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TECHNICAL NOTE

Toxicology

The use of lateral flow immunoassays for the detection of fentanyl in seized drug samples and postmortem urine

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Abstract

The opioid crisis has continued to progress in the United States and the rest of the world. As this crisis continues, there is a pressing need for a rapid and cost-effective method for detecting fentanyl. Recent studies have suggested that lateral flow immunoassays (LFIs) could fill this technology gap. These qualitative paper-based assays contain antibodies designed to react with fentanyl and provide positive or negative results within a matter of minutes. In this study, two different LFI configurations for the detection of fentanyl were examined (dipsticks and cassettes) for effectiveness of detection using seized drug samples and postmortem urine samples. In the current study, 44 seized drug samples (32 fentanyl positive, 12 fentanyl negative) and 14 postmortem urine samples (10 fentanyl positive, 4 fentanyl negative) were analyzed. All 32 fentanyl-containing seized drug samples and 10 postmortem fentanyl positive urine samples displayed positive LFI results with both LFI configurations. The fentanyl dipsticks displayed a sensitivity of 100%, a specificity of 75%, and an efficiency of 93.2% for seized drug samples and a sensitivity, specificity, and efficiency of 100% for postmortem urine. Analysis of the fentanyl cassettes displayed a sensitivity, specificity, and efficiency of 100% for seized drug samples and a sensitivity of 100%, a specificity of 75%, and an efficiency of 92.9% for postmortem urine samples. These data point to the utility of LFIs as a quick and low resource-dependent option for presumptive detection of fentanyl in real-world situations.

KEYWORDS
fentanyl, forensic chemistry, forensic toxicology, illicit drugs, lateral flow immunoassay, opioid, seized drugs

1 | INTRODUCTION

The continued prevalence of fentanyl in the illicit drug supply has caused additional risks to illegal drug users and ongoing safety concerns for first responders and forensic scientists [1–4]. The extreme toxicity of fentanyl is well known, with a potency estimated to be 50 times greater than heroin and 100 times greater than morphine [5,6]. In addition, fentanyl derivatives, such as carfentanil and ohmefentanyl, being introduced into the same illegal drug supply, are even more potent than fentanyl itself [7,8]. Due to the extreme toxicity of all of the fentanyl compounds, extra precautions are required when handling packages or containers of seized drugs whether in a field or laboratory setting. The common occurrence of fentanyl in seized drugs and the dire consequences of accidental exposure emphasize the need for rapid, low-cost and widely accessible detection technology for fentanyl and its derivatives.

A promising solution for rapid fentanyl detection is the use of paper-based lateral flow immunoassays (LFIs). Use of LFIs for the simple, rapid detection of various biological and chemical compounds has gained popularity in a variety of disciplines including, but not limited to, point-of-care medicine (e.g., pertussis, *streptococcus*) [9,10], agricultural surveillance (e.g., red fire ant, Grapevine Leafroll-Associated Virus) [11,12], biodefense (e.g., *Bacillus anthracis*, *Yersinia pestis*) [13,14], and more recently, forensic science (e.g., illegal drugs) [15,16]. These assays employ antibodies directed against the target (i.e., fentanyl), which are incorporated into the surface of the assay. A positive or negative determination can be made on liquid samples in a matter of minutes.

In the current study, the effectiveness of two different fentanyl LFI configurations (dipsticks and cassettes) in detecting fentanyl in seized street samples and in urine from suspected overdose cases was examined. These LFIs have been previously evaluated for limits of detection (LOD) and cross-reactivity with fentanyl derivatives in a laboratory setting [15]. The fentanyl LFI dipsticks were determined to have an LOD of 25 ng/mL, and the fentanyl LFI cassettes were determined to have an LOD of 75 ng/mL. In addition, both LFI configurations cross-reacted with several commonly encountered fentanyl derivatives (e.g., acetyl fentanyl, furanyl fentanyl, methyl fentanyl). In the previous study, it was also determined that the LFIs were able to detect fentanyl in biofluids (e.g., saliva, urine) of experimental rabbits exposed to fentanyl [15]. The intent of this study was to investigate the use of LFIs in operational settings for drug chemists and forensic toxicologists. Even though the LFIs are designed for and are labeled for use in different biological matrices (urine and saliva), the evaluation provides support for the potential of these LFIs to identify the presence of fentanyl in a rapid, cost-effective, and reliable way, in seized illegal drug samples and in postmortem urine of suspected overdose cases.

