Evaluating Urine Fentanyl Tests for Clinical Implementation

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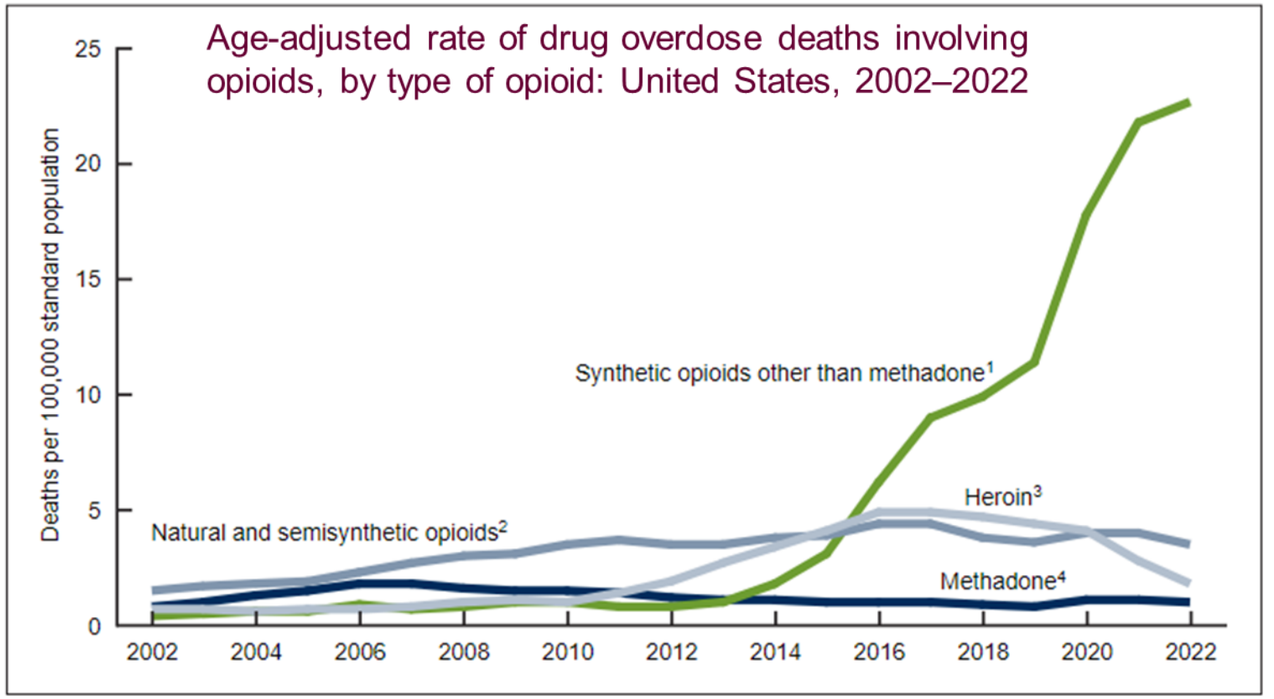
Disclosure

- None

Learning Objectives

- Understand the utilities of screening and confirmatory fentanyl testing in various clinical settings
- Compare different immunoassays, including point-of-care tests, currently available for urine fentanyl screening
- Evaluate analytical and clinical considerations for implementation of urine fentanyl immunoassays

Opioid overdose death in U.S.

