New Emit® II Plus 6-Acetylmorphine Assay* on the V-Twin®/Viva-E® Analyzers

N. Morjana, H. Yau, A. Dominowski, C. Schaible, Y.F. Zheng, D. Clark and G. Siefring

Siemens Healthcare Diagnostics Inc., Newark, Delaware 19702, USA.

SIEMENS

Abstract

Background. 6-Acetylmorphine (6-AM) is a heroin metabolite and its presence in urine specifically confirms the illicit use of heroin. A new Emit® II Plus 6-AM Assay for human urine screening is currently being developed on the V-Twin®/Viva-E® (Siemens) analyzers. The assay has a cutoff of 10 ng/mL. The assay reagents will provide qualitative and semi-quantitative results.

Methods. Precision was evaluated using the cutoff and +/- 25% controls according to CLSI EP5-A2. Recovery was studied by spiking 6-AM into human urine at levels that span the calibration range (0-20 ng/mL). On-instrument stability was assessed by testing the cutoff and +/- 25% controls over a 32-day period. Urine specimens were analyzed and the results compared to those from the GC/MS. Cross-reactivity with structurally related drugs was assessed at different cross-reactant concentrations.

Results. The qualitative repeatability CV's (rate) for the cutoff and +/- 25% controls ranged from 0.27-0.28 % and the within-lab CV's ranged from 0.97-1.25 %. The semi-quantitative repeatability (ng/mL) CV's ranged from 1.14-1.35 % and the within-lab CV's ranged from 3.50-5.05 %. The overlap distribution between the +/- 25% 6-AM controls and the cutoff was < 5%. The analytical sensitivity of the assay was found to be ≤ 1.5 ng/mL. Semi-quantitatively, the assay quantified 6-AM-spiked samples between 5-20 ng/mL within +/- 20 % of nominal values. At the 10 ng/mL cutoff, the percent agreement of specimens between the new assay and GC/MS was > 95 %. The assay reagents had minimal cross-reactivity with the structurally related drugs, morphine (0.02%), morphine-3-glucuronide (0.003%), morphine-6-Glucuronide (0.002%) and codeine (0.002%). The reagents are stable on-board the analyzer for at least 30 days.

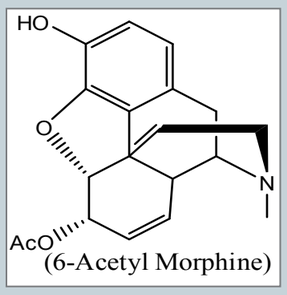
Conclusion. The new Emit® II Plus 6-AM Assay will be a suitable screening method for urine specimens in both qualitative and semi-quantitative analyses.

.

Introduction

6-Acetylmorphine (6-AM) is an active heroin metabolite and its presence in urine confirms the illicit use of heroin. 6-AM is rapidly metabolized by deacetylation to morphine, which is further metabolized by conjugation to morphine glucuronides. The half life of 6-AM is approximately 38 minutes and is detected in urine for only a few hours after heroin exposure. The Emit® 6-AM Assay is being developed on the V-Twin®/Viva-E® analyzers with application on other chemistry analyzers. It is intended for use in urine for qualitative and semi-quantitative testing of 6-AM. The assay has one cutoff, 10 ng/mL. The 10 ng/mL cutoff satisfies the new SAMHSA requirements for initial 6-AM testing.

The Emit® II Plus 6-AM Assay is a homogeneous enzyme immunoassay. It is based on competition between drug (6-acetylmorphine) in the sample and drug-labeled with glucose-6-phosphate dehydrogenase (G6PDH) for antibody binding sites. The enzyme conjugate activity decreases upon binding to the antibody. The unbound enzyme conjugate converts the oxidized nicotinamide adenine dinucleotide (NAD) in the Antibody Reagent to NADH and the change in the absorbance can be measured spectrophotometrically at 340 nm. Enzyme activity decreases upon binding to the antibody, allowing 6-AM concentrations in a sample to be measured in terms of G6PDH activity.



