μm particle size, 2.1 × 50 mm (waters; Milford, MA) with a column temperature of 25°C. The mobile phase conditions were set to run as a gradient with a flow rate of 0.45 mL/min for a run-time of 4.50 min. The mobile phase consisted of 5 mM ammonium formate (Sigma-Aldrich, St. Louis, MO) in nanopure water (pH 3.0; mobile phase A) and 0.05% formic acid (Sigma-Aldrich) in methanol (mobile phase B). The analytes were separated by selected precursor ions and then fragmented by collision with argon gas molecules for selected ion products. Two ion transitions were used to qualify and quantify each analyte, respectively, over an analytical measurement range of 2–5,000 ng/mL on the Waters Acquity TQD Ultra Performance Liquid chromatography–tandem mass spectrometer (LC–MS-MS) (Waters). Results were quantified using the QuanLynx software (Waters).

Sample preparation for amphetamines analysis

This clinical assay was designed to determine concentrations of amphetamine and methamphetamine. Twenty-five microliters of deuterated analogs of amphetamine (amphetamine-d11; Cerilliant) and methamphetamine (methamphetamine-d14; Cerilliant) as internal standards were pipetted into a 2-mL polypropylene 96-well PSCX SPE column (SPEware). Twenty-five microliters of urine specimen was transferred into the SPE column. To adjust the pH of the urine specimen, 750 μL of 0.1 M sodium phosphate buffer (pH 6.0) was added to the well for optimal adsorption of drug onto the column. The sample was loaded onto the column by adjusting the vacuum pressure to 1 drop per 4 s. The SPE column was then washed with 1 mL of nanopure water, 1 mL of 0.1 M acetic acid (made in-house) and 1 mL of methanol (HPLC grade; VWR International). The SPE column was then dried for 5 min at a pressure of 60 psi. Amphetamines were eluted off of the SPE column by gravity flow with 1 mL of methanol–ammonium hydroxide (95:5) (VWR International) into a clean 96-well plate. The plate was covered for direct injection onto the mass spectrometer. Calibrators (purchased from Cerilliant) and controls (prepared in-house) were prepared in the same manner as patient samples.

LC–MS-MS

Ten microliters of eluted samples were injected into an Acquity HSS C18 UPLC analytical column, 1.8 μm particle size, 2.1 × 50 mm (Waters) with a column temperature of 25°C. The mobile phase conditions were set to run as a gradient with a flow rate of 0.5 mL/min for a run-time of 3.00 min. The mobile phase consisted of 5 mM ammonium formate (Sigma-Aldrich) in nanopure water (pH 3.0; mobile phase A) and 0.05% formic acid (Sigma-Aldrich) in methanol (mobile phase B). The analytes were separated by selected precursor ions and then fragmented by collision with argon gas molecules for selected ion products. Two ion transitions were used to qualify and quantify each analyte, respectively, over an analytical measurement range of 200–5,000 ng/mL on the Waters Acquity TQD Ultra
Performance LC–MS-MS (Waters). Results were quantified using the QuanLynx software (Waters).

Method comparison
This study was approved by the Institutional Review Board of the University of Utah. Residual patient urine samples were confirmed positive or negative by mass spectrometry for cocaine, opiates and amphetamines. Urine samples were stored refrigerated for two weeks and then 139 samples were selected, de-identified and stored at −70 °C until analysis by immunoassay. Split sample analysis of these specimens were performed on both the Roche COBAS INTegra 400 plus and the Ortho Vitros 5.1 FS instruments. Qualitative results from the automated chemistry analyzers were compared to results from mass spectrometry analysis (GC–MS or LC –MS-MS). The accuracy of the two automated immunoassay systems was evaluated. Additional testing was performed for discrepant results between mass spectrometry and immunoassay.

Percent method agreement
Values were calculated by comparing the ratio between the true positive/negative results by immunoassay versus true (confirmed) positive/negative results. Results were converted to percentage.

