

PERFORMANCE EVALUATION OF THE ARK XYLAZINE ASSAY ON THE SIEMENS ATELICA DT 250 ANALYZER

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BACKGROUND

Xylazine is increasingly detected as an adulterant in illicit drug supplies and is associated with significant public health and forensic concerns. The ARK™ Xylazine Assay is a homogeneous enzyme immunoassay intended for the qualitative detection and/or semi-quantitative estimation of xylazine and its metabolites in human urine at a cutoff concentration of 10 ng/mL. The assay is intended for use on automated clinical chemistry analyzers as a rapid screening test. Semi-quantitative results may be used to determine appropriate specimen dilution for confirmatory testing or to establish laboratory quality control procedures. As with all screening immunoassays, positive results are preliminary and require confirmation by a more specific method, such as GC/MS or LC-MS/MS.

OBJECTIVES

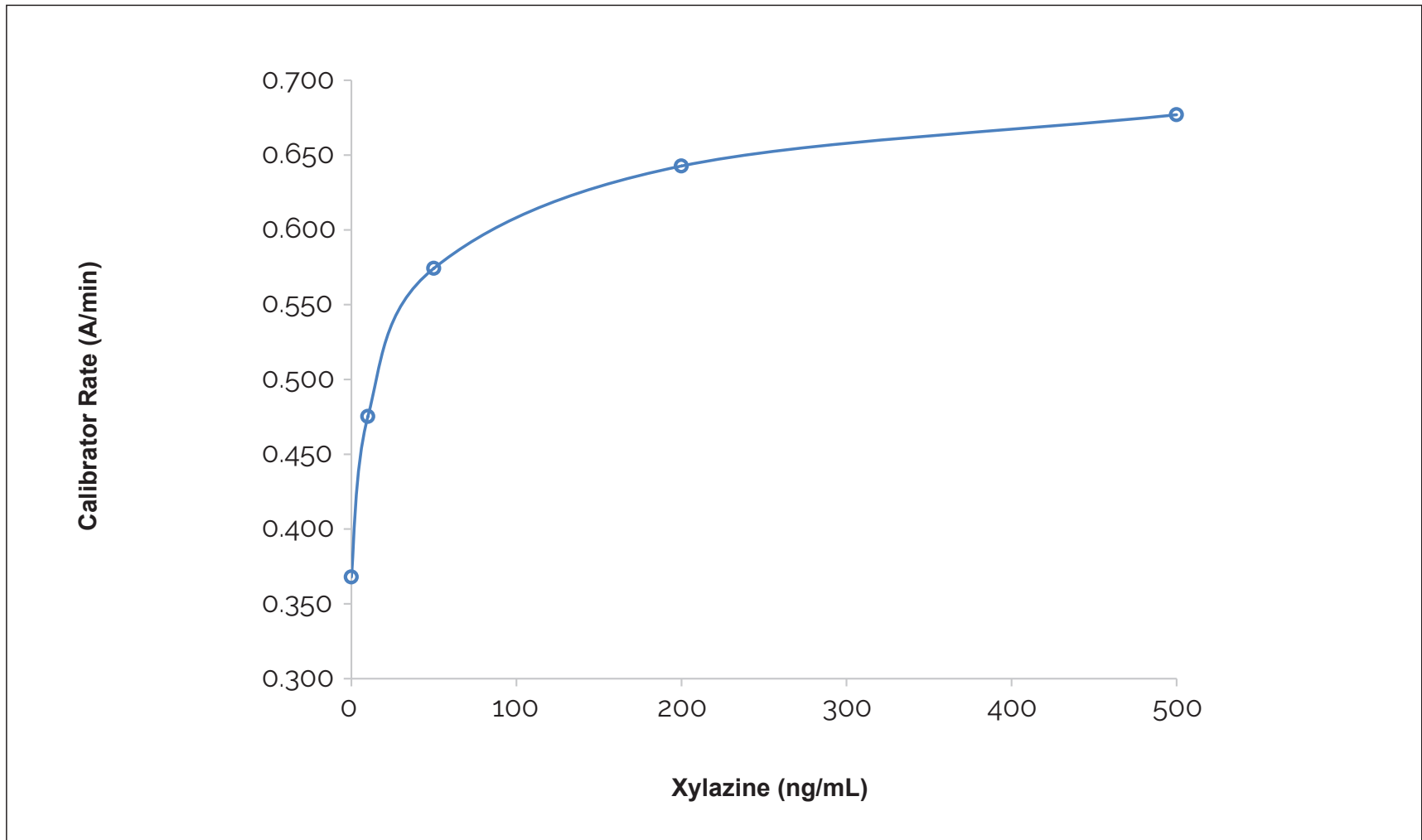
This study focused on the implementation of the ARK Xylazine Assay on the Siemens Atellica® DT 250 analyzer. The objective was to determine the analytical performance of the ARK Xylazine Assay on the Siemens Atellica DT 250 analyzer and compare its performance with that obtained on the Beckman Coulter AU 480 analyzer.

