

Achieving lower limits of detection using pre-existing immunoassay based screening techniques

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Abstract

AIT Laboratories has reviewed the need for more sensitive screening assays capable of achieving lower cutoff levels for several commonly encountered drugs using immunoassay based techniques. Immunoassay based screening techniques have traditionally been performed using unmodified, commercially available kits. These kits often lack the sensitivity required to detect low levels of highly potent drugs and toxins in biological specimens. AIT has developed an approach for improving cutoff levels to allow for detection of low, but forensically and clinically relevant, drug levels. While the ultimate goal of the project is to allow for screening of multiple specimen types, the initial feasibility study presented here has focused on urine specimens. Serially dilute calibrators of oxycodone, methadone, and benzodiazepines (nitrazepam), along with appropriate blanks and controls purchased from Microgenics (Freemont, CA) were analyzed using an Abbott Aeroset. The analytical suitability of lower screening cutoffs was evaluated through the replicate analysis (n=20) of calibrators prepared at +/- 25% of the investigated cutoff level. Multiple cutoffs were investigated for each drug class and selected cutoff levels showed excellent precision and accuracy indicating that lower cutoff levels can be achieved. Replicate analysis of oxycodone calibrators at concentrations of 37.5 and 62.5 ng/mL produced %CV's in the range of 4.0-4.7, while analysis of methadone calibrators at concentrations of 75 and 125 ng/mL produced %CV's in the range of 4.0-7.7 and replicate analysis of benzodiazepine (nitrazepam) calibrators at concentrations of 56.25 and 93.75 ng/mL produced %CV's in the range of 4.1-6.2. As a result of these studies, AIT Laboratories now employs an oxycodone screen cutoff of 50 ng/mL (vendor recommends 300 ng/mL), a methadone screen cutoff of 100 ng/mL (vendor recommends 300 ng/mL) and a benzodiazepine screen cutoff of 75 ng/mL (vendor recommends 200 ng/mL). Implementation of lower screen cutoffs will prove beneficial for the detection of highly potent drugs and toxins in biological specimens.

Keywords: Cutoff; immunoassay; screen

Experimental

Reagents

DRI Oxycodone, CEDIA Methadone, and CEDIA Benzodiazepine Assays were obtained from Microgenics (Freemont, CA).

Calibrators

CEDIA Negative, DRI Oxycodone 100 ng/mL, 300 ng/mL, 500 ng/mL, and 1,000 ng/mL Calibrators from Microgenics (Freemont, CA) were used for oxycodone calibration points.

CEDIA Negative, PPX/METD Cutoff (300 ng/mL), PPX/METD High (1,000 ng/mL) Calibrators from Microgenics (Freemont, CA), and a serially diluted Methadone 100 ng/mL (see "Sample Preparation") were used for methadone calibration points.

CEDIA Negative, CEDIA Multi-Drug Secondary (200 ng/mL), Multi-Drug Primary (300 ng/mL), Multi-Drug Intermediate (800 ng/mL), and Multi-Drug High (5,000 ng/mL) Calibrators from Microgenics were used for benzodiazepine calibration points.

Controls

DRI Oxycodone 375 ng/mL, CEDIA MGC Primary DAU (High QC-Methadone 375 ng/mL), and MGC Clinical DAU (High QC-Benzodiazepine 375 ng/mL) Controls from Microgenics (Freemont, CA) were used to dilute to desired concentrations used for validation purposes only.

Instrumentation

All methods were validated using the Abbott Aeroset (Figure 1). Instrument parameters are provided and may be modified accordingly (Tables 1-3).



Figure 1. Abbott Aeroset instrument used for all experiments

Assay name	QKCY
Reaction mode	RATE LIP
Wavelength-Prim/Sec	340-412
Read time-Main	20-24
Standard	S. Vol 10 µL
Reagent 1-QKCY	R. Vol 80 µL
Reagent 2-QKCY	R. Vol 80 µL
Calibration Mode	Linear
Blank/Calibration replicates	2-2
BLK-NEG	S. Vol 10 µL
C1-QKCY 100	S. Vol 10 µL
C2-QKCY 300	S. Vol 10 µL
C3-QKCY 500	S. Vol 10 µL
C4-QKCY 1000	S. Vol 10 µL

Table 1. Instrument parameters used for oxycodone assay

Assay name	METD
Reaction mode	RATE LIP
Wavelength-Prim/Sec	572-660
Read time-Main	30-33
Standard	S. Vol 4 µL
Reagent 1-METD	R. Vol 86 µL
Reagent 2-METD	R. Vol 86 µL
Calibration Mode	Linear
Blank/Calibration replicates	2-2
BLK-NEG	S. Vol 4 µL
C1-METD 100	S. Vol 4 µL
C2-PPX/METD CUTOFF	S. Vol 4 µL
C3-PPX/METD HIGH	S. Vol 4 µL

Table 2. Instrument parameters used for methadone assay

Assay name	BENZ
Reaction mode	RATE LIP
Wavelength-Prim/Sec	572-660
Read time-Main	30-33
Standard	S. Vol 2 µL
Reagent 1-BENZ	R. Vol 87 µL
Reagent 2-BENZ	R. Vol 87 µL
Calibration Mode	Linear
Blank/Calibration replicates	2-2
BLK-NEG	S. Vol 2 µL
C1-SEC (200 ng/mL)	S. Vol 2 µL
C2-PRIMARY (300 ng/mL)	S. Vol 2 µL
C3-INTER (800 ng/mL)	S. Vol 2 µL
C4-HIGH (5000 ng/mL)	S. Vol 2 µL

Table 3. Instrument parameters used for benzodiazepine assay

Sample Preparation

Calibrators

- Methadone 100 ng/mL was prepared by diluting 5 mL of Microgenics PPX/METD Cutoff calibrator with 10 mL of negative synthetic urine. Rotate for 10 minutes.