Percent positive method agreement
\[ \text{Percent positive method agreement} = \frac{\text{number of positive specimens (immunoassay)}}{\text{number of positive specimens (LC–MS-MS) × 100}} \]

Percent negative method agreement
\[ \text{Percent negative method agreement} = \frac{\text{number of negative specimens (immunoassay)}}{\text{number of negative specimens (LC–MS-MS) × 100}} \]

Results and Discussion
Results from the three-way method comparison are summarized for each drug category in Table I, and agreement between the assays is shown in Table II. The cocaine immunoassays were designed to detect benzoylecgonine at concentrations equal to or greater than 300 ng/mL. Forty clinical urine samples (12 positive, 28 negative) were tested by all methods. The Ortho Vitros and the Roche Integra cocaine assays have 100% cross-reactivity with benzoylecgonine and generated 13 false positive results each when compared to mass spectrometry analysis (GC–MS or LC –MS-MS). The accuracy of the two automated immunoassay systems was evaluated. Additional testing was performed for discrepant results between mass spectrometry and immunoassay.

Opiates
The opiate immunoassays are designed to detect codeine, morphine, hydrocodone, dihydrocodeine, hydromorphone, and to a lower extent, morphine-3-glucuronide. A positive result should trigger when the sum of one or more of these drug analytes is equal to or greater than the 2,000 ng/mL cutoff. Forty-five clinical urine samples (36 positive, nine negative) were tested by all methods. The Ortho Vitros Opiate assay generated one false negative result. The assay did not flag a positive result for a sample that was confirmed positive for hydrocodone (1,852 ng/mL), hydromorphone (20 ng/mL) and dihydrocodeine (168 ng/mL), which gave a combined total result of 2,040 ng/mL. The Vitros assay has the following percent cross-reactivity at 2,000 ng/mL: codeine (170.9%), hydrocodeine (133.3%), dihydrocodeine (96.6%), hydromorphine (56.3%), 6-acetylmorphine (43.7), heroin (39.2%) and morphine-3-glucuronide (19.6%). The false negative result could be due to poor antibody cross-reactivity; in addition, possible urine adulteration could impact immunoassay analysis. The urine specimens had creatinine concentrations in the normal range; however, urine adulteration was not tested.

The Roche Integra assay generated 13 false negative results for opiate patient samples. The 13 samples each contained hydrocodone (1,467–2,859 ng/mL), hydromorphone (7.3–481 ng/mL), and dihydrocodeine (101–609 ng/mL) and had a combined total concentration of >2,000 ng/mL. The positive patient samples that were confirmed by LC–MS-MS were within the 2,000 ng/mL cutoff concentration range for the immunoassay. The Roche assay has the following percent cross-reactivity at 2,000 ng/mL: codeine (129%), hydrocodeine (41%), dihydrocodeine (93%), hydromorphine (32%), 6-acetylmorphine (84%), heroin (65%) and morphine-3-glucuronide (42%). The false negative results were probably due to poor cross-reactivity, or possible urine adulteration could impact immunoassay results. Urine specimens had creatinine concentrations in the normal range; however, urine adulteration was not tested.

Amphetamines
The amphetamine immunoassays are designed to detect d-amphetamine, d-methamphetamine, and to a lesser extent l-amphetamine and l-methamphetamine. A positive result should trigger when the sum of one or more of these drug analytes is equal to or greater than the 1,000 ng/mL cutoff. Fifty-four clinical urine samples (39 positive, 15 negative) were tested by all methods. The Ortho Vitros assay generated four false negative results. The positive patient samples, confirmed by GC–MS for amphetamines, were within the 1,000 ng/mL cutoff concentration range for the immunoassay. The four patient samples that had false negative results were positive for amphetamine by GC–MS with values ranging from 1,029–1,250 ng/mL, which is greater than the assay cut-off. The Vitros Fusion assay has the following percent cross-reactivity at 1,000 ng/mL: d-methamphetamine (100%), d-amphetamine (90.9%), l-methamphetamine (43.5%) and l-amphetamine (10.0%). The false negative results could be due to poor antibody cross-reactivity to d-amphetamine/d-methamphetamine concentrations near the cut-off of the assay, or urine adulteration could impact immunoassay results. Urine specimens had

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Table I
Three-Way Instrument Method Comparison