Highlights

- Lateral flow immunoassays (LFIs) provide presumptive results on samples in a matter of minutes.
- LFIs detected fentanyl in "real world" seized drug samples.
- LFIs detected fentanyl postmortem urine from suspected fentanyl overdose cases.

2 | MATERIALS AND METHODS

2.1 | Fentanyl-specific LFIs

Two different configurations of fentanyl-specific LFIs, dipsticks (for urine) and cassettes (for saliva), were purchased from Express Diagnostics International (Blue Earth, MN; currently known as HealthCare America Corp.); both LFIs were extensively evaluated in a previous study [15]. Currently, these LFIs are labeled by the manufacturer as "For Forensic Use Only" and do not have approval for in vitro diagnostic use in the United States. Both types of LFIs were used as previously described with slight modifications accounting for the specified test matrix [15]. Both tests (dipsticks, cassettes) are competitive LFIs; following treatment with the test substance, the formation of a single band in the control area indicated a positive result (i.e., the presence of fentanyl above the LFI's LOD), whereas the formation of two bands (in the control area and test area), even if faint, indicated a negative result. If a single band forms only in the test area, the results were considered invalid or inconclusive [15,17]. These assays can also detect the presence of fentanyl or certain fentanyl analogs (e.g., acetyl fentanyl, butyryl fentanyl, crotonyl fentanyl, *p*-fluoroisobutyryl fentanyl, 2-furanyl fentanyl, β-hydroxythiofentanyl, methyl fentanyl) at or above the LOD for each assay/compound [15]. The specific concentration of fentanyl (or analog) is not indicated. Following testing, the results were noted and recorded by a digital camera.

2.2 | Seized drug case sample analysis

A total of 44 seized drug samples from the Anne Arundel County Forensic Services (Millersville, MD) were evaluated using both fentanyl LFIs (dipsticks, cassettes). Of these samples, 32 were determined to contain fentanyl and 12 were determined to be fentanyl-negative by gas chromatography–mass spectrometry (GC-MS) analysis. For the corresponding street samples, the LOD is less than 0.03 mg/mL for the GC/MS that was used to confirm the presence of illicit drugs. All case samples were examined with both LFI types by forensic chemists (licensed by the Maryland Department of Health) trained in using the LFIs and remained in the test laboratory's chain of custody. Specific cutting agents within the substances were not

fully reported for this study. Briefly, drug chemists diluted 10 mg of individual case samples in 1 mL HPLC-grade water (Fisher Scientific; Cat #: W5-1; Hampton, NH) and vortexed for 30 s prior to evaluation. LFI tests were then performed (dipsticks, cassettes) as stated in the Fentanyl-Specific LFIs section above and as previously described [15]. For the fentanyl dipsticks, 100 µL aliquots of each diluted sample were pipetted into the wells of a 96-well plate in triplicate. The LFIs were then dipped into the solution containing the diluted sample for 10–20 s as described in the manufacturer's protocol. Following this, the dipsticks were placed flat on a clean, non-porous surface [15]. For the fentanyl LFI cassettes, the tests were first placed flat and 120 µL of diluted sample (per the manufacturer's recommendation) was pipetted into the sample well of the test cassette [15]. All procedures were performed under the appropriate engineering controls (i.e., chemical fume hood) with the operators wearing the recommended personal protective equipment (i.e., gloves, eye protection). Both the dipsticks and cassettes were scored positive or negative 5–10 min following exposure to the diluted case samples as stated in the manufacturer's protocol. For data analysis, a single positive (of the three sample replicates) was considered a positive detection for that sample; this was the scoring approach due to variation in detection of fentanyl in samples at or near the LOD. As the sample reaches closer to the LFI's LOD, the tests will produce both positive and negative results. Any positive results would indicate the presence of fentanyl in the tested sample above the assays LOD. In addition, calculations were performed to determine sensitivity (ability to determine the presence of fentanyl), specificity (ability to determine the absence of fentanyl), and efficiency (ability to determine the presence or absence of fentanyl) of the fentanyl LFIs for each condition (either seized drugs or postmortem urine) [18]. These calculations were based on the presence of true positives (TP, correct indication of fentanyl), false positives (FP, incorrect indication of fentanyl), true negatives (TN, correct indication that no fentanyl is present), and false negatives (FN, results indicate that no fentanyl is present above the GC-MS detection cutoff). The formulas for these outputs are shown below:

- Sensitivity (%) = (TP)/(TP + FN) × 100
- Specificity (%) = (TN)/(TN + FP) × 100
- Efficiency (%) = (TN + TP)/(TN + TP + FN + FP) × 100

Finally, results were noted and documented with a digital camera.