QCs

- Oxycodone 37.5 ng/mL was prepared by diluting 1.5 mL of Microgenics Oxycodone 375 control with 13.5 mL of synthetic negative urine. Rotate for 10 minutes.

- Oxycodone 62.5 ng/mL was prepared by diluting 2.5 mL of Microgenics Oxycodone 375 control with 12.5 mL of synthetic negative urine. Rotate for 10 minutes.

- Methadone 75 ng/mL was prepared by diluting 5 mL of Microgenics MGC Primary DAU high control with 10 mL of synthetic negative urine. Rotate for 10 minutes.

- Methadone 125 ng/mL was prepared by diluting 3 mL of Microgenics MGC Primary DAU high control with 12 mL of synthetic negative urine. Rotate for 10 minutes.

- Benzodiazepine 56.25 ng/mL was prepared by diluting 2.25 mL of Microgenics MGC Clinical DAU high control with 12.75 mL of negative synthetic urine. Rotate for 10 minutes

- Benzodiazepine 93.75 ng/mL was prepared by diluting 2.25 mL of Microgenics MGC Clinical DAU high control with 12.75 mL of negative synthetic urine. Rotate for 10 minutes.

Methods

-The analytical suitability of lower screening cutoffs was evaluated through the replicate analysis (n=20) of calibrators prepared at +/- 25% of the investigated cutoff level.

- Investigated cutoff levels were 50 ng/mL for oxycodone (Table 4), 100 ng/mL for methadone (Table 5), and 75 ng/mL for benzodiazepines (Table 6).

Results and Discussion

Oxycodone assay	Low QC (37.5 ng/mL)	High QC (62.5 ng/mL)	Methadone assay (100 ng)	Low QC (75 ng/mL)	High QC (125 ng/mL)	Benzodiazepine assay (75 ng/mL cutoff)	Low QC (56.25 ng/mL)	High QC (93.75 ng/mL)
	40.1	65.3		75.0	120.4		56.8	97.6
	44.5	64.2		69.9	125.4		58.7	97.2
	40.1	65.2		74.5	120.7		56.7	100.2
	38.8	69.4		67.2	115.2		52.9	95.1
	40.1	65.0		66.2	121.5		54.1	91.1
	40.2	62.1		74.6	119.1		64.1	94.9
	39.4	73.4		66.0	109.8		61.6	95.5
	37.6	65.7		64.2	117.5		52.6	99.3
	40.3	65.8		76.9	121.4		55.6	95.1
	38.7	64.3		59.6	112.5		57.8	94.5
	40.5	66.4		62.0	110.9		53.8	103.6
	38.9	63.8		62.0	118.2		51.2	90.5
	42.5	61.1		67.7	112.6		59.2	90.1
	35.5	67.1		65.3	110.9		55.1	101.8
	42.8	66.8		68.8	109.9		50.9	100.7
	41.0	63.3		62.4	115.6		59.9	98.9
	38.9	66.2		64.4	111.9		56.4	100.8
	41.6	64.6		61.1	114.6		60.2	98.6
	40.0	64.6		63.3	121.4		59.2	91.3
	40.2	62.4		65.9	118.1		56.7	97.5
SD	1.9	2.7	SD	5.2	4.6	SD	3.5	3.9
%CV	4.8%	4.1%	%CV	7.7%	3.9%	%CV	6.2%	4.1%

Table 4. Precision data for oxycodone

Table 5. Precision data for methadone

Table 6. Precision data for benzodiazepines

Precision

Validation experiments indicated excellent method precision with %CVs ranging from 4.0% to 4.7% for oxycodone high and low QC's respectively, 3.9% to 7.7% for methadone high and low QC's respectively, and 4.0% to 6.1% for benzodiazepine high and low QC's, respectively. Precision experiments indicate that lower screening cutoffs can be implemented using commercially available immunoassay kits. Throughout all studies, %CV's associated with low QC quantities did not exceed 7.7% which is well within precision acceptance criteria.

Accuracy

Validation experiments also indicate a high degree of accuracy at the lower cutoffs values. Mean values determined for oxycodone low and high QC's were 40.1 ng/mL, and 65.3 ng/mL, representing accuracies of 93.5% and 100%, respectively. Mean quantitative values for methadone low and high QC's were 66.8 ng/mL, and 116.4 ng/mL, representing accuracies of 89.0, and 93.1%, respectively. Mean quantitative values for benzodiazepine low and high QC's were 56.6 ng/mL, and 96.7 ng/mL, representing accuracies of 100%, and 96.9%, respectively.

Conclusion

As a result of successful validation experiments, AIT Laboratories now employs an oxycodone screen cutoff of 50 ng/mL (vendor recommends 300 ng/mL), a methadone screen cutoff of 100 ng/mL (vendor recommends 300 ng/mL) and a benzodiazepine screen cutoff of 75 ng/mL (vendor recommends 200 ng/mL). Implementation of lower screen cutoffs will prove beneficial for the detection of highly potent drugs and toxins in biological specimens.

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