

PERFORMANCE EVALUATION OF SECOND-GENERATION ARK URINE DRUG TESTING ASSAYS ON THE SIEMENS ATELICA DT 250 ANALYZER

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INTRODUCTION

The ARK™ Fentanyl II, Ethyl Glucuronide II, and Ketamine II Assays are homogeneous immunoassays intended for the qualitative detection of their respective analytes in human urine using automated clinical chemistry analyzers. The Ethyl Glucuronide II and Ketamine II Assays also support semi-quantitative estimation to aid specimen dilution for confirmatory testing and laboratory quality control. All assays provide preliminary analytical results that require confirmation by GC/MS or LC-MS/MS.

Enhancements to immunoassays for fentanyl, ethyl glucuronide, and ketamine yield superior sensitivity, specificity, help to improve product attributes and reagent consistency. Second-generation homogeneous enzyme immunoassays are designed to enhance analytical performance, reagent consistency, and overall robustness in high throughput urine drug screening.

OBJECTIVES

This study focused on the product improvement of three ARK Diagnostics homogeneous immunoassays for Fentanyl, Ethyl Glucuronide and Ketamine and their implementation on the Siemens Atellica DT 250 Analyzer. The objective was to determine the analytical performance of each ARK homogeneous immunoassay on the Siemens Atellica DT 250 analyzer and compare their performance with that obtained on the Beckman AU680 analyzer.

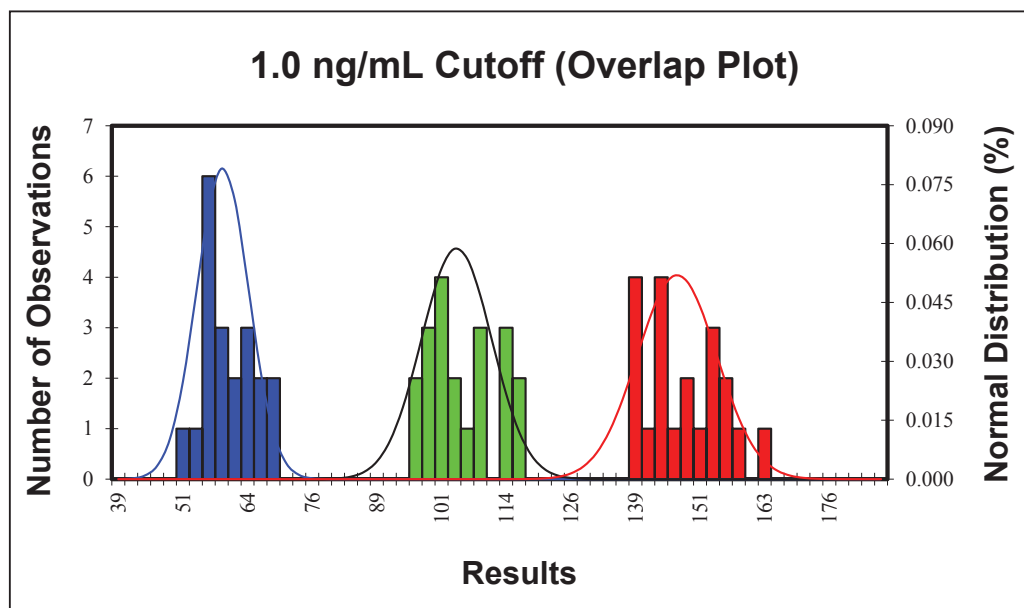
METHODS

Studies were performed at ARK Diagnostics (Fremont, CA) and followed Clinical Laboratory and Standards Institute (CLSI) guidelines. CLSI creates consensus based laboratory standards and guidance recognized by laboratories, accreditors, and government agencies to improve testing, speed up product commercialization, and streamline regulatory clearance. Validation testing included precision, method comparison (vs. Beckman AU680 analyzer) with at least 50 positive and 50 negative specimens, analytical recovery, sample carryover, and onboard reagent stability.

