Toxicology

Comparative assessment of fentanyl assay performance indicators

How will false negatives or missed positives impact your lab? An assessment of time, cost and credibility

In 2021, 107,622 fatalities in the United States were attributed to drug overdose, an average of just under 295 deaths per day. Approximately 66% of overdose deaths were attributable to opioids, driven by a rise in illicit fentanyl.[1]

The opioid crisis

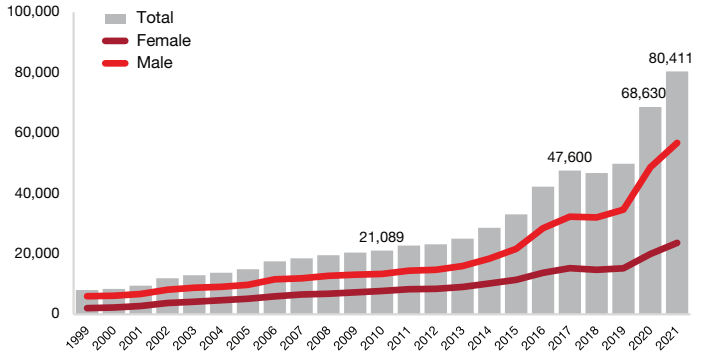
Fentanyl is a synthetic opioid that is 50 to 100 times more powerful than heroin or prescription opioids.[1] As a prescription drug, fentanyl is used to treat severe pain, such as late-stage cancer or following surgery, and is often applied via skin patches. As an illegal drug manufactured in labs, the synthetic is often distributed in a powder or liquid form.

- Due to its cheap production cost and availability, fentanyl is commonly used to adulterate heroin, cocaine, methamphetamines and other street narcotics, leading to the user unknowingly ingesting a substance laced with fentanyl[1]
- With a potency 100 times more powerful than morphine, the substance to which heroin metabolizes, only a trace amount of fentanyl is required to produce the drug effect[1]
- The high potency significantly increases risk of overdose, with average fentanyl levels as low as 3.9 ng/mL* required for a lethal dose[2]
- Fentanyl is colorless, odorless and tasteless, and is nearly impossible to detect without a chemical test

With a surge in intentional and unintentional use of fentanyl, methods to detect fentanyl that exhibit ***high sensitivity, accuracy*** and ***reproducibility*** are increasingly important to address and curb the fentanyl crisis.

*in urine, measured by radioimmunoassay[2]

Figure 1. Opioid-related* overdose deaths in the U.S., all ages by gender, 1999-2021[3]



* Among deaths with drug overdose as the underlying cause the "any opioid" subcategory was determined by the following ICD-10 multiple cause-of-death codes: natural and semi-synthetic opioids (T40.2), methadone (T40.3), other synthetic opioids (other than methadone), or heroin (T40.1).

Performance indicators

Though fentanyl cutoffs are under discussion with key guidance bodies like SAMHSA (Substance Abuse and Mental Health Services Administration), DoT (Department of Transportation) urine guidances and EWDTS (European Workplace Drug Testing Society), a definitive and standardized recommendation is not currently published. Without standardized guidelines, assessment of the efficacy of fentanyl assays should be gated by performance indicators that can be compared across products and consistently evaluated with regard to reliability, performance, cost and time efficiency.

To assess accuracy and reproducibility across products, three performance indicators should be considered:

- **Clinical sensitivity** - Able to detect fentanyl in urine samples in sub-nanogram ranges, the assay should offer a detection window that considers norfentanyl, the primary metabolite. With high clinical sensitivity, laboratories can expect to minimize false negative results and save the cost and time of separate confirmation tests.

- **Specificity and robustness** - The assay should be specific and robust enough to minimize cross-reactions with other opioids or false-positives due to common co-medications, and should be reliable enough to ensure a consistently accurate result with minimal to no false negatives. Again, this performance indicator can save a lab testing facility both the time and the cost of confirmation testing.
- **Precision/reproducibility** - Better reproducibility and precision in the assay indicate more reliable results, with an assay that can be trusted to reliably repeat findings.