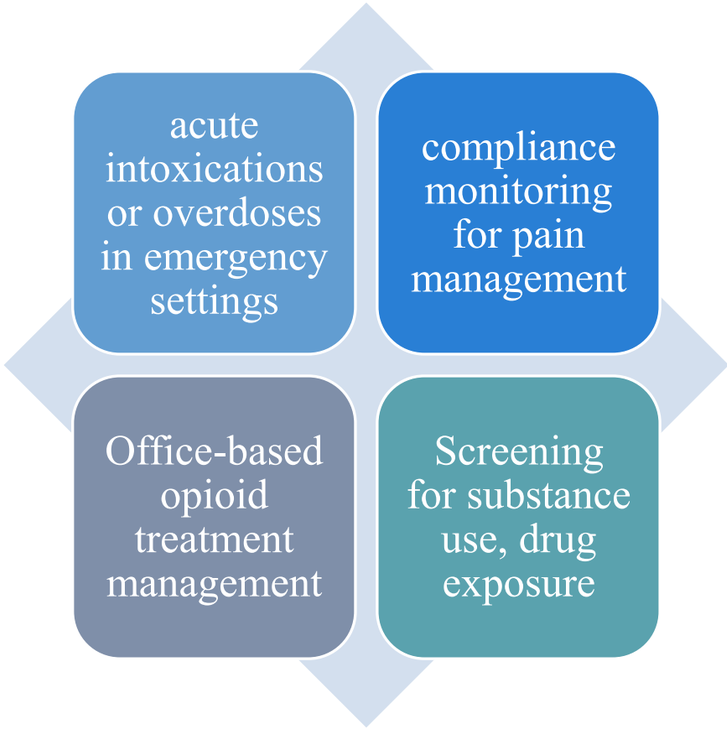


Adapted from Spencer, Merianne R. et al. "Drug Overdose Deaths in the United States, 2002-2022", no. 491, 2023. https://stacks.cdc.gov/view/cdc/135849

Fentanyl testing provides essential information in various clinical settings

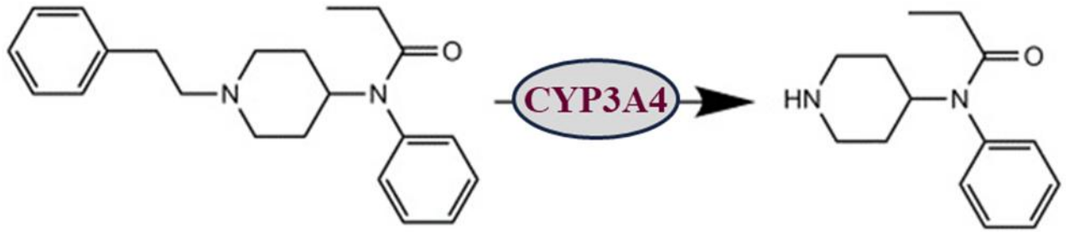
- Fentanyl is a potent synthetic opioid, about 100x more potent than morphine and 50x more potent than heroin.
- Fentanyl is often laced to other illicit drugs unbeknownst to users.

Adapted from https://www.dea.gov/resources/facts-about-fentanyl



Fentanyl metabolism

- Fentanyl is primarily metabolized in the liver to inactive norfentanyl.
- Less than 1% of the parent drug is metabolized to other inactive compounds.
- Intestinal metabolism of fentanyl to norfentanyl is also catalyzed by CYP3A4 (at a half rate of liver metabolism).
- Fentanyl immunoassays are usually designed to detect fentanyl and/or norfentanyl.



Fentanyl Norfentanyl

Fentanyl excretion

- Fentanyl is mainly excreted in urine (75%) and feces (9%), primarily as norfentanyl (<10% excreted as intact molecule).
- Fentanyl is highly lipophilic, with variable half-life depending on the duration of administration.
- Reported plasma half-life and urine detection window are:
  - Fentanyl: 3-12 hrs; 1-3 days
  - Norfentanyl: 9-10 hrs; 1-3 days

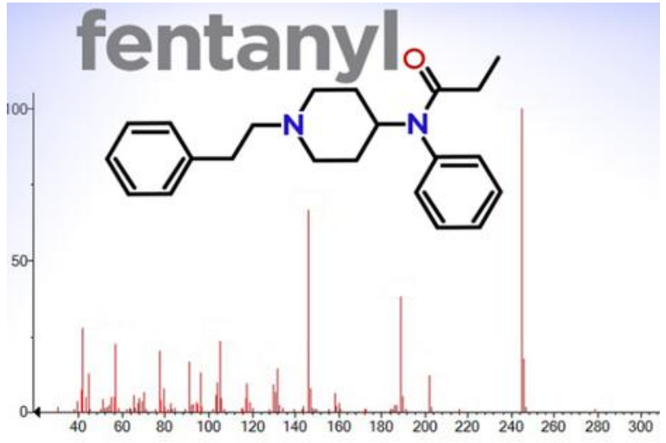
Regular use of fentanyl, as seen in patients with opioid use disorder, can lead to prolonged clearance of fentanyl (7 days) and norfentanyl (13 days).