2.3 | PostMortem urine analysis

A forensic toxicologist at the State of Delaware Department of Safety and Homeland Security, Division of Forensic Science (Delaware DSHS DFS) examined postmortem urine samples from previously concluded cases with both LFI configurations (dipsticks, cassettes). All case samples remained in Delaware DSHS DFS laboratory's chain of custody. Of the 14 urine case samples selected for examination, 10 were confirmed fentanyl-positive and four were confirmed fentanyl-negative. The presence or absence of fentanyl (and other compounds of interest) was determined from toxicology reports that used enzyme-linked immunosorbent assay (ELISA) for preliminary drug screening and GC-MS and/or liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis for confirmatory drug analysis in peripheral blood. In addition, GC-flame ionization detection (FID) was used to determine the presence of ethanol and other volatiles in the peripheral blood; urine was also analyzed with this procedure when the blood screened positive. Preliminary identification of cannabinoids by ELISA was not confirmed through GC-MS analysis. Finally, the urine samples from each selected case were analyzed to confirm the presence or absence of fentanyl by GC-MS; the LOD for this analysis was 1 ng/mL; and the results were qualitatively reported as only as fentanyl positive or fentanyl negative (i.e., fentanyl in excess of the LOD). In a typical medical examiner's investigation, the concentration of fentanyl in the blood is considered the causative factor in determining the cause of death. All urine samples were warmed to room temperature prior to evaluation with either LFI. For the fentanyl dipstick tests, 0.5 mL of urine from individual cases was pipetted into 12 × 75 mm test tubes in a test tube rack in quintuplet prior to evaluation. For the fentanyl cassette tests, approximately 120 µL of urine were dropped into the sample well of the test cassette using a bulb pipette. For data analysis, a single positive LFI (of the five sample replicates) was considered a positive detection for that sample as explained in the previous section. Sensitivity, specificity, and efficiency were determined as stated above. For each test run, positive [2 µg/mL fentanyl, (Cerilliant Corp.)] and negative controls (no substances added) diluted in Certified Drug Free Urine (UTAK Laboratories, Inc.; Cat #: 88121-CDF(L)LTR; Lot #: C3969; Valencia, CA) were run in either duplicate or triplicate. Finally, the results were recorded and documented using a digital camera.

3 | RESULTS

3.1 | Evaluation of fentanyl-containing case samples

Drug chemists from Anne Arundel County Forensic Services analyzed street samples (n = 44) obtained during law enforcement seizures using LFIs (dipsticks, cassettes) (Table 1). These samples were divided into two categories: fentanyl-containing samples (n = 32) and non-fentanyl samples (n = 12). The fentanyl-containing samples included in this study were typically mixtures of various substances including precursors (4-ANPP), opiates (codeine, heroin, morphine), opioids (acetyl fentanyl), stimulants (caffeine, cocaine), and cutting agents (quinine). Only one of the fentanyl-containing samples was straight fentanyl unmixed with other substances. Prior to examination using the fentanyl LFIs, 10 mg of the individual samples was diluted in 1 mL water and characterized as stated above. Both fentanyl LFIs (dipsticks, cassettes) successfully identified fentanyl in all fentanyl-positive case samples (Table 1); no false-negative results were reported. In the fentanyl-negative samples, false-positive results were displayed in three of the 12 cases tested with the fentanyl dipstick LFIs; the common ingredient of these