Assay Reaction

AB + DRUG + DRUG-G6PDH -----> AB-DRUG + AB-DRUG-G6PDH + DRUG-G6PDH
(inactive) (active)

DRUG-G6PDH
Glucose-6-phosphate + NAD+ ----------> 6-Phosphogluconolactone + NADH + H+
(absorbs at 340 nm)

Where: AB = Anti-6-Acetylmorphine antibody
DRUG = 6-Acetylmorphine
DRUG-G6PDH = Drug-glucose-6-phosphate dehydrogenase conjugate

Assay Features

The Emit® II Plus 6-AM Assay is currently under development and has the following features:

- Cutoff level: 10 ng/mL
- Two-reagent system: Antibody Reagent (liquid) & Enzyme Reagent (powder)
- Liquid calibrators: 0, 5, 10, 15 and 20 ng/mL.
- Qualitative and semi-quantitative results
- Reagents able to be used on other analyzers
- Linearity (1.1 - 20 ng/mL)
- At least 32 days of on-instrument stability

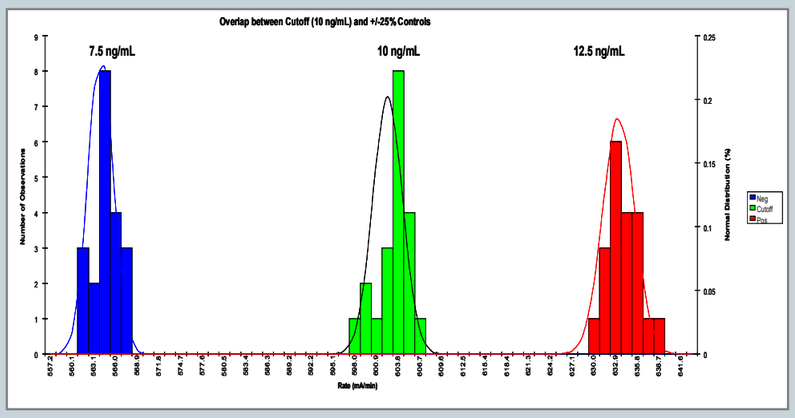
Performance data was collected on the Viva-E® analyzer.

Analytical Sensitivity

The analytical sensitivity is defined as the lowest analyte concentration that can be distinguished from the zero calibrator with a confidence level of 95%. It was determined as the analyte concentration that corresponds to the mean negative calibrator rate plus two SDs. The analytical sensitivity is 1.1 ng/mL.

Overlap at the Cutoff

For the 10 ng/mL cutoff, there was 0.0 % rate overlap for the respective 6-AM control values of 7.5 and 12.5 ng/mL (+/- 25% of the cutoff). Twenty replicates (N=20) were run on each level.



Qualitative Comparative Analysis

Comparative analysis was performed with 105 urine specimens using the 6-AM Assay on the Viva-E® analyzer and GC/MS. A specimen that gave a rate equal or greater than the cutoff was interpreted as positive. A specimen that gave a rate less than the cutoff was interpreted as negative.

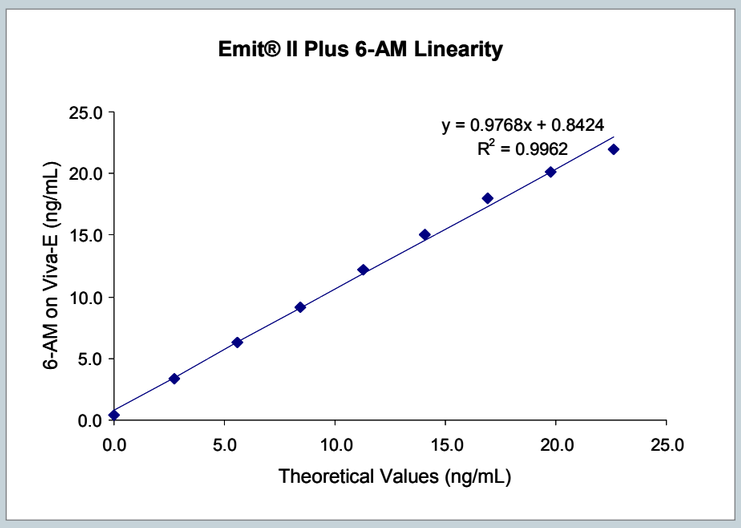
		Reference Method GC/MS	
		+	-
Emit® II Plus 6-AM Assay	+	49	1
	-	0	55

>99% Agreement

Analytical Recovery / Linearity

Recovery was tested by spiked sample analysis on the Viva-E® analyzer. 6-Acetylmorphine was spiked to various levels in urine and compared to nominal values. Linearity was tested by patient sample analysis on the Viva-E® analyzer.

