B-336: Comparison of the LZI Fentanyl Enzyme Immunoassay with ARKII and SEFRIA Fentanyl Assays on Beckman AU Analyzer

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BACKGROUND

Fentanyl is a fast-acting synthetic opioid that can be prescribed for severe pain or found in illicit drugs. Routine clinical screening of fentanyl use is often performed on automated immunoassay analyzers, by measuring fentanyl and/or its primary metabolite, norfentanyl, in urine. Limited data has been published on performance of the relatively new LZI fentanyl enzyme immunoassay, which detects both fentanyl and norfentanyl but calibrated against norfentanyl. In this study, we evaluated the performance of LZI fentanyl assay on Beckman AU analyzer, in comparison to ARKII and SEFRIA assays calibrated against fentanyl.

METHODS

The LZI and ARKII fentanyl assays were evaluated on Beckman AU 5800 analyzer according to manufacturer's instructions in our clinical laboratory. The LZI assay uses a cutoff value of 5 ng/mL for norfentanyl, while the ARKII assay uses a cutoff value of 1 ng/mL for fentanyl. The SEFRIA assay was performed on Beckman AU analyzer in a reference laboratory, with a cutoff value of 2 ng/mL for fentanyl. A qualitative comparison study was performed on a total of 42 de-identified urine samples, which were consecutively collected from clinical fentanyl screening test orders. Fentanyl screening results from the LZI and SEFRIA assays were available for all 42 samples, with ARKII results available for 28 out of the 42 samples. Results from LC-MS/MS confirmatory test performed in a reference laboratory (cutoff value of 1 ng/mL for either fentanyl or norfentanyl) was also available on samples with discrepant LZI results when compared to either SEFRIA or ARKII results.

RESULTS

Table 2. Comparison Result Summary

LZI Assay (cutoff: 5 ng/mL norfentanyl)	SEFRIA (cutoff: 2 ng/mL fentanyl)		ARKII (cutoff: 1 ng/mL fentanyl)		
	Total: 42	**Positive**	**Negative**	**Total: 28**	**Positive**	**Negative**
	Positive	10	7**	**Positive**	10	0
	Negative	0	25***	**Negative**	1*	17
	Agreement	83.3%		**Agreement**	96.4%	

Note: the above table groups the SEFRIA block (Total:42 / Positive / Negative) and the ARKII block (Total:28 / Positive / Negative) side by side with the LZI Assay row labels.

* Confirmed negative by LC-MS/MS (<1 ng/mL for fentanyl and norfentanyl)

** All confirmed positive by LC-MS/MS (>=1 ng/mL for fentanyl or norfentanyl); with SEFRIA results below cutoff but above 1 ng/mL

*** Borderline negative on LZI assay for one of the samples: LC-MS/MS positive for norfentanyl (<1.0 ng/mL fentanyl; 3.3 ng/mL norfentanyl)

Table 1. Precision Study

Instrument 1	Mean (cutoff=100)	Within-day Imprecision	Between-day Imprecision	Portion above Cutoff
High QC	143	5.3%	8.9%	100%
Calibrator	106	9.7%	10.4%	58.8%
Low QC	78	9.3%	13.9%	0%
Instrument 2	**Mean (cutoff=100)**	**Within-day Imprecision**	**Between-day Imprecision**	**Portion above Cutoff**
High QC	150	8.4%	12.4%	100%
Calibrator	110	8.6%	7.0%	75%
Low QC	81	4.8%	7.0%	0%
Acceptable:		YES (<10%)	YES (<15%)	YES

Table 3. Discrepancy Among Immunoassays vs. LC-MS/MS Confirmatory Results

SEFRIA (cutoff: 2 ng/mL fentanyl)	ARKII (cutoff: 1 ng/mL fentanyl)	LZI (cutoff: 5 ng/mL norfentanyl)	LCMS (cutoff: 1 ng/mL fentanyl or norfentanyl)		
			Result	**Fentanyl**	**Norfentanyl**
NEG	NEG	NEG	POS	<1.0 ng/ml	3.3 ng/ml
NEG	POS	NEG	NEG	<1.0 ng/ml	<1.0 ng/ml
NEG	POS	POS	POS	<1.0 ng/ml	20.5 ng/ml
NEG	POS	POS	POS	<1.0 ng/ml	6.7 ng/ml
NEG	POS	POS	POS	1.6 ng/ml	22.1 ng/ml
NEG	POS	POS	POS	2.8 ng/ml	89.1 ng/ml
NEG	N/A	POS	POS	2.8 ng/ml	>1000.0 ng/ml
NEG	N/A	POS	POS	1.1 ng/ml	21.1 ng/ml
NEG	N/A	POS	POS	<1.0 ng/ml	23.7 ng/ml

> No false positive result was observed for the LZI assay in this study, although only samples with discrepant immunoassay results were sent for LC-MS/MS testing. One false positive result was observed by ARKII assay.

> High false negative rates seen in SEFRIA assay is likely due to the cutoff being 2 ng/mL.

> Overall, immunoassay is less sensitive than LC-MS/MS method.

CONCLUSIONS

- Overall, the Beckman AU LZI fentanyl assay's analytical performance around the cutoff value is acceptable.

- In this study, there was no false positive result reported by LZI assay and 17 out of 18 positive samples were correctly identified, with a calculated sensitivity of 94%, specificity 100%, positive predictive value 100% and negative predictive value 96%.

- The limitation of the study is small sample volumes and not all results were confirmed by LC-MS/MS method. The false positive and false negative rate may be under-estimated.

- In conclusion, the Beckman AU LZI fentanyl assay with a cutoff value of 5 ng/mL calibrated against norfentanyl has shown comparable performance as existing fentanyl assays (i.e. ARKII) and can be implemented for routine clinical screening of fentanyl.

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