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A lot testing protocol for quality assurance of fentanyl test strips for harm reduction applications

Hirudini Fernando[1], Anita Amate[1], Kathleen L. Hayes[1], Heather D. Whitehead[1], Charlie Desnoyers[1], Emmanuel Uzobuife[1], Madison S. Denchfield[1], Braden Whitelatch[1] and Marya Lieberman[1*]

Abstract

Fentanyl test strips (FTS) are lateral flow immunoassays that were originally designed and validated for detecting low concentrations of fentanyl in urine. Some FTS are now being marketed for the harm reduction purpose of testing street drugs for the presence of fentanyl. This manuscript provides a simple protocol to assess whether different brands and lots of fentanyl test strips perform adequately for use in drug checking. The results gathered from this protocol will document problems with particular lots or brands of FTS, help buyers choose from among the array of products, provide feedback to manufacturers to improve their products, and serve as an early warning system for ineffective products.

Introduction

Fentanyl test strips (FTS) are competitive lateral flow immunoassay tests designed for detection of ng/mL levels of fentanyl in human urine; when used for this purpose, they are regulated by national medical regulatory authorities (MRAs) as medical devices. As discussed in the accompanying Commentary article [1], there has been a rapid uptake of FTS for drug checking, which involves different sample preparation methods, matrices, concentrations, and interferences than urine testing. Due to the rapid expansion of the drug checking market, there is also a risk that bad manufacturing or distribution practices could lead to high failure rates or unreliable performance in some brands or lots of FTS. We argue that these risks can be mitigated by a coordinated community lot checking program.

This manuscript has two main aims: first, to offer a minimal protocol to assess sensitivity, usability, and interferences for different brands or lots of FTS, and second, to provide a scientific framework for evaluating and communicating the results. The protocol proposed for testing focuses on product characteristics that are important for drug checking applications. Our goal is to fill a void in the regulation of FTS that are being marketed and used for drug checking applications, not to compete with or supersede any governmental regulatory functions. In particular, we do not intend this protocol to evaluate the suitability of FTS for use in urinary drug testing, as national medical regulatory agencies such as the Food and Drug Administration (FDA) or Health Canada already regulate products for that application. We also do not intend this protocol for evaluation of seized drug

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*Correspondence:
Marya Lieberman
mlieberm@nd.edu
[1] Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556, USA

samples in a forensic context, as our proposed methodology is not designed to answer forensic questions.

The main characteristics that make a product suitable for drug checking are:

(1) Adequate information is provided on the packaging of each strip to identify the brand, manufacturer, and lot or batch;
(2) The packaging protects the product adequately for field use;
(3) Instructions suitable for drug checking applications are provided;
(4) The FTS can detect fentanyl at 200 ng/mL in deionized (DI) water;
(5) The FTS does not give false positives for water, or for common interferences at concentrations relevant to sample preparation methods used for drug checking.

The proposed lot checking protocol is highlighted in Table 1; briefly, information is collected to record who did the testing, what brand/lot was evaluated, and whether the packaging and instructions were suitable for drug checking applications. Next, the results of assessing five strips with a true positive sample of 200 ng/mL fentanyl and five strips with a true negative sample are recorded. Optionally, results from up to seven known FTS interferences are recorded starting at a concentration of 2 mg/mL and proceeding to concentrations of 0.7, and 0.2 mg/mL if a false positive result is observed. While street fentanyl is usually present as an HCl salt, commercial solid fentanyl standards are often the citrate salt; for fair comparison of these chemical forms, the concentration of fentanyl for this protocol is always expressed as the concentration of the free base.