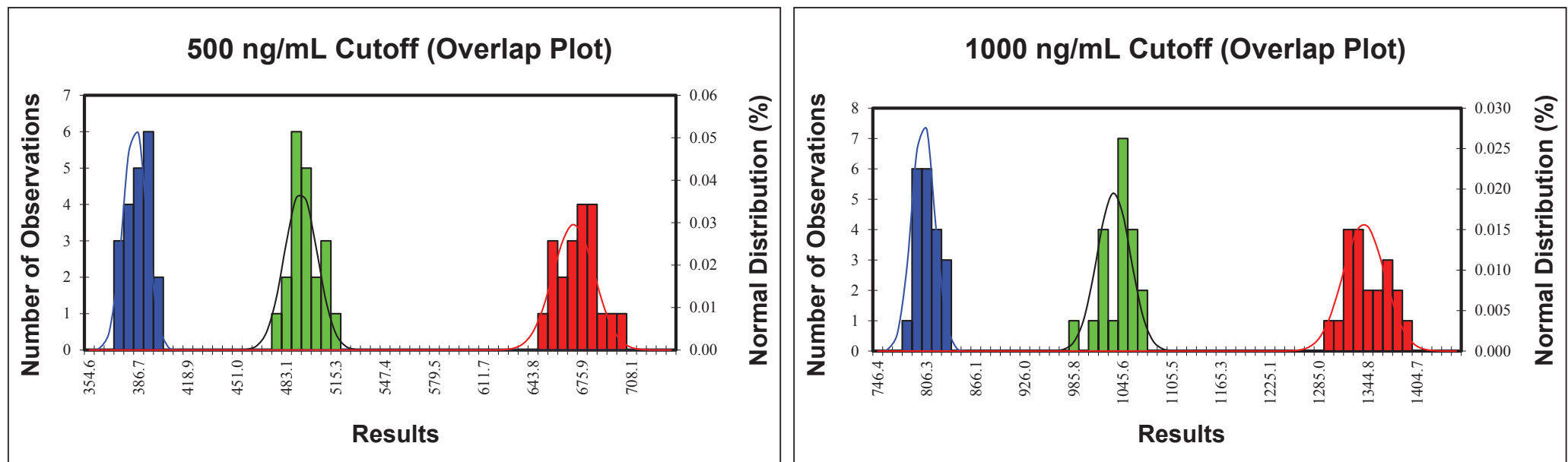
HISTOGRAM OVERLAP ANALYSIS

Histogram analysis was used to evaluate the frequency distribution of values for each assay-specific Negative Control, Cutoff Calibrator, and Positive Control. Twenty replicates of each sample were tested. The resulting measurement distributions showed no overlap among the Negative Control, Cutoff Calibrator, and Positive Control for all three assays. The ARK Fentanyl II Assay was tested qualitatively, with the 1.0 ng/mL cutoff assigned as 100 normalized units. The ARK Ethyl Glucuronide II Assay and ARK Ketamine II Assay were tested semi-quantitatively.