Fentanyl testing: specimen types

- Urine: higher concentration, easy to collect, reasonable detection window (within 24hr up to 72hr)
- Blood (up to 12hr)
- Saliva (1-3 days, not as reliable)
- Hair (up to 3 months)
- Meconium, umbilical cord

Urine fentanyl testing methods

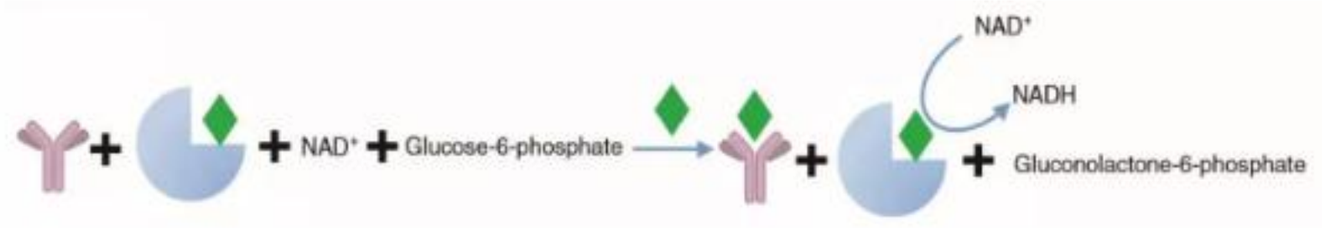
- Immunoassays: high throughput, fast TAT, cost effective, widely available
- Mass spectrometry-based assays: highly sensitive and specific, suitable for confirmation or screening
  - Gas chromatography, liquid chromatography
  - Standard, high-resolution
  - Targeted, untargeted



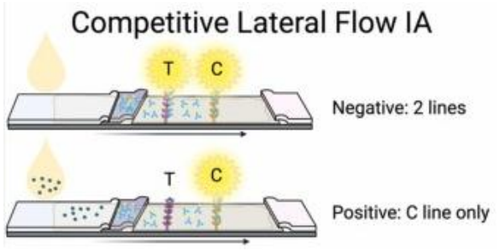
Overview of urine fentanyl immunoassays (IAs)

Urine fentanyl immunoassays (IAs

- Immunoassays (competitive):
  - Homogeneous enzyme immunoassay (IA); photometric-IA on automated chemistry analyzers

