PRELIMINARY PERFORMANCE OF THE ARK™ HIGH SENSITIVITY OPIATES ASSAY ON THE BECKMAN COULTER® AU680

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Introduction

ABSTRACT

Background

Three separate homogeneous immunoassays are commercially available for the detection of Morphine/Codeine, Hydrocodone/Hydromorphone and Oxycodone/Oxymorphone. A new High Sensitivity (HS) Opiates Assay for human urine screening has been developed by ARK Diagnostics, Inc. The ARK™ HS Opiates Assay is designed to detect Morphine/Codeine, Hydrocodone/Hydromorphone and Oxycodone/Oxymorphone in a single assay when calibrated with dihydrocodeine at a cutoff level of 300 ng/mL. The benefit provided by this assay is the consolidation of three assays into one, thereby minimizing cost, labor, and eliminating the need for multiple channels on the analyzer. The assay consists of ready-to-use liquid reagents that provide qualitative and semi-quantitative results. The data presented in this study was generated on the Beckman Coulter AU680 System.

Methods

The ARK™ HS Opiates Assay is a liquid stable, homogeneous enzyme immunoassay, intended for the qualitative and/or semi-quantitative determination of opiates in human urine at a cutoff concentration of 300 ng/mL dihydrocodeine on automated clinical chemistry analyzers. Two reagents, calibrators (0, 300, 1000, 2000, and 4000 ng/mL) and controls (225 and 375 ng/mL) compose the test system. The 300 ng/mL calibrator is the cutoff for distinguishing "positive" from "negative" samples. Fifty negative and 44 opiates positive specimens from a pain clinic were analyzed and the results compared to those of LC-MS/MS. Precision over 5 days, histogram overlap analysis of controls and cutoff, analytical recovery and linearity, limit of blank and detection, and specificity (cross-reactivity with opioids) were evaluated on the Beckman Coulter AU680. Analytical recovery was studied by spiking dihydrocodeine into human urine at levels that span the assay range (50-4000 ng/mL).

Method Comparison

Fifty negative and 44 opiates-positive specimens from a pain clinic were analyzed qualitatively and semi-quantitatively by ARK™ HS Opiates Assay and by LC-MS/MS. The ARK™ HS Opiates Assay used 300 ng/mL dihydrocodeine as the cutoff concentration. Results showed overall agreement 100.0% clinical specificity and 95.2% clinical sensitivity.

ARK™ HS Opiates		LC-MS/MS	
		(+)	(-)
	(+)	42	0
	(-)	2*	50

* Two (2) samples, positive by LC-MS/MS and negative by ARK™ HS Opiates Assay with the 300 ng/mL Cutoff, contain morphine below 50 ng/mL by LC-MS/MS.

Precision

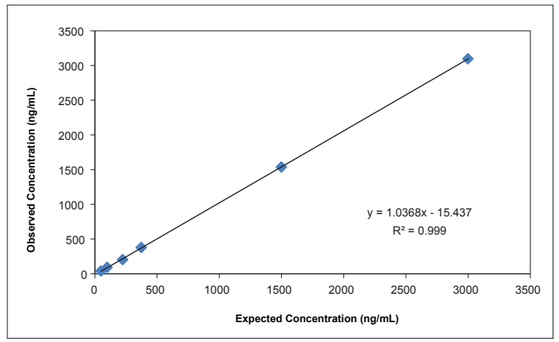
Controls (225 and 375 ng/mL) and Cutoff (300 ng/mL) were tested in quadruplicate twice per day for 5 days (N=40). Semi-quantitative mode of analysis was evaluated. Precision data were calculated according to the Clinical Laboratory Standards Guideline Protocol EP05-A3.

Samples N=40		Within Run		Total	
Dihydrocodeine (ng/mL)	Mean (ng/mL)	SD	CV (%)	SD	CV (%)
225	226.3	8.2	3.6	13.3	5.9
300	301.3	13.0	4.3	13.3	4.4
375	380.6	15.9	4.2	21.3	5.6

Analytical Recovery and Linearity

Analytical recovery and linearity were performed by adding concentrated dihydrocodeine into drug-free negative urine. Test sample concentrations were 50, 100, 225, 375, 1500 and 3000 ng/mL. Semi-quantitative percent nominal values ranged from 81.7 % to 103.2 %.

Target (ng/mL)	Mean (ng/mL)	SD	CV (%)	Recovery (%)
50	40.9	3.8	9.2	81.7
100	92.3	3.9	4.2	92.3
225	204.9	8.9	4.3	91.0
375	379.5	11.0	2.9	101.2
1500	1536.5	73.5	4.8	102.4
3000	3096.6	113.4	3.7	103.2



Specificity – Target Opiate Cross-reactants

The seven listed opiates were prepared in drug-free negative urine. Their corresponding concentration approximately equivalent to the 300 ng/mL dihydrocodeine cutoff was investigated using a dose-response curve.