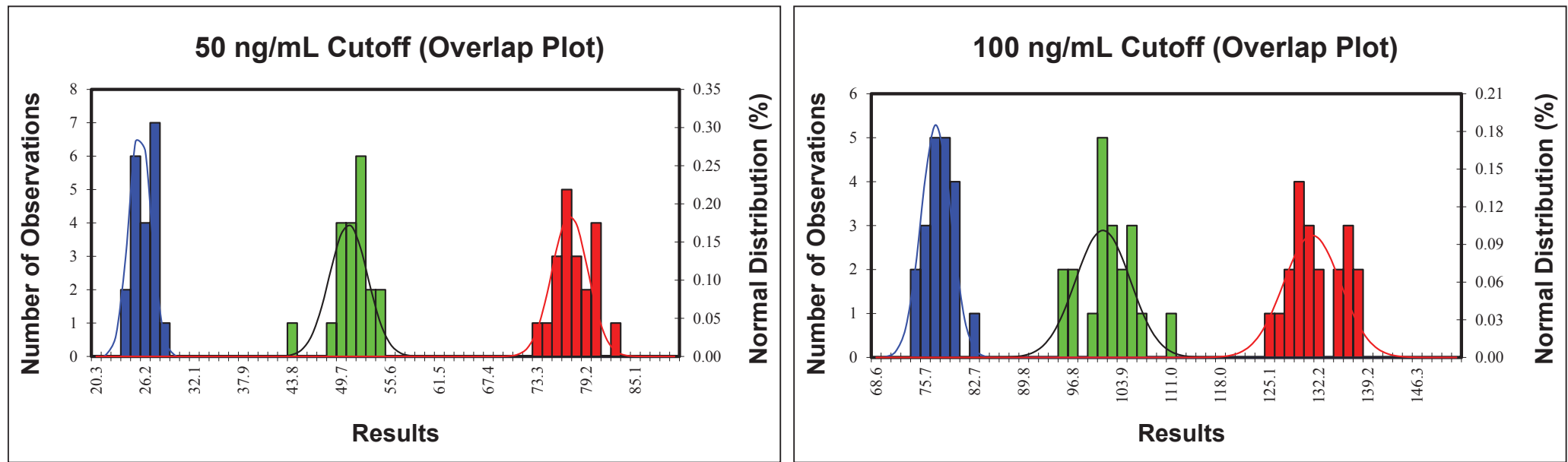
ARK Fentanyl II Assay



ARK Ethyl Glucuronide II Assay



ARK Ketamine II Assay



PRECISION

Negative pooled human urine samples were separately supplemented with the target analyte to prepare analyte-specific sample sets spanning concentrations of ±25%, ±50%, ±75%, and ±100% around the respective cutoff concentration for each assay. Mean, standard deviation (SD), and coefficients of variation (%CV) for repeatability (within-run) and total within-laboratory precision were calculated.

ARK Fentanyl II Assay

Samples were tested qualitatively in quadruplicate twice a day for 5 days. The 1.0 ng/mL cutoff was assigned as 100 normalized units, such that qualitative results ≥100 were positive and qualitative results <100 were negative. Precision met acceptance criteria, with correct qualitative results with >95% confidence intervals.

Fentanyl (ng/mL)	N	Mean Qual. Units	Precision Results	Repeatability (Within-Run Precision)		Within-Laboratory (Total Precision)	
				SD	CV (%)	SD	CV (%)
0.00	40	-5.28	40 Negative	5.764	N/A	6.078	N/A
0.25	40	20.68	40 Negative	6.152	29.8	7.540	36.5
0.50	40	44.95	40 Negative	6.699	14.9	7.492	16.7
0.75	40	71.48	40 Negative	7.542	10.6	7.678	10.7
100 (Cutoff)	40	91.68	36 Negative, 4 Positive	6.171	6.7	6.852	7.5
1.25	40	118.83	39 Positive, 1 Negative	11.305	9.5	11.967	10.1
1.50	40	136.47	40 Positive	8.652	6.3	9.315	6.8
1.75	40	156.95	40 Positive	6.355	4.0	7.038	4.5
2.00	40	177.64	40 Positive	7.302	4.1	7.426	4.2

ARK Ethyl Glucuronide II Assay

Samples were tested semi-quantitatively in duplicate twice a day for 20 days. Total precision ranged from 2.2% to 6.3% CV.