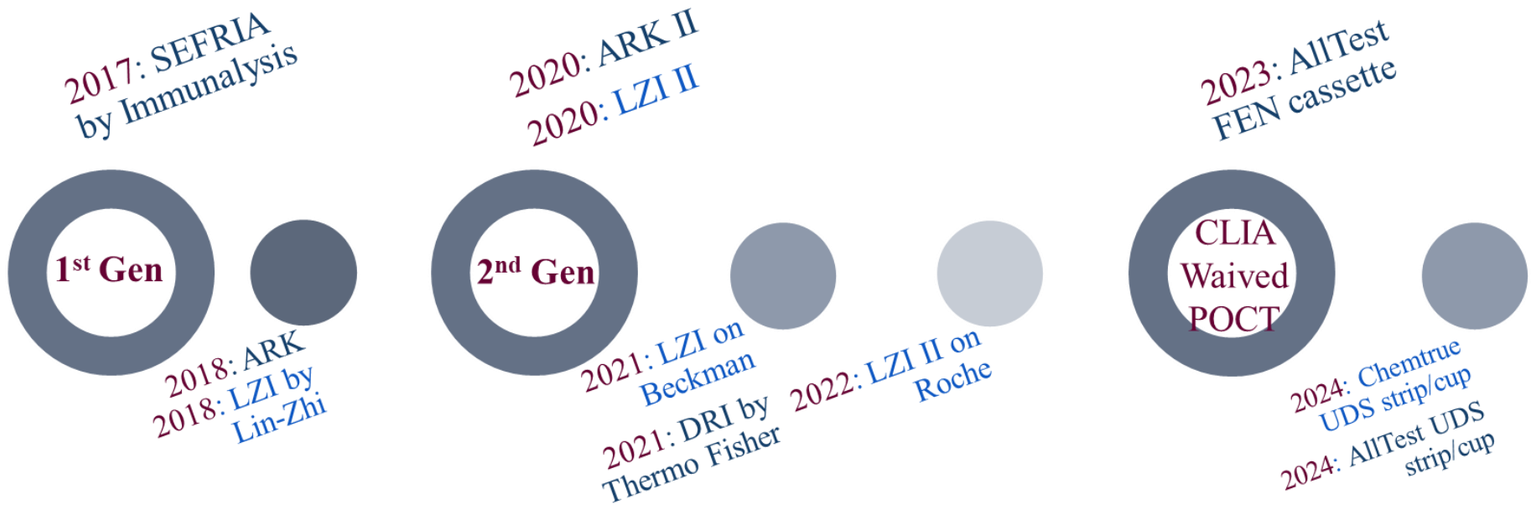


  - Competitive binding lateral flow immunochromatographic assay; test strip



Adapted from Uljon, Sacha. “Advances in fentanyl testing.” Advances in clinical chemistry vol. 116 (2023): 1-30. doi:10.1016/bs.acc.2023.05.004

Urine fentanyl IA: FDA-approved test options



LZI or LZI II: calibrated against norfentanyl; SEFRIA, ARK, ARK II: calibrated against fentanyl

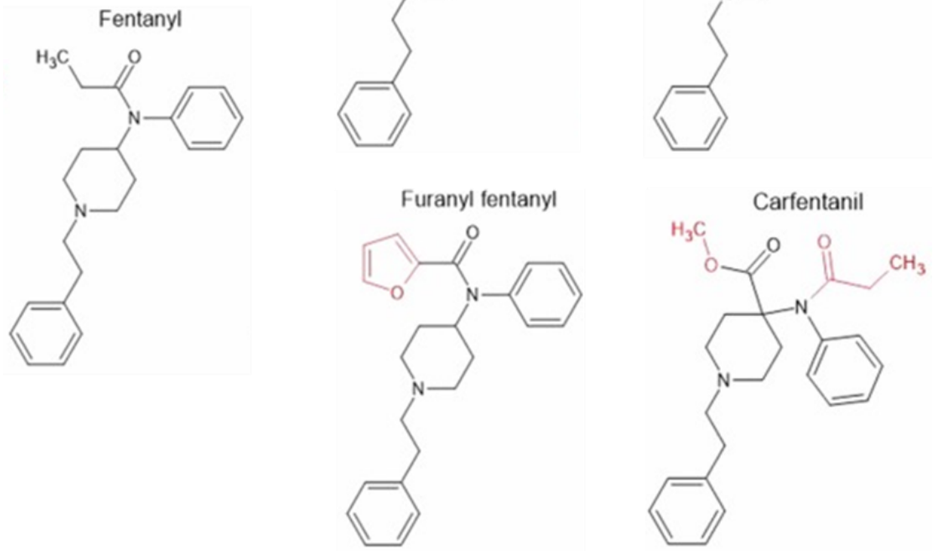
Urine fentanyl IA: key features

	SEFRIA	ARK	ARK II	LZI	LZI II (FEN2)	AllTest POCT	Chemtrue POCT
Detect	FEN	FEN	FEN	NorFEN	NorFEN	FEN	NorFEN
Cutoff (ng/mL)	1	1	1	5	5	1	5
Cross-reactivity with fentanyl (FEN)	100%	100%	100%	200% (2.5 ng/mL)	132% (3.8 ng/mL)	100%	50% (10 ng/mL)
Cross-reactivity with norfentanyl (NorFEN)	0.005%	3% (30 ng/mL)	7% (15 ng/mL)	100%	100%	<0.001%	100%
Cross-reactivity with fentanyl analogs	Varies depending on the compound and the assay Refer to package inserts for details						

Fentanyl and its analogs

Common fentanyl analogs (reported potency in relevant to fentanyl):