TABLE 1 Analysis of seized drugs from Anne Arundel County Forensic Services

Fentanyl-Positive Identification by GC-MS	Anne Arundel County Lab Samples (n = 32)	# Positive Samples/Total (%)	
		LFI Dipsticks	LFI Cassettes
Fentanyl	**1**	**3/3 (100)**	**3/3 (100)**
Fentanyl, 4-ANPP	**1**	**3/3 (100)**	**3/3 (100)**
Fentanyl, 4-ANPP, 6-MAM, Acetylcodeine, Heroin, Quinine	**1**	**3/3 (100)**	**3/3 (100)**
Fentanyl, 4-ANPP, Caffeine, Diphenhydramine, Heroin	**1**	**3/3 (100)**	**3/3 (100)**
Fentanyl, 4-ANPP, Caffeine, Etizolam (small amount), Quinine	**1**	**3/3 (100)**	**3/3 (100)**
Fentanyl, 4-ANPP, Caffeine, Heroin, Quinine, Tramadol	**2**	**3/3 (100)[a]**	**3/3 (100)[a]**
Fentanyl, 4-ANPP, Caffeine, Quinine, Tramadol	**1**	**3/3 (100)**	**3/3 (100)**
Fentanyl, 4-ANPP, Diphenhydramine, Etizolam, Procaine, Quinine	**1**	**3/3 (100)**	**3/3 (100)**
Fentanyl, 4-ANPP, Diphenhydramine, Quinine	**1**	**3/3 (100)**	**3/3 (100)**
Fentanyl, 4-ANPP, Quinine	**1**	**3/3 (100)**	**3/3 (100)**
Fentanyl, 4-ANPP, Quinine, Tramadol	**1**	**3/3 (100)**	**3/3 (100)**
Fentanyl, Acetyl Fentanyl, Caffeine	**1**	**3/3 (100)**	**3/3 (100)**
Fentanyl, 6-Acetylcodeine, Diphenhydramine, Heroin, Quinine	**1**	**3/3 (100)**	**3/3 (100)**
Fentanyl (small amount), Caffeine, Heroin, Quinine	**1**	**3/3 (100)**	**3/3 (100)**
Fentanyl, Caffeine, Lidocaine	**2**	**3/3 (100)[a]**	**3/3 (100)[a]**
Fentanyl, Caffeine, Quinine	**2**	**3/3 (100)[a]**	**3/3 (100)[a]**
Fentanyl, Cinchonidine, Quinine	**1**	**3/3 (100)**	**3/3 (100)**
Fentanyl, Cocaine	**1**	**3/3 (100)**	**3/3 (100)**
Fentanyl, Diphenhydramine, Heroin, Lidocaine, Quinine	**1**	**3/3 (100)**	**3/3 (100)**
Fentanyl, Heroin, Quinine	**1**	**3/3 (100)**	**3/3 (100)**
Fentanyl, Quinine	**5**	**3/3 (100)[a]**	**3/3 (100)[a]**
Fentanyl, Quinine, Sorbitol	**1**	**3/3 (100)**	**3/3 (100)**
Fentanyl, Quinine, Sorbitol, Tramadol	**2**	**3/3 (100)[a]**	**3/3 (100)[a]**
Fentanyl, Quinine, Tramadol (trace amount)	**1**	**3/3 (100)**	**3/3 (100)**
Fentanyl-Negative Identification by GC-MS	**Anne Arundel County Laboratory Samples (n = 12)**	**# Positive Samples/Total (%)**	
		LFI Dipsticks	**LFI Cassettes**
Acetaminophen (Tablet), Oxycodone	1	0/3 (0)	0/3 (0)
Benzenamine, Gabapentin Lactam	1	0/3 (0)	0/3 (0)
Benzoylecgonine, Cocaine, Tropacocaine	1	**3/3 (100)[b] False Positive**	0/3 (0)
Cocaine	2	0/3 (0)[a]	0/3 (0)[a]
Cocaine HCl	1	**3/3 (100)[b] False Positive**	0/1(0)[c]
Cocaine, Levamisole	1	0/3 (0)	0/3 (0)
Cocaine, Tetramisole	1	**3/3 (100)[b] False Positive**	0/3 (0)
Etizolam	1	0/3 (0)	0/3 (0)
Oxycodone	3	0/3 (0)[a]	0/3 (0)[a]

The results are expressed as the number of positive tests over the total number of tests examined; the percentage of positive test results is shown in parentheses. Results in bold indicate 100% detection of fentanyl.

Abbreviations: 4-ANPP, 4-anilino-N-phenethylpiperidine; 6-MAM, 6-monoacetylmorphine; GC-MS, gas chromatography–mass spectrometry, n, number of experimental replicates.