METHODS

Performance studies were conducted 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, analytical recovery, method comparison, high sample dilution, sample carryover, and calibration curve and onboard reagent stability. Following CLSI guidelines, precision was assessed using a five-day study design. Analytical recovery was evaluated using drug free urine samples spiked with certified xylazine reference material across the assay range. Method comparison included 60 negative and 50 positive patient specimens according to the cutoff concentration analyzed using both the Atellica DT 250 analyzer and the reference Beckman Coulter AU480 analyzer.

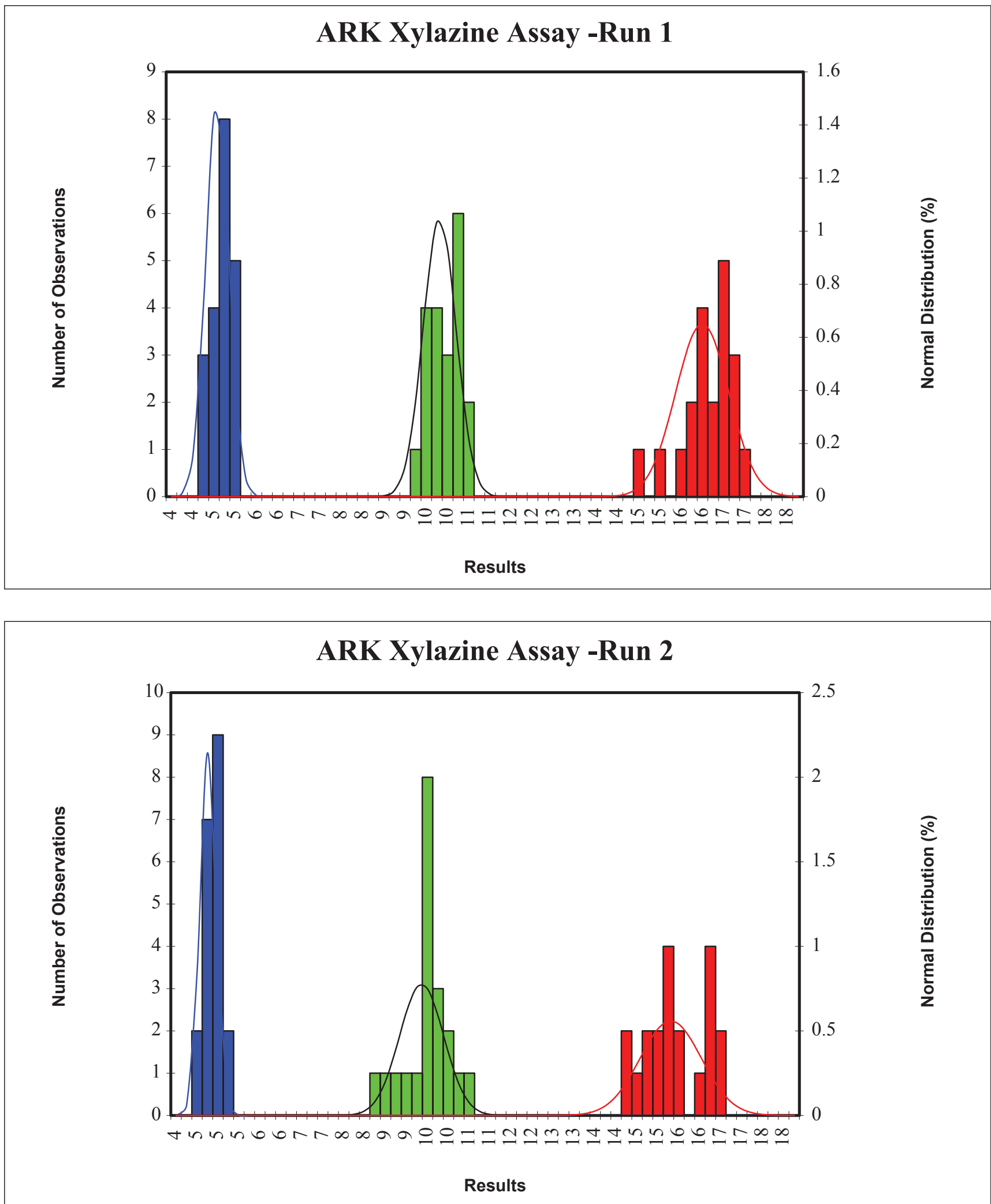
CALIBRATION CURVE

The assay system uses 5 calibrators which contain xylazine at concentrations of 0, 10, 50, 200, and 500 ng/mL.



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. Two analytical runs of twenty replicates for each QC sample (Low control, Cutoff calibrator, and High Control at 5, 10, and 15 ng/mL respectively) were assayed. The resulting measurement distributions showed no overlap among the Negative Control, Cutoff Calibrator, and Positive Control.



PRECISION