- Acetyl fentanyl (0.3)
- Para-fluorofentanyl (0.3)
- Furanyl fentanyl (7)
- Carfentanil (30-100)



Adapted from Giltner, Angela et al. “Fentanyl analog trends in Washington D.C. observed in needle-exchange syringes.” Forensic science international vol. 338 (2022): 111393. doi:10.1016/j.forsciint.2022.111393

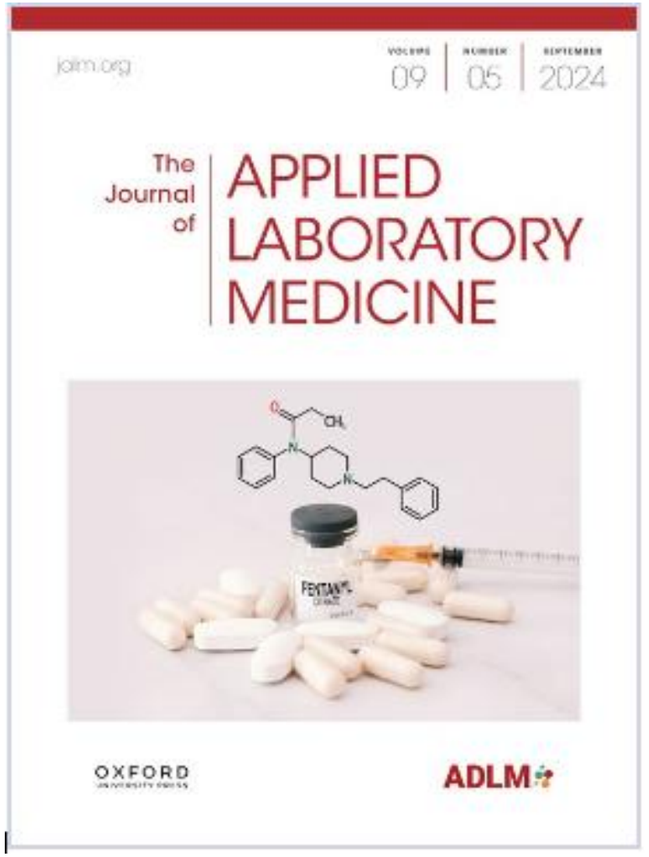
Recent studies

Evaluate analytical performance using MS-based method as the reference:

- Lam KHB et al and Militello L et al: LZI II (FEN2) and ARK II on Roche Cobas
- Mills CM et al: SEFRIA and ARK II on Abbott Architect
- Laryea ET et al: Rapid test vs SEFRIA and ARK II on Abbott Alinity c
- Uljon S et al: SEFRIA, LZI II (FEN2) and ARK II on low positive specimens

Discuss potential causes or explanations for false positive or negative results

Understand result interpretation challenges



https://academic.oup.com/jalm/issue/9/5

Recent studies: performance review

	SEFRIA (n=2)	ARK II (n=3)	LZI II (n=2)	AllTest POCT (n=1)
Sensitivity (Sn.)	95-96%	90-96%	89-97%	84%
Specificity (Sp.)	82-97%	94-99%	99-100%	87%
Negative Predictive Value (NPV)	95-99%	95-99%	86-97%	85%
Positive Predictive Value (PPV)	79-84%	90-94%	98-100%	87%
False Positive Rate (FPR, 1-Sp.)	3-18%	1-6%	0-0.6%	3%
Overall Diagnostic Efficiency	88-97%	95-99%	96-98%	86%