The concentrations of the test samples in the protocol are relevant to the lowest concentrations of fentanyl and highest concentrations of interferants that would reasonably occur with common field sample preparation methods that are used for drug checking. Many sites perform FTS testing on cooker residue from injectable opioids [2, 3]. We argue that if a strip can detect fentanyl at 200 ng/mL, it should be fit for analysis of fentanyl in cooker residues. The goal of testing the cooker residue is to discover fentanyl that is present in the drug at or below a level that could cause an overdose. A typical preparation

Table 1 Fentanyl test strip metadata (link to password-protected data entry form: https://tinyurl.com/FTSgoodbad)

A. Who did the testing?
Name, contact information, affiliation, potential conflicts of interest (association with or funding by FTS manufacturers/distributors or law enforcement agencies)
B. Where did the FTS come from?
Stated brand name, lot number, expiration date
Evidence that the product is being marketed for drug checking applications
Manufacturer, distributor, price paid, other supply chain information
C. Based on your experience in using FTS for drug checking, is the packaging suitable for drug checking applications?
Does the foil packet list the manufacturer, distributor, brand name, product name, lot number, and expiration date?
Are the strips packaged adequately (eg, sealed foil packet with desiccant)?
Are directions for use available?
Are directions for use adequate?
Does the user need a cell phone or computer to view the directions?
Are the directions for sample prep suitable for drug checking applications?
Are the instructions for reading results clear?
What is the claimed limit of detection (LOD) for fentanyl?
What information is provided about interferences?
Are there other problems with the packaging–bad printing, incompletely sealed package, unreasonable claims?
D. Do the strips work correctly?
a. FENT1-FENT5: Does this batch of FTS detect fentanyl at 200 ng/mL? Test 5 strips. A positive result requires a strong control line and no visible test line
b. WAT1-WAT5: Does this batch of FTS give clear negative results with water? Test 5 strips. A negative result requires easily visible control and test lines
c. Interferences: (optional) Does this batch of FTS give false positive results with common interferences? We suggest testing each of the following drugs: diphenhydramine HCl, procaine HCl, lidocaine HCl, levamisole HCl, methadone HCl, methamphetamine HCl, and MDMA HCl, at concentrations of 2.0 mg/mL. If a false positive is observed for a substances, the strips should then be tested at concentrations of 0.7 mg/mL and 0.2 mg/mL
Documentation: upload lab notes and photographs of the FTS

for a dose of an injectable opioid is 10 milligrams (mg) of bulk material dissolved in one milliliter (ml) of water. If the bulk material contains 1% fentanyl by weight (%w/w), the fentanyl dose would be 0.1 mg, which is below the quantity associated with fatal overdose risk [4–7] but may cause perceptible effects to the user. Assuming 10 µl of the injection (about a fifth of a drop) are left in the cooker, there would be 0.1 mg of the bulk drug, or 0.001 mg (1000 nanograms) of fentanyl. Addition of 1 mL of water to the cooker will result in a fentanyl concentration of 1000 ng/mL. The 200 ng/mL level chosen for our "true positive" standard sample would give an additional margin of error. False positives from cutting agents such as diphenhydramine are unlikely for cooker residues, because the concentrations of interferences will be in the neighborhood of 0.1 mg/mL or less. Likely interferences should be evaluated at a concentration of 0.2 mg/mL to provide an additional safety margin for samples prepared from cooker residues.

Another common sample preparation method is to mix powdered drugs or crushed pills with some quantity of water. Directions in the literature [8] and in the instructions of different brands of FTS vary greatly, but in cases where a larger mass of the drug material would be ingested (eg, cocaine could be used in gram quantities), it is desirable to be able to identify fentanyl present at less than 0.1% w/w. The directions for preparation of solutions of powders or crushed pills produce solutions of the bulk drug at concentrations between 0.2 and 10 mg/mL. For samples that are prepared by diluting one 10 mg scoop of powder with 5 mL (or about 1 teaspoon or bottle cap full) of water, the nominal concentration of the powder is around 2 mg/mL (2,000,000 ng/mL for the bulk drug, 2000 ng/mL for a component present at 0.1%). For samples prepared with one 10 mg scoop diluted with 15 mL (1 tablespoon) water, the nominal concentration of the powder is 0.7 mg/mL (700,000 ng/mL for the bulk drug, 700 ng/mL for a component present at 0.1%). The concentrations of fentanyl in these solutions would be well above the 200 ng/mL level for a clinically dangerous percentage of fentanyl in the drug material, so false negatives are not the main concern. However, false positives are a concern because many bulk drugs or common cutting agents are potential interferences and could be present at relatively high concentrations [9]. Interferences should be tested at 2.0 mg/mL and 0.7 mg/mL for these sample preparation methods. 10 mg/mL interference levels should be assessed only if a site uses a 10 mg/mL nominal concentration for sample preparation.

