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Performance Evaluation of the Emit II Plus Oxycodone, Buprenorphine, and 6-Acetylmorphine Assays on the Atellica DT 250 System

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Background

Oxycodone, buprenorphine, and heroin are members of the opiate class of drugs prescribed for pain relief. All three act as narcotic analgesics and are associated with a high potential for abuse and addiction. These drugs are metabolized by the liver into secondary metabolites that are often pharmacologically active and can be further metabolized into conjugated glucuronides.

The Emit II Plus Oxycodone (Oxy), Buprenorphine (Bup), and 6-Acetylmorphine (6-AM, a primary heroin metabolite) assays can be used to detect the presence of these drugs in human urine at cutoffs of 100 and 300 ng/mL (Oxy), 5 ng/mL (Bup) and 10 ng/mL (6-AM). These assays provide both qualitative and semiquantitative results. Protocols for these assays have been developed on the new Atellica DT 250 System. The data presented here were generated on the Atellica DT 250 System and the Viva-E/V-Twin/Viva-ProE Systems.

The Emit II Plus assays are homogeneous enzyme immunoassays. They are based on competition with drug in the sample and drug labeled with glucose-6-phosphate dehydrogenase (G6PDH) for antibody-binding sites. The unbound drug-enzyme conjugate converts the oxidized nicotinamide adenine dinucleotide (NAD+) in the antibody reagent to NADH, and the change in absorbance can be measured spectrophotometrically. Enzyme activity decreases upon binding to the antibody, allowing drug concentrations in a sample to be measured in terms of G6PDH activity.

Assay reaction

$$\text{AB} + \text{DRUG} + \text{DRUG-G6PDH} \longrightarrow \text{AB-DRUG} + \underset{\text{(inactive)}}{\text{AB-DRUG-G6PDH}} + \underset{\text{(active)}}{\text{DRUG-G6PDH}}$$

$$\text{Glucose-6-phosphate} + \text{NAD+} \xrightarrow{\text{DRUG-G6PDH}} \text{6-phosphogluconolactone} + \underset{\text{(absorbs at 40 nm)}}{\text{NADH}} + \text{H}^+$$

where:

AB = anti-drug antibody

DRUG = drug of interest

DRUG-G6PDH = drug-glucose-6-phosphate dehydrogenase conjugate

The oxycodone and buprenorphine assays can be run as either qualitative or semiquantitative assays. The 6-AM assay is a qualitative assay.

Assay features

Table 1. Features of the Oxycodone, Buprenorphine, & 6-Acetylmorphine assays

	Oxycodone	Buprenorphine	6-Acetylmorphine
Cutoff Level	100 ng/mL 300 ng/mL	5 ng/mL	10 ng/mL
Two reagent system	requires no reconstitution or working reagent preparation	requires no reconstitution or working reagent preparation	R2 requires reconstitution
Calibrators (ng/mL)	0 100 300 500 1000	0 2.5 5 15 25	0 5 10 15 20
Controls	±25% of the cutoff 75 & 125 ng/mL (100 ng/mL cutoff) 225 & 375 ng/mL (300 ng/mL cutoff)	±40% of the cutoff 3 & 7 ng/mL	±25% of the cutoff 7.5 & 12.5 ng/mL
Results	Qualitative & Semiquantitative	Qualitative & Semiquantitative	Qualitative & Semiquantitative
Analyte(s)	Comparable detection for oxycodone & oxymorphone	Comparable detection for buprenorphine & norbuprenorphine	Detection of 6-acetylmorphine (major metabolite of heroin)
Assay measuring interval	50–400 ng/mL (100 ng/mL cutoff) 75–1000 ng/mL (300 ng/mL cutoff)	0.8–25 ng/mL	1.6–20 ng/mL

Materials and Methods

Repeatability and within-lab precision were assessed for all three assays using EMIT II plus calibrators and reagents on a single Atellica DT 250 system per CLSI EP05-A3 guideline. The Assays were evaluated in duplicate in two runs/day using one reagent lot for 20-days (n = 80 samples/evaluation). The oxycodone assay was evaluated both qualitatively and semiquantitatively at two cutoffs (100 ng/mL and 300 ng/mL) and at a single cutoff for the buprenorphine (5 ng/mL) assay. A single qualitative cutoff was evaluated for the 6-AM (10 ng/mL) assay. Samples comprised a control at each assay's cutoff(s) and positive- and negative-controls at least 25% greater than or less than the cutoff.