[a]Represents duplicate results from multiple samples of the same substances.

[b]Represents false-positive results.

[c]Inconclusive results observed for test replicates.

TABLE 2 Analysis of postmortem urine

Case#	Preliminary Analysis (Peripheral Blood) ELISA	Confirmatory Analysis (Peripheral Blood) GC-MS or LC-MS/MS for Drugs and GC-FID for Alcohol/Volatiles	Fentanyl Conc. (ng/mL) (Peripheral Blood) GC-MS	Fentanyl Urine Analysis GC-MS	# Positive Tests/Total (%)	
					LFI Test Strips	LFI Test Cassettes
Fentanyl Positive Cases						
1	**Fentanyl, Diphenhydramine, Tramadol**	**Fentanyl, Amitriptyline, Nortriptyline, Tramadol**	**34**	**Positive**	**5/5 (100)**	**5/5 (100)**
2	**Fentanyl, Opiate, Benzodiazepines, Cannabinoids, Diphenhydramine**	**Fentanyl, Morphine**	**6.5**	**Positive**	**5/5 (100)**	**5/5 (100)**
3	**Fentanyl, Opiate**	**Fentanyl**	**15**	**Positive**	**5/5 (100)**	**5/5 (100)**
4	**Fentanyl, Cocaine, Opiate, Oxycodone, Tramadol**	**Fentanyl, Benzoylecgonine, Cocaine, Ecgonine Methyl Ester, Morphine, Tramadol, Ethanol**	**17**	**Positive**	**5/5 (100)**	**5/5 (100)**
5	**Fentanyl, Benzodiazepines, Cocaine, Diphenhydramine**	**Fentanyl, Benzoylecgonine, Cocaine, Ecgonine Methyl Ester, Cyclobenzaprine**	**18**	**Positive**	**5/5 (100)**	**5/5 (100)**
6	**Fentanyl, Cannabinoids, Cocaine, Opiate**	**Fentanyl, Benzoylecgonine, Cocaine, Ecgonine Methyl Ester, 6-Monoacetylmorphine, Morphine**	**7.4**	**Positive**	**5/5 (100)**	**5/5 (100)**
7	**Fentanyl, Cocaine**	**Fentanyl, Benzoylecgonine, Cocaine, Ecgonine Methyl Ester**	**11**	**Positive**	**5/5 (100)**	**5/5 (100)**
8	**Fentanyl, Cocaine, Opiate**	**Fentanyl, Benzoylecgonine, Cocaine, Ecgonine Methyl Ester**	**6.2**	**Positive**	**5/5 (100)**	**5/5 (100)**
9	**Fentanyl, Cannabinoids**	**Fentanyl**	**9.8**	**Positive**	**5/5 (100)**	**5/5 (100)**
10	**Fentanyl, Cocaine**	**Fentanyl, Benzoylecgonine, Cocaine, Ecgonine Methyl Ester**	**13**	**Positive**	**5/5 (100)**	**5/5 (100)**
Fentanyl-Negative Cases						
11	Benzodiazepines	Oxazepam, Temazepam, Ethanol	N/A	Negative	0/5 (0)	0/5 (0)
12	None Detected	N/A	N/A	Negative	0/5 (0)	0/5 (0)
13	Cannabinoids, Cocaine	Benzoylecgonine, Cocaine, Ecgonine Methyl Ester, Acetone	N/A	Negative	0/5 (0)	**5/5 (100)**[a] **False Positive**
14	Amphetamine, Cannabinoids, Methamphetamine	Amphetamine, Methamphetamine	N/A	Negative	0/5 (0)	0/5 (0) Very Faint Test Lines

The results are expressed as the number of positive tests over the total number of tests examined; the percentage of positive results is shown in parenthesis. Tests with 100% detection of fentanyl are shown in bold. Confirmation of compounds detected in peripheral blood was based on GC-MS or LC-MS/MS identification for drugs and GC-FID for alcohol/volatiles. Analysis of urine by GC-MS was performed to confirm the presence or absence of fentanyl in urine samples; a positive result was considered the detection of fentanyl above the LOD (1 ng/mL) for the GC-MS analysis. Confirmatory GC-MS analysis was not performed for cannabinoids. Positive and negative controls were performed in either duplicate or triplicate for each test run (data not shown).