This protocol requires access to solutions of fentanyl and optionally, interferences. The interferences include four legal pharmaceuticals, which are readily available to testers, and solid forms of three illicit substances, which can be obtained legally by testers in the US whose labs are licensed by the Drug Enforcement Agency (DEA). Testing sites may be able to arrange access to reference materials and equipment by partnering with a local academic or forensic lab. Safety and disposal issues for controlled substances must be addressed at each site. The materials and methods section provides suggestions that sites can adapt into site-specific standard operating procedures.

Information is currently being collected and shared in a password-protected Google form (link: https://tinyurl.com/FTSgoodbad). Participants can register and receive the password by contacting the corresponding author. Registered participants can view and download the responses. The spreadsheet feeds a simple, publicly viewable dashboard (link to dashboard: https://tinyurl.com/LotResults) and a list of products where testing information is needed.

Materials and methods

Equipment and supplies

Supplies needed for sample preparation are a 2–20 µL automatic pipet, a 100–1000 µL automatic pipet, pipet tips, an analytical balance (should read to 0.1 mg place) and calibration weights as recommended by manufacturer. The analytical balance should be checked against calibration weights once a year or any time the balance is moved. The pipettes should be checked by weighing dispensed water once a year. For lot checking, supplies needed are the appropriate test solutions (~1 mL portions in Eppendorf tubes), clean paper or paper towels to lay the strips on, a timer, a cell phone camera, and internet connectivity to upload results. Use of a visual score card, such as the VSCGC40 (Lateral Dx, UK), is recommended for scoring of faint lines.

Safety and storage issues for 200 ng/mL fentanyl solution

Aliquots of 1.5 mL of the 200 ng/mL fentanyl solution, a quantity suitable for testing 10 strips, can be dispensed into labeled, dated Eppendorf tubes or small vials. Each aliquot contains a total of 0.3 µg of fentanyl and can be stored in a refrigerator for up to 16 months. As a benchmark, the amounts of fentanyl citrate in typical oral formulations for breakthrough pain range from 200 to 1600 µg, and doses of 50–100 µg are used for pain relief in dental surgery. To reduce the risk of diversion or accidental ingestion, a bittering agent such as denatonium benzoate (Bitrex®) can be added to the fentanyl solution at a concentration of 1 ppm (threshold for bitter taste is 0.05 ppm). At 1 ppm, denatonium benzoate does not interfere with the FTS, but renders the solutions extremely unpalatable.

Waste disposal

DEA-licensed research labs can include leftover solutions containing controlled substances in their regular waste stream. Other sites can use a commercial drug disposal product such as Deterra® pouches, or adhere to the published FDA guidance for disposal of drugs outside a lab or pharmacy take-back setting [10].

Preparation of true positive fentanyl solution

The 200 ng/mL fentanyl sample can be prepared from an analytical reference standard of fentanyl or from solid fentanyl salts. Reference standards at 0.1 or 1.0 mg/mL in methanol are available to labs that do not have DEA licensing to purchase controlled substances. Alcohols can interfere with FTS results at concentrations above 10%. However, dilution of the fentanyl standard to 200 ng/mL dilutes the methanol to below 0.2%, so it does not interfere. Labs with DEA licensing can use solid fentanyl citrate or fentanyl HCl; these compounds have large difference in formula weight due to the counterions. The reported fentanyl concentration should be based on the concentration of the free base. The instructions below compensate for the presence of the citrate or chloride counterion. The source of the fentanyl standard and its lot number and expiration date should be recorded in the lab notes. Tap water that contains high levels of divalent cations ("hard" water) is known to reduce the affinity of the antibody for its hapten. Therefore, deionized (DI) or distilled water should be used for preparing the fentanyl samples.