Recovery was assessed using urine samples on the Atellica DT 250 system. Samples were prepared by spiking known concentrations of oxycodone, buprenorphine, or 6-acetylmorphine into drug-free urine and recovery was compared to nominal values. Spiked samples were evaluated across the assay range for oxycodone, buprenorphine, and 6-acetylmorphine at target levels across the assay range.

A method comparison study was conducted to establish the qualitative concordance between the Atellica DT 250 assay results and the results generated by the predicate Viva-E/V Twin system.
A minimum of 100 patient samples were evaluated for each assay on each of the two systems.
A specimen yielding a rate equal to– or greater than the cutoff was interpreted as positive.
A specimen yielding a rate less than the cutoff was interpreted as negative.

On-instrument stability was determined quantitatively using a single reagent lot on the Atellica DT 250 system in alignment with the CLSI EP25 ED2:2003 guideline. The cutoff calibrators and control levels were assayed periodically over a 60-day period for Oxycodone and Buprenorphine, and over a 30-day period for 6-Acetylmorphine.

Results

Precision

Repeatability and within-lab precision were determined in qualitative (rate) and semiquantitative (ng/mL) modes for Oxycodone and Buprenorphine and in qualitative (rate) mode for 6-Acetylmorphine on the Atellica DT 250 system. All three assays demonstrated SDs and %CVs meeting performance acceptance criteria for both repeatability and within-lab precision.

Table 2. Oxycodone qualitative precision (n=80 for each cutoff level).

Sample	Level (ng/mL)	Mean (mA/min)	Repeatability		Within Lab	
			SD	%CV	SD	%CV
100 ng/mL cutoff						
Negative Control	75	374	1.8	0.5	3.9	1.1
Cutoff	100	392	1.8	0.4	3.9	1.0
Positive Control	125	415	2.5	0.6	4.8	1.2
300 ng/mL cutoff						
Negative Control	225	390	2.5	0.6	4.6	1.1
Cutoff	300	414	2.0	0.5	4.6	1.1
Positive Control	375	440	1.6	0.4	4.6	1.0

Table 3. Oxycodone semiquantitative precision (n=80 for each cutoff level).

Sample	Level (ng/mL)	Mean (ng/mL)	Repeatability		Within Lab	
			SD	%CV	SD	%CV
100 ng/mL cutoff						
Negative Control	75	83	1.7	2.0	2.5	3.0
Cutoff	100	99	1.7	1.7	2.7	2.7
Positive Control	125	123	2.8	2.3	3.9	3.2
300 ng/mL cutoff						
Negative Control	225	210	7.5	3.6	10.6	5.0
Cutoff	300	286	6.5	2.3	12.0	4.2
Positive Control	375	380	6.4	1.7	15.0	3.9

Table 4. Buprenorphine qualitative precision

Sample	Level (ng/mL)	Mean (mA/min)	Repeatability		Within Lab	
			SD	%CV	SD	%CV
Negative Control	3	320	1.6	0.5	2.1	0.7
Cutoff	5	344	1.6	0.5	2.3	0.7
Positive Control	7	364	2.3	0.6	3.0	0.8