Precision was determined as described in CLSI Protocol EP5-A3. Negative pooled human urine samples were supplemented with xylazine to prepare samples spanning concentrations of ±25%, ±50%, ±75%, and ±100% around the cutoff concentration. Each sample was assayed in quadruplicate twice a day for 5 days for a total of 40 replicates. Mean determinations of xylazine, standard deviations (SD), and coefficients of variation (%CV) for repeatability (within-run) and total within-laboratory precision were calculated.

Sample	N	Mean (ng/mL)	Within Run		Between Day		Total (Within Lab)	
			SD	CV (%)	SD	CV (%)	SD	CV (%)
2.5 ng/mL	40	2.09	0.091	4.4	0.100	4.8	0.101	4.8
5.0 ng/mL	40	4.96	0.180	3.6	0.183	3.7	0.184	3.7
7.5 ng/mL	40	7.98	0.303	3.8	0.287	3.6	0.314	3.9
10.0 ng/mL	40	10.92	0.380	3.5	0.441	4.0	0.448	4.1
12.5 ng/mL	40	13.99	0.415	3.0	0.460	3.3	0.498	3.6
15.0 ng/mL	40	16.19	0.750	4.6	0.719	4.4	0.750	4.6
17.5 ng/mL	40	19.30	0.699	3.6	0.707	3.7	0.732	3.8
20.0 ng/mL	40	21.71	0.879	4.0	1.027	4.7	1.108	5.1

ANALYTICAL RECOVERY

Negative pooled human urine was supplemented with xylazine drug at 6.0, 9.0, 12.0, 20.0, 60.0, 150.0, and 400.0 ng/mL. Two analytical runs of three replicates of each sample were assayed. Results of the six replicates were averaged and compared to the theoretical target concentration. Percent recovery was calculated.