Abbreviations: ELISA, enzyme-linked immunosorbent assay; GC-FID, gas chromatography–flame ionization detection; GC-MS, gas chromatography–mass spectrometry; LC-MS/MS, liquid chromatography–tandem mass spectrometry; LFI, lateral flow immunoassay.

[a]Represents false-positive results.

samples was cocaine. It is possible that there could be trace amounts of fentanyl in the samples that were undetected by the GC-MS analysis. Additional verification that fentanyl or a fentanyl analog was not present at detectable levels in the false-positive samples was completed by running the GC-MS datafiles through deconvolution software (AMDIS) using high sensitivity settings. Following examination of case samples with the fentanyl dipstick LFI, the sensitivity was calculated to be 100%. Also, the specificity was calculated to be 75% and the efficiency was calculated to be 93.2%. When the cassette LFIs were tested against fentanyl-negative samples, no false positives were indicated; however, it is interesting to note that inconclusive results were observed for two of the three replicates when examining a sample of cocaine HCl. The sensitivity, specificity, and efficiency were calculated to be 100% for the cassette LFIs in this study. The sensitivity, specificity, and efficiency results for both the dipstick and cassette LFIs for seized drug samples are summarized in Table 3.

3.2 | Evaluation of postmortem urine from medical examiner cases

A total of 10 fentanyl-positive and 4 fentanyl-negative cases were analyzed for this study. The range of fentanyl concentrations detected in peripheral blood for was 6.2–34 ng/mL with a mean and standard deviation of 13.79 ± 8.26 ng/mL for fentanyl-positive cases included in this study. The median peripheral blood fentanyl concentration for these cases was 12 ng/mL. In addition, Case #4 (fentanyl-positive) and Case #11 (fentanyl-negative) were both positive for ethanol in peripheral blood at concentrations of 0.017 and 0.032 g/dL, respectively. Case #13 (fentanyl-negative) was positive for acetone in peripheral blood (0.015 g/dL) and urine (0.019 g/dL). A forensic toxicologist from the Delaware DSHS DFS analyzed urine from 14 postmortem cases with both the fentanyl LFIs (dipstick, cassette). Ten of the analyzed cases were fentanyl-positive, and four were fentanyl-negative as determined through ELISA and GC-MS analysis of peripheral blood (Table 2). In addition, the urine from each case was examined for the presence or absence of fentanyl above the GC-MS LOD (1 ng/mL) and were reported in Table 2. In eight of the 10 fentanyl-positive cases, multiple other drugs (including alcohol) were identified in the peripheral blood. In addition to fentanyl, these cases contained anti-depressants (amitriptyline, nortriptyline), opioids/opioid metabolites (6-monoacetylmorphine, morphine, oxycodone, tramadol), and stimulants/metabolites (benzoylecgonine, cocaine, ecgonine methyl ester). Even though cannabinoids were not confirmed by GC-MS analysis of the peripheral blood, these compounds were found in preliminary ELISA tests. Urine from fentanyl-positive cases displayed positive results (five replicates for each case) with both the fentanyl LFI dipsticks and cassettes (Table 2); no false negatives were reported. There is some separation of results between the dipsticks and cassettes when evaluating the fentanyl-negative case urine. In the four cases examined, the fentanyl LFI dipsticks produced negative results as expected (five replicates for each case). For these samples, the sensitivity, specificity, and efficiency were 100%. When the LFI cassettes were used, there was a false positive reported (Case #13, all five LFI replicates were positive) and a case (Case #14) that produced very faint test lines. The appearance of a second test line (at any intensity) is what the manufacturer indicates as a negative result (or below the LOD). Analysis of the results from the fentanyl cassette LFIs displayed a sensitivity of 100%, a specificity of 75%, and an efficiency of 92.9%. The sensitivity, specificity, and efficiency results for both the dipstick and cassette LFIs for postmortem urine are summarized in Table 3.

4 | DISCUSSION

Given the enormity of the opioid crisis and the frequency with which fentanyl is observed in mixtures of street drugs, the need for rapid, simple, and cost-effective fentanyl detection technology is urgent. Since LFIs are a proven, low-cost detection technology, the work described in this manuscript is a first step toward understanding whether use of a fentanyl-specific LFI could work to test real-world mixtures encountered by a forensic laboratory or medical examiner. By testing both actual street drug mixtures and urine from drug overdose victims with two common LFI configurations (a dipstick for urine and a cassette for saliva), we were able to describe the utility of the two configurations in different mixture matrices, from the perspective of both a controlled substance and medical examiner's laboratory.