Preparation of 200 ng/mL fentanyl from 1 mg/mL fentanyl standard in methanol: Pipet 10 µL of standard into 990 µL of DI water, mix well. Concentration is now 10 µg/mL. Pipet 20 µL of this into 980 µL of DI water; concentration is now 200 ng/mL. Label and date; solution may be stored for at least 16 months in refrigerator.

Preparation of 200 ng/mL fentanyl from 0.1 mg/mL fentanyl standard in methanol: 10 µL of standard into 90 µL of DI water, mix well. Concentration is now 10 µg/mL. 20 µL of this into 980 µL of DI water; concentration is now 200 ng/mL. Final methanol concentration is 0.2% v/v. Label and date; solution may be stored for at least 16 months in refrigerator.

Preparation of 200 ng/mL fentanyl from fentanyl salts: Fentanyl citrate or fentanyl HCl are powerful opioids, and the lab should have appropriate SOPs, engineering controls, and personal protective equipment in place. Narcan should be available and the lab technician should not work alone. Weigh 10 mg of fentanyl citrate, preferably by difference, and add 0.636 mL water for every 1.0 mg of fentanyl citrate to make a 1.0 mg/mL primary stock solution of fentanyl. If fentanyl hydrochloride reference material is used, add 0.902 mL water for every 1.0 mg of fentanyl HCl to make the 1.0 mg/mL primary stock solution of fentanyl. Label and date. This primary stock is good for at least 16 months in refrigerated storage.

Preparation of 200 ng/mL fentanyl from 1.0 mg/mL fentanyl standard: Pipet 10 µL of the 1.0 mg/mL primary standard solution into 990 µL of DI water, mix well. Concentration is now 10 µg/mL. Add 20 µL of the 10 µg/mL secondary standard to 980 µL of deionized (DI) water and mix well; concentration is now 200 ng/mL. Label and date; solution may be stored for at least 16 months in refrigerator.

Preparation of true negative samples

True negative samples consist of plain water. The type of water used and whether it is "hard" or "soft" water should be recorded in the "lab notes" section of the form.

Preparation of interferent solutions

Interferants are divided into two groups: non-controlled and controlled substances. Diphenhydramine HCl, procaine HCl, lidocaine HCl, and levamisole HCl are available from chemical suppliers as high-purity chemicals and as primary or secondary pharmaceutical reference materials. These drugs should be tested as their hydrochloride salt form, as the free base forms are less soluble in water. The concentrations are calculated as the mg/mL of the HCl form. To prepare the sample for testing, an analytical balance should be used to weigh approximately 40 mg of the HCl salt, to which the necessary number of mL of DI water is added to obtain a 2 mg/mL solution. This solution can then be diluted 1:2 with DI water to obtain a 0.7 mg/mL solution and 1:9 to obtain a 0.20 mg/mL solution.

Methadone HCl, methamphetamine HCl, and MDMA HCl are controlled substances that are available in pure, solid form only to labs with DEA licenses. The solutions must be prepared from pure solids. Analytical reference standards, such as 1 mg/mL solutions in methanol or acetonitrile, cannot be used to prepare the interference standards, because these solutions will not be diluted enough and the organic solvent will interfere. The concentrations of the interferences are calculated as the mg/mL of the HCl form.

All three of these substances are chiral. Methadone HCl and MDMA HCl should be used in the racemic form. Methamphetamine HCl is available as the D, L or racemic form. In street drugs, methamphetamine is preferred (and most common) as the D isomer, but some chemical syntheses produce racemic products. The D isomer is preferred for interference testing, but sites may choose to test the racemic form instead. Specify the source, chirality, and lot number used; if seized drugs or street drugs

are used, record what you know about the purity of the sample from other analyses.

Preparation of 2.0 mg/mL solution: weigh 40 mg of solid and add 20 mL of DI water, mix well. All the solid should dissolve. Label, date, store in fridge.

Preparation of 0.7 mg/mL solution from 2 mg/mL aqueous solution: Add 300 µL of the 2 mg/mL solution to 600 µL of DI water.

Preparation of 0.20 mg/mL solution from 2 mg/mL aqueous solution: Add 100 µL of the 2 mg/mL solution to 900 µL of DI water.