Target (ng/mL)	Mean (ng/mL)	SD	CV (%)	Recovery (%)
6.0	5.93	0.216	3.6	98.8
9.0	9.17	0.238	2.6	101.9
12.0	12.53	0.328	2.6	104.4
20.0	21.62	0.389	1.8	108.1
60.0	61.30	0.659	1.1	102.2
150.0	139.20	5.277	3.8	92.8
400.0	388.12	24.362	6.3	97.0

HIGH SAMPLE DILUTION

High sample dilution performance was evaluated using manually prepared serial dilutions of pooled negative human urine supplemented with xylazine at concentrations of 100, 1,000, and 10,000 ng/mL. Serial 1:10 dilutions were prepared using ARK Xylazine Calibrator A as the diluent. Each dilution was analyzed in two analytical runs with three replicates per run. The mean of the six replicates was compared with the theoretical target concentration, and percent recovery was calculated.

Concentration (ng/mL)	Dilution Factor	Recovery (%)
100	10 (1:10)	100.6
1,000	100 (1:10 × 2)	104.5
10,000	1000 (1:10 × 3)	101.8

METHOD COMPARISON

A total of 60 negative and 50 positive patient specimens were tested. Samples that exhibited a value equal to or greater than the cutoff calibrator (10 ng/mL xylazine) were considered positive, and samples that exhibited values lower than the cutoff calibrator were considered negative (relative to the respective cutoff). Measurements of the ARK Xylazine Assay performed on the Siemens Atellica DT 250 were compared to the measurements on the reference analyzer, Beckman Coulter AU 480.

		Beckman Coulter AU480	
		(+)	(-)
Siemens Atellica DT 250	(+)	50	1 ¹
	(-)	0	59

¹The false positive sample was near the 10 ng/mL cutoff concentration, measuring 9.4 ng/mL on the AU 480 and 10.2 ng/mL on the Atellica DT 250

Sensitivity (true positive rate) = 100.0%
Specificity (true negative rate) = 98.3%

SAMPLE CARRYOVER

A low sample (negative urine) was used to measure carryover from a potential high sample containing 6,000 ng/mL of xylazine. Five sets of high and low samples were aliquoted in the following sequence: H₁H₂H₃L₁L₂L₃. Each sample cup was tested in a single replicate. No sample carryover was detected.

High Sample	Low Sample	Cutoff	Semi-quantitative Result	Qualitative Result
6,000 ng/mL	0 ng/mL	10 ng/mL	0.06 – 0.40 ng/mL	Negative

CALIBRATION AND ONBOARD REAGENT STABILITY

Fresh reagents were stored on-board the Atellica DT 250 throughout the study. Reagents were uncapped at the beginning of the day and capped at the end of the day. QCs were tested in duplicate at each of these time points. The value of each QC level was considered, if the value of each QC level was not within their established QC limits, the calibration was considered invalid. The established QC limits were set as ±15% around the mean level for the high control (15 ng/mL) and ±1.5 ng/mL for the low control (5 ng/mL). Calibration stability was maintained for up to 14 days and onboard reagent stability for up to 30 days.

RESULTS

Total precision ranged from 3.6% to 5.1% CV for concentrations above 10 ng/mL. Analytical recovery ranged from 92.8% to 108.1% across concentrations of 6.0 to 400.0 ng/mL. Method comparison demonstrated strong qualitative agreement with a sensitivity of 100.0% and specificity of 98.4% relative to the 10 ng/mL cutoff. Dilution studies showed recoveries between 100.6% and 104.5% for samples diluted up to 1:1000. No measurable sample carryover was observed from high concentration samples up to 6000 ng/mL. Calibration stability was maintained for up to 14 days and onboard reagent stability for up to 30 days.

DISCUSSION

The performance data indicate that the ARK Xylazine Assay is highly dependable on the Siemens Atellica DT 250 analyzer, maintaining strong agreement with the reference method. These results support implementation of the assay as an automated screening method for xylazine detection in forensic and toxicology laboratories.

REGULATORY STATUS

For Criminal Justice and Forensic Use Only in the U.S.