Ethyl Glucuronide (ng/mL)	N	Mean (ng/mL)	Repeatability (Within-Run Precision)		Between Day		Within-Laboratory (Total Precision)	
			SD	CV (%)	SD	CV (%)	SD	CV (%)
125.0	80	119.13	7.170	6.0	3.684	3.1	7.449	6.3
250.0	80	259.78	7.758	3.0	6.719	2.6	9.642	3.7
375.0	80	391.63	8.003	2.0	4.838	1.2	8.674	2.2
500.0 (Cutoff)	80	526.92	8.458	1.6	8.754	1.7	11.781	2.2
625.0	80	660.85	12.552	1.9	13.281	2.0	17.427	2.6
750.0	80	786.77	18.016	2.3	13.292	1.7	21.255	2.7
875.0	80	937.82	20.350	2.2	18.994	2.0	26.569	2.8
1000.0 (Cutoff)	80	1058.90	24.523	2.3	18.828	1.8	29.249	2.8
1250.0	80	1333.24	31.916	2.4	25.916	1.9	41.154	3.1
1500.0	80	1569.93	39.357	2.5	42.168	2.7	54.907	3.5
1750.0	80	1849.35	58.907	3.2	50.568	2.7	71.870	3.9

ARK Ketamine II Assay

Samples were tested semi-quantitatively in duplicate twice a day for 20 days. Total precision ranged from 3.3% to 5.6% CV for concentrations ≥50.0 ng/mL.

Ketamine (ng/mL)	N	Mean (ng/mL)	Repeatability (Within-Run Precision)		Between Day		Within-Laboratory (Total Precision)	
			SD	CV (%)	SD	CV (%)	SD	CV (%)
12.5	80	13.08	1.208	9.2	1.624	12.4	1.633	12.5
25.0	80	25.01	1.553	6.2	2.140	8.6	2.278	9.1
37.5	80	38.45	2.190	5.7	2.442	6.4	2.448	6.4
50.0 (Cutoff)	80	50.28	1.852	3.7	2.548	5.1	2.561	5.1
62.5	80	61.30	2.660	4.3	3.381	5.5	3.395	5.5
75.0	80	73.32	3.836	5.2	4.113	5.6	4.119	5.6
87.5	80	84.49	3.058	3.6	4.104	4.9	4.125	4.9
100.0 (Cutoff)	80	98.61	4.245	4.3	4.864	4.9	4.881	4.9
125.0	80	124.39	4.533	3.6	5.730	4.6	5.749	4.6
150.0	80	151.48	4.752	3.1	6.099	4.0	6.115	4.0
175.0	80	176.83	6.230	3.5	6.467	3.7	6.780	3.8
200.0	80	205.26	5.995	2.9	6.790	3.3	6.814	3.3

ANALYTICAL RECOVERY

Negative pooled human urine samples were separately supplemented with the target analyte to prepare analyte-specific sample sets spanning the assay range for each assay. Two separate semi-quantitative analytical runs were performed for each assay, with three replicates of each concentration per run (N=6). Results were averaged and compared to the theoretical target concentration.

ARK Fentanyl II Assay

Because the ARK Fentanyl II Assay is a qualitative assay, analytical recovery was not tested.

ARK Ethyl Glucuronide II Assay

Recovery ranged from 103.1% to 109.0% for concentrations tested from 200.0 to 1400.0 ng/mL.

Ethyl Glucuronide (ng/mL)	Mean (ng/mL)	SD	CV (%)	Recovery (%)	N
200.0	206.48	12.027	5.8	103.2	6
400.0	412.27	10.008	2.4	103.1	6
800.0	848.93	11.569	1.4	106.1	6
1000.0	1086.65	32.534	3.0	108.7	6
1200.0	1303.58	21.154	1.6	108.6	6
1400.0	1526.45	14.246	0.9	109.0	6

ARK Ketamine II Assay

Recovery ranged from 104.8% to 112.0% for concentrations tested from 20.0 to 400.0 ng/mL.

Ketamine (ng/mL)	Mean (ng/mL)	SD	CV (%)	Recovery (%)	N
20.0	22.40	1.470	6.6	112.0	6
50.0	53.39	1.145	2.1	106.8	6
100.0	104.78	2.951	2.8	104.8	6
200.0	217.80	7.662	3.5	108.9	6
300.0	319.56	10.617	3.3	106.5	6
400.0	443.37	25.073	5.7	110.8	6

METHOD COMPARISON

Unaltered, de-identified clinical human urine specimens were analyzed for each assay. Results obtained by the Siemens Atellica DT 250 analyzer were compared to results from the reference Beckman Coulter AU680 analyzer. Percent sensitivity (true positive) and percent specificity (true negative) were calculated.