In discussion of how different commercially-available assays measure up to these performance indicators, facilities undertaking these tests should consider the following: *What is the cost of false negatives in your lab?* and, *What are the ramifications of missing positive results?* These questions should be evaluated with respect to not only cost and time, but also to the lab's reputation and credibility.

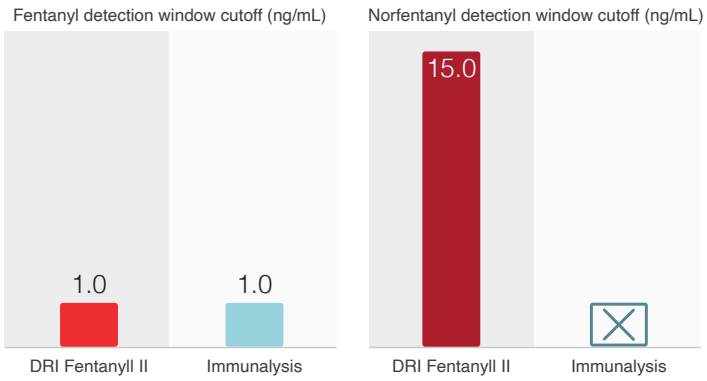
Comparative assessment

This paper evaluates documentation and research published and available for the two commercial fentanyl assays currently offered on the market: the ARK Fentanyl II Assay/DRI™ Fentanyl II Assay and the Fentanyl Urine SEFRIA™ by Immunalysis.

Clinical sensitivity

With the continuing rise in both intentional and unintentional fentanyl use, an assay for the detection of fentanyl and norfentanyl with high clinical sensitivity has become increasingly important. Even in cases where clinical symptoms indicate fentanyl use and the need for naloxone as an antidote, clinical confirmation of the presence of fentanyl can support timely and appropriate care.[4]

Figure 2. Fentanyl and norfentanyl detection window cutoffs for two assays[11,12]

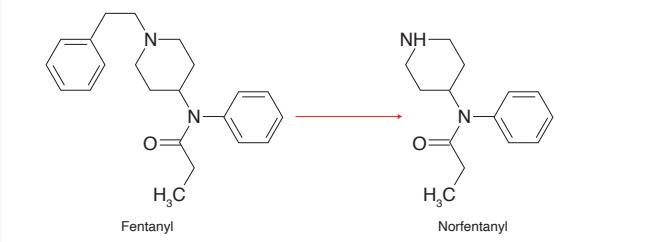


Although urine fentanyl concentrations present in cases involving overdose or death vary widely among individuals, concentrations of fentanyl in urine were as low as 3.9 ng/mL (on average; n=112) in intravenous abuse fatalities.[2] As a result, a clinical need for screening methods that can detect fentanyl in urine samples at or below single-digit ng/mL concentrations, and norfentanyl around 10 ng/mL, has been asserted.[5]

Detection window

In defining a cutoff concentration, the pharmacokinetics and the analytical performance of the assay need to be taken into consideration. Pharmacokinetics dictate the length of time

Figure 3. Fentanyl and metabolites

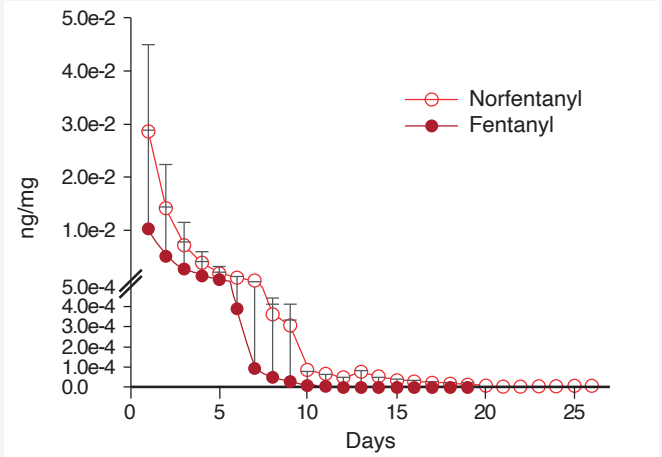


Fentanyl metabolizes into predominantly norfentanyl; less than 1% metabolizes to despropionylfentanyl and other metabolites.[7]

Selecting for an assay with high fidelity for the parent drug and primary metabolite is paramount. In a retrospective study, both fentanyl and norfentanyl were concurrently detected in 93.8% of positive results (n=4,366). The median concentration of fentanyl was 34 ng/mL, whereas the median concentration for norfentanyl was 265.3 ng/mL.[8] In the same retrospective study, 6.1% of positive results were for norfentanyl *only* (n=283).[8] These results should lead lab facilities to ask:

What is the impact of false negative results?