Overall, both LFI configurations (dipsticks, cassettes) performed with a sensitivity of 100% in the complex street drug mixtures tested, and from the urine samples from overdose victims. No false negatives were reported with either the dipsticks or cassettes in either type of sample. It is interesting to note that three false positives were observed with the dipstick LFIs in the street drug samples. All of these false-positive results with the dipstick contained cocaine as one of the ingredients although three additional samples containing cocaine did not give false-positive results. While testing the cassette LFIs, a single overdose victim's urine (Case #13) gave a consistent

TABLE 3 Summary of results for sensitivity, specificity, and efficiency of the LFIs (dipstick, cassette) used in this study

	Sensitivity (%)	Specificity (%)	Efficiency (%)
Seized drug samples (n = 44)			
Fentanyl Dipstick LFI	100	75	93.2
Fentanyl Cassette LFI	100	100	100
Postmortem urine samples (n = 14)			
Fentanyl Dipstick LFI	100	100	100
Fentanyl Cassette LFI	100	75	92.9

Abbreviations: LFI, lateral flow immunoassay; n, number of experimental replicates.

false-positive reading over five replicates; this sample also tested positive for cocaine. In addition, a sample from another victim (Case #14) resulted in faint test bands (slight false positive) on the cassette LFIs; however, did not test positive for cocaine. While the reason(s) for the false-positive results is not entirely clear, there are several possibilities that could contribute alone or in concert to the failure of the tests. For the dipsticks, it is possible that the presence of cocaine (or some other undetected component) caused a change in pH that may have affected the results of the assays. Although optimal pH is not indicated in the manufacturer's protocol for the LFI, it is known that alterations in pH are known to affect how an antibody binds to its specified target. In the case of the cassette LFIs, the reason could be slightly different. These assays were designed for the composition (i.e., proteins, pH) and viscosity of saliva, and it is possible that something different about those two urine samples allowed the samples to flow differently across the test line; therefore, altering the results. In addition, since Case #13 was positive for acetone in peripheral blood (0.015 g/dL) and urine (0.019 g/dL), it is possible that the acetone or a metabolic breakdown product in the urine was able to dislodge the fentanyl bound on the test line in the cassette, therefore, giving a false-positive result. Finally, for both the dipsticks and cassettes, it is possible that another undetected component in the sample competed with the labeled fentanyl bound on the test line, causing the blank test line (false positive). There is evidence for these types of occurrences in immunoassays. A recent publication has shown that certain antibiotics (e.g., levofloxacin) can induce false positives in immunoassays designed to detect opiates in urine samples [19,20]. In these urine samples, it is unknown whether antibiotics are present in the samples; it is certainly possible that the false positives observed in this study could have contained one or more of these interfering substances.

First responders, crime scene examiners, and forensic scientists are all in need of rapid and cost-effective procedures for the identification of fentanyl; based on the recent data, LFIs seem to fill this need. In addition, LFI configurations can be safely used at a crime scene or in a controlled laboratory environment as a presumptive test for the presence of fentanyl. In comparison with historical chemical color tests, LFI configurations are less subjective and more specific for fentanyl. These configurations can also produce rapid results (within minutes) and do not utilize any harsh or caustic chemicals. Also, these configurations could be used by Medical Examiner personnel during the course of a death investigation to provide preliminary results to the investigators. For other applications, some states require the collection of urine for suspected driving under the influence (DUI) cases. It is possible that these LFIs could be used as a preliminary screen for these cases. In addition, Customs and Border Protection Officers inspect and seize goods that are suspected of being used to conceal contraband coming from overseas. LFIs could be used in this situation by swabbing the outside of packages/containers to determine if fentanyl is hidden in a package or shipping container. Overall, these LFI configurations have the potential to assist first responders and investigators for numerous applications.

5 | CONCLUSIONS

These data point to the utility of LFIs as a quick, low resource-dependent, and low-cost option for presumptive detection of fentanyl at a crime scene, in an operationally relevant situation, or in a resource austere environment for forensic identification. These assays could be deployed to provide an additional detection tool for the appropriate end users.

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