Spiked 6-AM (ng/mL)	Recovery (ng/mL)	Recovery (%)
0	0.4	N/A
2.5	3.3	132
5	5.7	114
7.5	8.3	111
10	11.0	110
12.5	13.6	109
15	16.3	109
17.5	18.7	107
20	21.7	109



On-instrument Stability

On-board stability was determined quantitatively on the Viva-E® analyzer. The 10 ng/mL cutoff and +/- 25% commercial control levels were assayed over 32 day period. The 6-AM Assay had at least 32 days of stability on the Viva-E® analyzer.

Time (day)		Control 1	Cutoff	Control 2
0 Time	Mean (ng/mL)	6.9	10.6	12.7
	SD	0.11	0.07	0.13
	CV%	1.6	0.7	1.1
32 Days	Mean (ng/mL)	6.8	10.3	13.1
	SD	0.09	0.21	0.19
	CV%	1.3	2.0	1.4
% Change		-1.5%	-2.90%	3.10%

Precision

Repeatability and within-lab precision was determined in qualitative (rate) and semi-quantitative (ng/mL) modes according to CLSI-EP5-A2. Precision was determined by assaying the cutoff and controls on 20 days, 2 runs per day in replicates of 2 (N= 80).

Qualitative Precision (mA/min)

	Level	Mean (mA/min)	Repeatability		Within-Lab	
			SD	%CV	SD	%CV
Control 1	7.5 ng/mL	577.8	1.50	0.27	7.0	1.25
Cutoff	10.0 ng/mL	590.5	1.66	0.28	6.5	1.11
Control 2	12.5 ng/mL	621.9	1.65	0.27	6.0	0.97

Semi- quantitative Precision (ng/mL)

	Level	Mean (ng/mL)	Repeatability		Within-Lab	
			SD	%CV	SD	%CV
Control 1	7.5 ng/mL	8.7	0.12	1.35	0.43	5.05
Cutoff	10.0 ng/mL	11.3	0.14	1.26	0.48	4.23
Control 2	12.5 ng/mL	14.2	0.16	1.14	0.50	3.50

Effect of Endogenous Substances

The following substances do not interfere in the assay when tested at +/- 25% at the cutoff.

Potential Interferent	Concentration Tested
Acetone	1.0 g/dL
Ethanol	1.0 g/dL
Oxalic Acid	0.1 g/dL
Ascorbic Acid	1.5 g/dL
Conjugated Bilirubin	2.0 mg/dL
Unconjugated Bilirubin	2.0 mg/dL
Hemoglobin	115 mg/dL
Glucose	2.0 g/dL
Sodium Chloride	6.0 g/dL
Urea	6.0 g/dL
Creatinine	0.5 g/dL
IgG	0.5 g/dL
Human Serum Albumin	0.5 g/dL
Riboflavin	7.5 mg/dL

Specificity

Concentrations of compounds that produce a negative response vs. the 10 ng/mL cutoff calibrator.

Compounds	Concentration Tested (ng/mL)
Dextromethorphan	100,000
Dihydrocodeine	500,000
Heroin	80
Hydrocodone	300,000
Hydromophone	10,000
Imipramine	200,000
Levorphanol	10,000
Morphine-3-Glucuronide	600,000
Morphine-6-Glucuronide	600,000
Normorphine	100,000
Buprenorphine	1,000,000
Codeine	500,000
Meperidine	800,000
Morphine	100,000
Nalorphine	100,000
Norcodeine	600,000
Oxycodone	400,000
Oxymorphone	80,000
Naloxone	300,000
Naltrexone	300,000

Conclusions

The Emit® II Plus 6-AM Assay, currently under development, is a convenient method for detecting the heroin metabolite (6-acetylmorphine) in urine. It should be a suitable screening method for urine specimens at cutoff level 10 ng/mL for both the qualitative and semi-quantitative analyses. The results on precision, overlap, recovery, sensitivity, specificity, interfering substances and on-instrument stability were found to be acceptable.

*Product under development - not available for sale
Product availability subject to meeting country specific registration and language requirements.

www.siemens.com/diagnostics