Figure 4. Fentanyl and norfentanyl elimination[9]



- The detection window for fentanyl is 1 to 3 days for single use, rising to an average of 7 to 13 days for chronic opioid use[9,10]
- Norfentanyl is present at 5 to 10 times the parent drug and has a longer detection window than fentanyl[6]

Table 1. Cross-reactivity comparison[11,12]

Manufacturer/Assay	ARK™ Diagnostics, Inc. ARK Fentanyl II Assay/DRI™ Fentanyl II Assay		Immunalysis Fentanyl Urine SEFRIA™	
	Concentration approximately equivalent to the cutoff (ng/mL)	Cross-reactivity (%)*	Concentration approximately equivalent to the cutoff (ng/mL)	Cross-reactivity (%)*
Fentanyl	1.0	100	1	100
Metabolites				
Norfentanyl	15	7	20,000	0.005
Acetyl fentanyl	1.1	90.91	1.0	100
Isobutyryl fentanyl	1.1	90.91	Not available	-
Acrylfentanyl	1.3	76.92	Not available	-
Butyryl fentanyl	1.4	71.43	0.8	125
Furanyl fentanyl	1.5	66.67	Not available	-
Para-fluoro fentanyl	1.5	66.67	Not available	-
Para-fluorobutyryl fentanyl (P-FBF)	1.9	52.63	Not available	-
Valeryl fentanyl	2.3	43.48	Not available	-
Acetyl norfentanyl	12.1	8.26	Not available	-
Sufentanil	2,362	0.04	175	<0.57
Methoxyacetyl fentanyl	Not available	-	Not available	-

*Cross-reactivity percentage = (Cutoff concentration/Concentration approximately equivalent to the 1.0 ng/mL cutoff) x 100

after drug administration when the drug can be detected in the sample, as well as the expected concentration of the drug in the defined patient sample. Higher cutoff values correspond to shorter detection windows, defined as the range of time after the drug administration when the substance can be detected.

Fentanyl rapidly metabolizes into norfentanyl (Figure 3), and both parent drug (10%) and metabolites are excreted into urine. Due to a shorter detection window for fentanyl in urine (Figure 4),[9] a lower cutoff value and detection of norfentanyl extends the detection window for a positive indication of the use of fentanyl.

Per a 2023 study, data from a needle exchange program in Washington, D.C., demonstrates a growing awareness that "proper identification of these [fentanyl] analogs is crucial to guide further treatment".[13] The DRI Fentanyll II assay also demonstrates comprehensive analog cross-reactivity, with proven capabilities to detect the emergence of synthetic fentanyl analogs (Table 1).

Table 2. Robustness against potential co-medications[11]

Compound	Concentration tested (ng/mL)	Compound	Concentration tested (ng/mL)
Buprenorphine	100	Naltrexone	100
Codeine	100	Norbuprenorphine	100
Dextromethorphan	100	Norcodeine	100
Dihydrocodeine	100	Normorphine	100
EDDP	100	Noroxycodone	100
Hydrocodone	100	Oxycodone	100
Hydromorphone	100	Oxymorphone	100
Levorphanol	100	Tapentadol	100
Methadone	100	Thioridazine	100
Morphine	100	Tramadol	100
Morphine-3-glucuronide	100	Trazodone	100
Naloxone	100	Diphenhydramine	100

Specificity and robustness

The assay's specificity should be evaluated as a marker of its ability to correctly detect fentanyl or its metabolites in a urine sample, without returning a false positive due to the presence of other co-medications or substances in the sample. Robustnessness is key for a fentanyl testing assay due to increasing use of fentanyl to adulturate common street drugs.