Table 5. Buprenorphine semiquantitative precision

Sample	Level (ng/mL)	Mean (ng/mL)	Repeatability		Within Lab	
			SD	%CV	SD	%CV
Negative Control	3	3.1	0.12	3.9	0.17	5.5
Cutoff	5	4.9	0.17	3.5	0.20	4.1
Positive Control	7	6.4	0.15	2.3	0.21	3.3

Table 6. 6-Acetylmorphine qualitative precision

Sample	Level (ng/mL)	Mean (mA/min)	Repeatability		Within Lab	
			SD	%CV	SD	%CV
Negative Control	7.5	473	2.3	0.5	6.1	1.3
Cutoff	10.0	496	2.1	0.4	5.6	1.1
Positive Control	12.5	514	1.7	0.3	4.6	0.9

Recovery

All three assays meet performance acceptance criteria for sample recovery.

Table 7. Oxycodone Sample Recovery

Oxycodone 100 ng/mL Cutoff			Oxycodone 300 ng/mL Cutoff		
Target Concentration (ng/mL)	Mean Recovery (ng/mL)	Mean Recovery (%)	Target Concentration (ng/mL)	Mean Recovery (ng/mL)	Mean Recovery (%)
50	52	104	100	104	104
75	74	99	200	215	108
100	94	94	225	226	100
125	113	90	300	332	111
300	228	114	400	412	103
400	298	99	500	565	113
400	378	95	600	658	110
			800	810	101
			1000	920	92

Table 8. Buprenorphine and 6-Acetylmorphine Sample Recovery

Buprenorphine 5 ng/mL Cutoff			6-Acetylmorphine 10 ng/mL Cutoff		
Target Concentration (ng/mL)	Mean Recovery (ng/mL)	Mean Recovery (%)	Target Concentration (ng/mL)	Mean Recovery (ng/mL)	Mean Recovery (%)
2.0	2.1	105	2.5	2.8	112
3.0	3.1	103	5.0	5.0	100
4.0	3.9	98	7.5	7.2	96
5.0	4.7	94	10.0	9.4	94
8.0	7.7	96	12.5	11.5	92
12.0	11.0	92	15.0	14.0	93
18.0	17.7	98	17.5	16.4	94
22.0	21.5	98	18.0	17.2	96
25.0	23.4	94			

Method Comparison

The percent agreement was 100% between the assays on the Atellica DT 250 and the Viva systems, which exceeded the predefined minimal concordance.

Table 9. Method comparison concordance tables for Oxycodone, Buprenorphine, and 6-Acetylmorphine on the Atellica DT 250 and Viva Systems

A. Oxycodone 100 ng/mL cutoff (n = 107 native samples)

		Viva-E	
		Neg	Pos
Atellica DT 250	Neg	39	0
	Pos	0	68

Agreement = 100%

B. Oxycodone 300 ng/mL cutoff (n = 112 native samples)

		Viva-E	
		Neg	Pos
Atellica DT 250	Neg	65	0
	Pos	0	47

Agreement = 100%

C. Buprenorphine (n = 108 native samples)

		Viva-E/V-Twin	
		Neg	Pos
Atellica DT 250	Neg	53	0
	Pos	0	55

Agreement = 100%

D. 6-Acetylmorphine (n = 111 native samples)

		Viva-E	
		Neg	Pos
Atellica DT 250	Neg	56	0
	Pos	0	55

Agreement = 100%

On-instrument stability

The Oxycodone and Buprenorphine assays demonstrated at least 56 days of stability on the Atellica DT 250 System, and the 6-Acetylmorphine assay demonstrated at least 28 days of stability on the Atellica DT 250 System.

Conclusions

The Emit II Plus Oxycodone, Buprenorphine, and 6-Acetlymorphine assays on the new Atellica DT 250 System are suitable screening methods for urine specimens for both qualitative and semiquantitative analyses. These assays meet all performance acceptance criteria when tested for precision, recovery, method comparison, and on-instrument stability.

All CLSI Guidelines can be found on the CLSI website: https://clsi.org

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