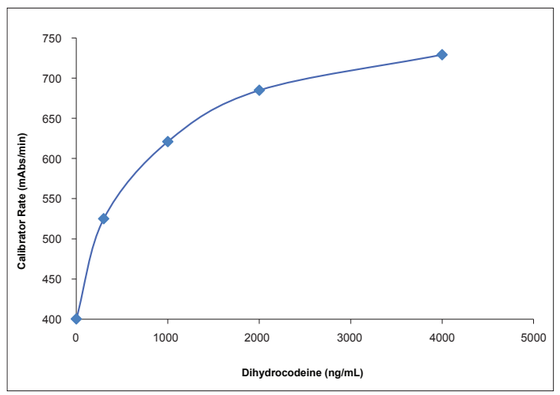
Compound	ng/mL	Compound	ng/mL
Hydromorphone	45	Morphine	110
Hydrocodone	47	Codeine	260
Oxycodone	48	Morphine-6-β-Glucuronide	346
Oxymorphone	48	Oxymorphone-3-β-Glucuronide	639
Levorphanol	100	Morphine-3-β-Glucuronide	676
Hydromorphone-3-β-Glucuronide	106		

Specificity – Opioid Cross-reactivity

All opioid compounds tested were added to drug-free negative urine. If 100,000 ng/mL produced a positive result, their corresponding concentration approximately equivalent to the 300 ng/mL dihydrocodeine cutoff was investigated using a dose-response curve.

Compound	ng/mL	Compound	ng/mL
Ethyl morphine	209	Noroxymorphone	40,648
6-Acetylmorphine	244	Nalorphine	50,000
Diacetylmorphine (heroin)	265	Naloxone	50,000
6-Acetylcodeine	548	Dextromethorphan	>100,000
Thebaine	3,228	Naloxegol	>100,000
Norhydrocodone	15,789	Naltrexone	>100,000
Noroxycodone	31,444	Normorphine	>100,000

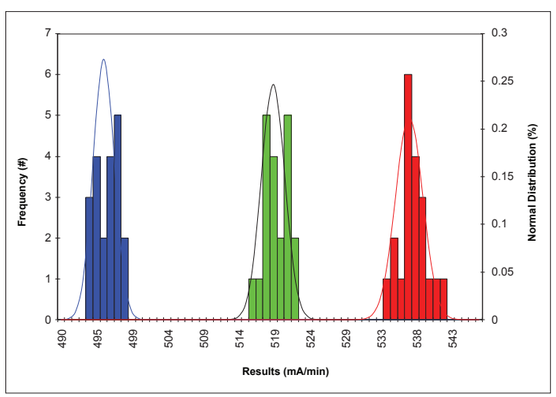
Semi-Quantitative Calibration Curve



ARK™ HS Opiates Assay Calibration Range: 0 to 4000 ng/mL

Histogram Overlap Analysis (Qualitative Analysis)

Frequency of distribution of dihydrocodeine values for each sample is shown by histogram analysis. Twenty (20) replicates each of Negative Control (225 ng/mL), Cutoff Calibrator (300 ng/mL), and Positive Control (375 ng/mL) were assayed together in a single run. The distributions of measurements did not overlap.



Limit of Blank and Detection

The following characteristics were determined according to CLSI EP17-A2. The LoB and LoD were evaluated by testing 60 replicates of drug-free negative urine (BLANK) and 60 replicates of 10.00 ng/mL dihydrocodeine. The LoB was determined using the non-parametric method.

The LoD was calculated using the following formula, where μ is the mean of the blank measurement, and SD refers to the standard deviation of the blank measurement. The constant used to calculate the LoD is 1.645/(1-(1/4*f))), where f is the degrees of freedom of the estimated standard deviation.
In this case, a value of 1.673 was used to calculate LoD.

Limit of Detection (LoD) = LoB + 1.673 SD

The grand mean, root mean square standard deviation (RMS SD), LoB, and LoD results are summarized in the table below.

Nominal (ng/mL)	Mean (ng/mL)	RMS SD	Limit of Blank (ng/mL)	Limit of Detection (ng/mL)
0	0.66	0.59	1.46	5.59
10	5.64	2.47		

Conclusions

The ARK HS Opiate Assay on the Beckman Coulter AU680 System is a sensitive opiates screening method for urine specimens at a cutoff level of 300 ng/mL, providing qualitative and semi-quantitative analysis of morphine, codeine, hydrocodone, hydromorphone, oxycodone, and oxymorphone in a single assay.

REGULATORY STATUS

In Development