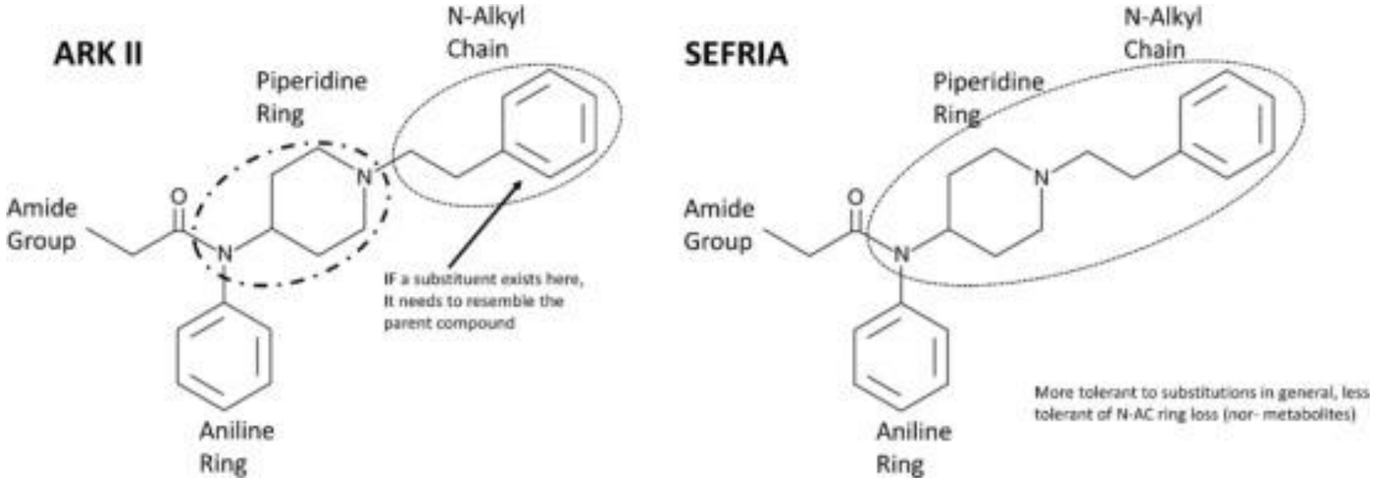
Positivity can be seen in samples with FEN and/or NorFEN less than claimed detection cutoffs.

Analytical considerations on result interpretation

- Definition of positive result
  - Generally: Detection of fentanyl and/or norfentanyl (major metabolite) indicating fentanyl use
  - Extended: Detection of fentanyl analogs indicating non-fentanyl related opioid use
    - It is rare to identify fentanyl analogs without the co-occurrence of fentanyl;
    - Require comprehensive MS-based method (e.g., untargeted, extended panel).
- Cross-reactivity to fentanyl analogs can lead to the so called “false positive” results, or on the other hand, contribute to an “increased sensitivity” at the same time.

Cross-reactivity with fentanyl analogs

Of the 58 analogs tested at ≤ 100 ng/mL,
51 detected by the ARK II assay and 57 detected by the SEFRIA assay.



Williams, Grace R et al. “Detection of 58 fentanyl analogs using ARK fentanyl II and Immunalysis fentanyl immunoassays.” Clinical biochemistry vol. 113 (2023): 45-51. doi:10.1016/j.clinbiochem.2023.01.001

Other potential cause of false results

- False positive from other cause
  - Interference from other illicit substance (intentional or unintentional use)
  - Interference from prescribed or over-the-counter (OTC) medications

Interference was observed in LZI and LZI II assays with Dextromethorphan at 40,000 ng/mL.

Risperidone is detected by the SEFRIA fentanyl assay at 2,500 ng/mL, trazodone at 10,000 ng/mL, and labetalol at 15,000 ng/mL; while all three compounds are not detected by the ARK II fentanyl assay at 100,000 ng/mL.

Other potential cause of false results

- False positive from other cause
  - Interference from other illicit substance (intentional or unintentional use)
  - Interference from prescribed or over-the-counter (OTC) medications
- False negative:
  - Analytical: lower sensitivity or cross-reactivity of fentanyl or norfentanyl
  - Pre-analytical: diluted urine, adulteration, improper storage/transportation

Clinical considerations and analytical evaluation for implementation

Clinical considerations

Clinical need: screening vs confirmation; targeted population vs all comers

Desired TAT: immediately, hours, days

Desired accuracy: potential impact of false positive or negative results

Implementation considerations

Core lab IA

- Higher sensitivity and specificity
- Lower cost/test
- High throughput
- Results available within 1hr from specimen receiving but could take hours depending on the transportation time
- Easy to conduct reflex or add-on for confirmatory test