ARK Fentanyl II Assay

Method comparison showed 100.0% sensitivity and 100.0% specificity at the 1.0 ng/mL cutoff.

	Beckman Coulter AU680 (+)	Beckman Coulter AU680 (-)	Sensitivity	Specificity
Siemens Atellica DT 250	(+)	70	100.0%	100.0%
	(-)	0		

ARK Ethyl Glucuronide II Assay

Method comparison showed 100.0% sensitivity and 100.0% specificity at both the 500.0 ng/mL and 1000.0 ng/mL cutoffs.

	Beckman Coulter AU680 (+)	Beckman Coulter AU680 (-)	Sensitivity	Specificity
500.0 ng/mL Cutoff	(+)	80	100.0%	100.0%
Siemens Atellica DT 250	(-)	0		
1000.0 ng/mL Cutoff	(+)	58	100.0%	100.0%
Siemens Atellica DT 250	(-)	0		

ARK Ketamine II Assay

Method comparison showed 100.0% sensitivity and 100.0% specificity at the 50.0 ng/mL cutoff and 100.0% sensitivity and 98.7% specificity at the 100.0 ng/mL cutoff.

	Beckman Coulter AU680 (+)	Beckman Coulter AU680 (-)	Sensitivity	Specificity
50.0 ng/mL Cutoff	(+)	62	100.0%	100.0%
Siemens Atellica DT 250	(-)	0		
100.0 ng/mL Cutoff	(+)	56	100.0%	98.7%
Siemens Atellica DT 250	(-)	0		

*The false positive sample was near the 100.0 ng/mL cutoff concentration, measuring at 99.6 ng/mL on the AU680 and 105.8 ng/mL on the Atellica DT 250.

SAMPLE CARRYOVER

Negative pooled human urine samples were separately supplemented with the assay-specific target analyte to prepare high concentration carryover samples for each assay. Carryover was assessed by testing five consecutive "H₁H₂H₃L₁L₂L₃" sample sequences, where each "H" represents a replicate of the high-concentration sample and each "L" represents a replicate of the low-concentration (negative) sample. Carryover was considered undetectable if all "L" negative sample replicates did not produce a false positive relative to the assay-specific cutoff.

Assay	Assay Cutoff (ng/mL)	Carryover Result
ARK Fentanyl II Assay	1.0	No carryover up to 3,000 ng/mL
ARK Ethyl Glucuronide II Assay	500.0 and 1000.0	No carryover up to 100,000 ng/mL
ARK Ketamine II Assay	50.0 and 100.0	No carryover up to 100,000 ng/mL

ONBOARD REAGENT STABILITY

Fresh reagents were stored onboard the Atellica DT 250 throughout the study. Reagents were uncapped at the beginning of the day and capped at the end of the day. Quality controls were tested in duplicate, and control value drift over time was assessed using linear regression analysis with an acceptance criterion of ±10%. Onboard reagent stability was determined to be up to 30 days for the ARK Fentanyl II Assay, ARK Ethyl Glucuronide II Assay, and ARK Ketamine II Assay.

DISCUSSION

The second-generation ARK urine drug testing assays demonstrated robust analytical performance on the Siemens Atellica DT 250 analyzer, yielding results comparable to the reference platform. These findings support the use of ARK's assays on the Atellica DT 250 analyzer for labs needing high quality, accurate drug screening for fentanyl, ethyl glucuronide or ketamine.

REGULATORY STATUS

ARK Fentanyl II Assay

- FDA cleared
- CE marked under IVDR

ARK Ethyl Glucuronide II Assay

- For Criminal Justice and Forensic Use Only in the U.S.
- CE marked under IVDR

ARK Ketamine II Assay

- For Criminal Justice and Forensic Use Only in the U.S.
- CE marked under IVDR