<table>
<thead>
<tr>
<th>Test</th>
<th>Analytes Detected</th>
<th>Cutoff ng/mL</th>
<th>Confirmation by MS</th>
<th>Ortho Vitros® 5,1 FS</th>
<th>Roche Integra® 400 plus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>TP = 12</td>
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<td></td>
</tr>
<tr>
<td>Cocaine (GC–MS)</td>
<td>Benzoylecgonine</td>
<td>300</td>
<td>TP = 36</td>
<td>TP = 35</td>
<td>TP = 39</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Opiates (LC–MS–MS)</td>
<td>Codeine</td>
<td>2000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Morphine</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>Dihydrocodeine</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Hydromorphone</td>
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<tr>
<td>Amphetamines (GC–MS)</td>
<td>Amphetamine</td>
<td>1000</td>
<td>TP = 39</td>
<td>TP = 35</td>
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</tr>
<tr>
<td></td>
<td>Methamphetamine</td>
<td></td>
<td>TN = 15</td>
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<td></td>
<td></td>
<td>FN = 4</td>
<td>FN = 6</td>
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</tbody>
</table>

(1P) = true positive; (TN) = true negative; (FP) = false positive; (FN) = false negative; (MS) = mass spectrometry

Table II
Percent (%) Method Agreement

<table>
<thead>
<tr>
<th>Drug</th>
<th>Cutoff Value (ng/mL)</th>
<th>Vitros 5,1 % Positive agreement</th>
<th>Vitros 5,1 % Negative agreement</th>
<th>Vitros 5,1 % Overall agreement</th>
<th>Integra 400 % Positive agreement</th>
<th>Integra 400 % Negative agreement</th>
<th>Integra 400 % Positive agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocaine (GC–MS)</td>
<td>300</td>
<td>100</td>
<td>77</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Opiates (LC–MS–MS)</td>
<td>2000</td>
<td>97.2</td>
<td>100</td>
<td>99.6</td>
<td>96.9</td>
<td>100</td>
<td>98.3</td>
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<tr>
<td>Amphetamines (GC–MS)</td>
<td>1000</td>
<td>89.7</td>
<td>100</td>
<td>94.9</td>
<td>84.6</td>
<td>100</td>
<td>88.2</td>
</tr>
</tbody>
</table>

% Positive Method Agreement = (number of positive specimens (immunoassay)/number of positive specimens (LC–MS–MS) * 100)
% Negative Method Agreement = (number of negative specimens (immunoassay)/number of negative specimens (LC–MS–MS) * 100)

despite creatinine concentrations in the normal range; however, urine adulteration was not tested.

The Roche Integra assay generated six false negative results. Five of the six patient samples were confirmed positive by GC–MS for amphetamines (1,029–1,283 ng/mL) and one was confirmed positive for methamphetamine (9,965 ng/mL). The Roche Integra assay generated one false positive result that was confirmed by GC–MS with a result of 809 ng/mL, which is below the 1,000 ng/mL cutoff. The Roche assay has the following cross-reactivity at 1,000 ng/mL: dl-amphetamine (57%), dl-methamphetamine (0.5%), l-methamphetamine (4.4%) and l-amphetamine (4.0%). The false negative results could be due to poor cross-reactivity to d- or l-amphetamine and d- or l-methamphetamine. Furthermore, it is possible that urine adulteration could impact immunoassay results. The false positive result could result from cross-reactivity to other metabolites or sympathomimetic amines, as stated in the package insert.

Discussion and Conclusions

Overall, the Ortho Vitros platform had better agreement with mass spectrometry results than the Roche Integra for opiates and amphetamines (Table II). The Roche Integra assay had low cross-reactivity to hydrocodone and hydromorphone and false negative results were generated in patient samples that had hydrocodone and hydromorphone. The Roche Integra assay also had low cross-reactivity to d-methamphetamine. It seems that for the assay to detect a positive for amphetamines, the d-amphetamine concentration should be higher than 1,000 ng/mL. Both immunoassays had poor cross-reactivity to l-amphetamine and l-methamphetamine, which are metabolites of selegiline, a drug commonly used to treat Parkinson's disease. The presence of the L-isomers of amphetamines could provide an explanation for the false negative results.

Based on our data, the Vitros Fusion analyzer generated fewer false positive and false negative results for opiate and amphetamine testing and was selected as the platform for urine drugs screening at our hospital.

References