POCT

- Lower sensitivity and specificity
- Higher cost/test
- Low throughput
- Results available within minutes from specimen collection
- Higher logistic burden to conduct reflex or add-on for confirmatory test

Assay selection and evaluation

Identify feasible options

- Core lab IA: available instrumentation
- POCT: waived vs non-waived options

Collect assay information and understand:

- claimed sensitivity: cut-offs, cross-reactivity to fentanyl or norfentnayl
- assay specificity: cross-reactivity to fentanyl analogs, interference from other substances

Design validation study and set acceptable criteria

- Sample selection
- Reference method

Our experience

Example 1: Core lab IA implementation

- Existing chemistry analyzer: Beckman AU5800
- Available fentanyl reagent options: ARKII and LZI
- Cost saving: ~$40 less per test
- TAT improvement: 2-3 days → hours
- Main users: ED, outpatient clinics, opioid treatment programs

Core lab IA: comparison study

Reference lab: SEFRIA; In-house: LZI and ARKII on the same analyzer

Table 2. Comparison Result Summary

	SEFRIA (cutoff: 2 ng/mL fentanyl)			ARKII (cutoff: 1 ng/mL fentanyl)	
LZI Assay (cutoff: 5 ng/mL norfentanyl)	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%	

* 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)

Manar S, George B, Huang R*. Clinical Impact of Implementing Urine Fentanyl Testing in A Safety Net Healthcare System, 22nd International Congress of Therapeutic Drug Monitoring and Clinical Toxicology, Sep. 17th, 2024

Core lab IA: discordance results

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.

- Sensitivity: 94%
- Specificity: 100%
- Negative predictive value: 96%
- Positive predictive value: 100%

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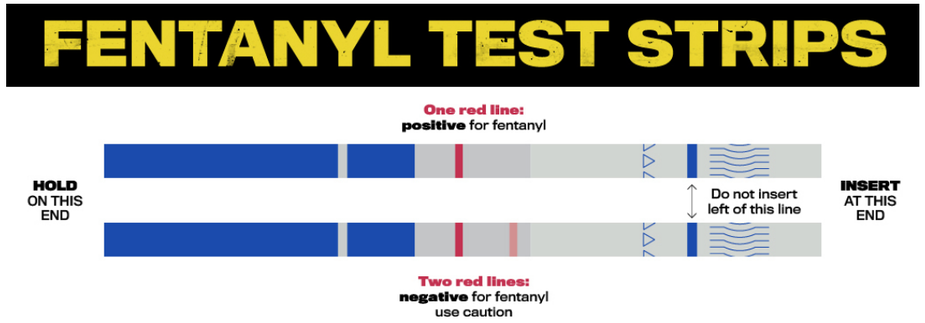
Example 2: POCT implementation evaluation

Business case justification for the use of urine fentanyl POCT in office-based opioid treatment (OBAT) settings:

- Needs assessment
- Cost analysis/budgeting
- IT integration: test ordering, result reporting, reflex testing
- Policy and procedure establishment
- Regulatory compliance/quality management



POCT: preliminary performance assessment

Waived POCT Fentanyl Test Strip (cutoff: 5 ng/mL norfentanyl)	LZI core lab assay (cutoff: 5 ng/mL norfentanyl)			LC-MS* (cutoff: 5 ng/mL norfentanyl)		
	Total: 48	Positive	Negative	Total: 93	Positive	Negative
	Positive	14	4**	Positive	32	3
	Negative	1**	29	Negative	0	58
	Agreement	89.6%		Agreement	96.8%	
	False positive	22.2%		False positive	8.6%	

* Information from IFU

** Further investigation by LC-MS could not be performed.

Other practical considerations

Reflex to confirmatory test

- Automatic: targeted vs all
- Add-on by providers

Select confirmatory method(s)

- In-house vs send-out
- FEN/NorFEN only vs targeted panel vs untargeted

Risk mitigation of false or discrepant results

- Expect discordant or unexpected results
- Ensure the communication channel is open and efficient between clinicians and labs

Conclusion

- Assess clinical needs
- Compare options
- Evaluate analytical performances
- Implementation considerations
  - pre-analytical
  - post-analytical
  - POCT specific

Thank you

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