In a study comparing the performance of two fentanyl screening immunoassays against liquid chromatography-tandem mass spectrometry (LC-MS/MS) as the gold standard, the clinical sensitivity and specificity for detection of fentanyl exposure were 100% and 96% for the DRI Fentanyl II assay.[14]

The DRI Fentanyl II assay shows good robustness against common co-medications. Opioids, structurally similar compounds, and functional analogs were found negative at the concentrations tested with the DRI Fentanyl II Assay (Table 2).

Although the DRI Fentanyl II assay demonstrates superior performance for the screening of non-pharmaceutical fentanyl (NPF), it should be noted that it does not perform well in the detection of fentanyl derivatives, such as alfentanil, sufentanil

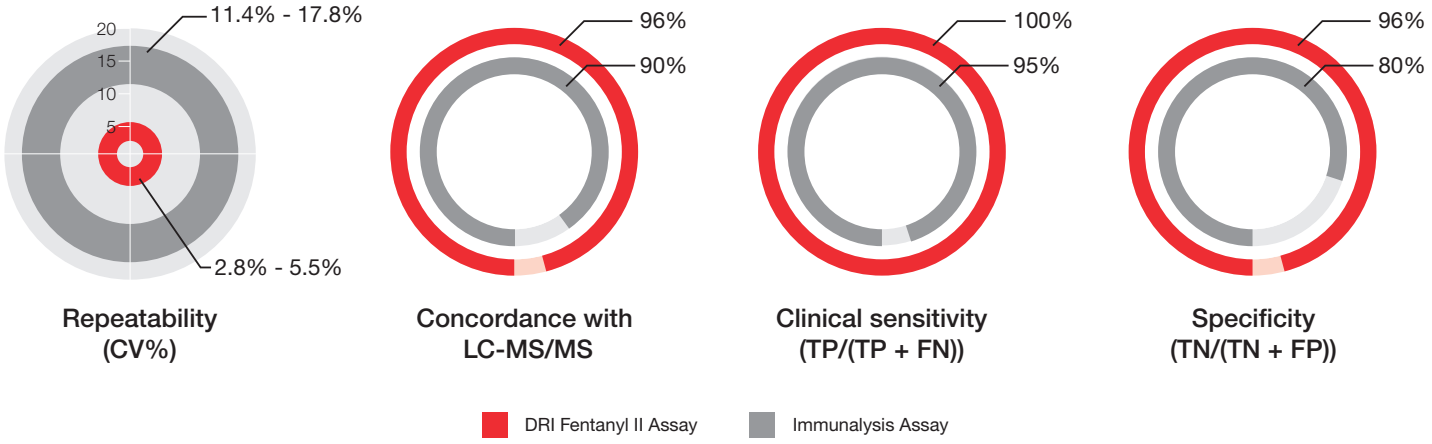
and remifentanil, which are medically used for sedation and pain management.[15] This appears to be consistent with the performance of the current fentanyl screening assays available on the market.

Precision and reproducibility

In comparative performance evaluations, the DRI Fentanyl II assay demonstrated superior precision with respect to reproducibility and total imprecision and better concordance with LC-MS/MS results than the Immunalysis assay.[14,16]

- Better precision for the DRI Fentanyl II assay indicates superior precision and more reliable results
- Significantly better accuracy using the DRI fentanyl II assay gives confidence that no positive samples were missed, and minimal false positive results were returned

Figure 5. Comparison of performance indicators[14,16]



The ability of a product to reliably perform and return consistent results can save labs time and cost by reducing the need for LC-MS/MS confirmation tests.

Conclusion

Overall, the DRI Fentanyl II assay matches or demonstrates superior performance against the Immunalysis assay with regard to clinical sensitivity to fentanyl, precision and reproducibility and cross-reactivity to opioids or common co-medications. Performance comparisons in peer-reviewed publications and product claims published in product literature reinforce these key performance indicators and reveal a clear leader: the DRI Fentanyl II assay (Figure 5).

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