General testing methodology

At each site, at least 5 strips should be tested for the true positive and true negative samples, and optionally, one strip for each of the interferences, so full testing at a site will require up to 31 strips. Strips should ideally be tested at 2–5 sites, as described in the Data Analysis section.

Testing temperature should be 20–25 °C. Immerse the base of the strip in the solution for 15–30 s, as directed by the product instructions, then remove the strip and let it lie flat on a clean paper towel to develop. Strips should be read and photographed within the manufacturer's recommended time frame (generally between 3 and 15 min, but check the instructions).

Fentanyl test strips are competitive lateral flow strips. If there is no fentanyl present (negative result) the test line should be visible. If there is more than 200 ng/mL fentanyl present (positive result), the test line should not be visible. As noted in manufacturer instructions, any visible line, even a very faint one, should be counted as a negative result. Figure 1 shows a portion of a visual score card, which contains printed lines of different intensity. The line indicated as a value of 1 is often too faint for visual recognition, and lines with intensities 1 or 2 are likely to be misread in poor lighting, so these outcomes should be noted.

Use of a standard visual score card, such as the Lateral Dx VSCGC40, is recommended. It will make the reading process more reproducible from person to person and help to convey what is meant by a "faint" line.

Data analysis

The following recommendations are based on published guidelines for sampling strategies and acceptance criteria for pharmaceuticals and other medical products [11]. Although we would like every test strip to work perfectly, perfection is not a reasonable expectation for any product. We define a "good quality" product as one in which at least 95% of the strips give a positive result for 200 ng/mL fentanyl, and at least 95% of the strips give a negative result for water. The 95% criterion is a common analytical metric and some manufacturers explicitly define their limit of detection as the fentanyl concentration for which 95% of the strips give a positive response. Newton et al. note that random sampling can accurately identify the prevalence of bad quality samples in a pool of good quality samples, but a prohibitively large number of samples (about 390) is required [11]. Since our goal is simply to decide whether a given lot of FTS should be accepted for use by HROs, our recommended data analysis strategy for both the positive samples and the negative samples is based on a combination of a fast screen of 10 strips, followed by analysis of 15 additional strips using a lot quality assurance criterion for lot acceptance.

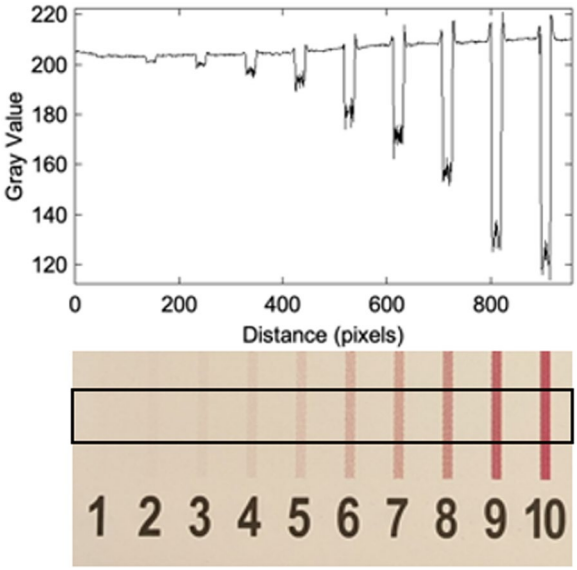

Fig. 1 Visual score card. Top: integrated grayscale intensity of the 10 lines shown in the bottom panel. Bottom: cropped section of visual score card showing the region that was integrated in the top panel

(1) Fast screen: The goal of the fast screen is to quickly identify products that don't work. The chance that a certain number of errors will be observed in a given number of tests is calculated using the binomial theorem. Ten strips from the selected lot are used to test 200 ng/mL fentanyl. If the accuracy is 95% or better, the error rate of the product should be less than 5%. If ten strips from a lot are tested and the error rate for the lot is 0.05, the chance of observing 0 errors is 59.9%, the chance of 1 error is 31.5%, the chance of 2 errors is 7.5%, and the chance of three or more errors is 1.1%. Thus, if three or more errors are observed, the chance that the lot's error rate is at or below the target error rate is less than 1.1%, and the lot should be immediately rejected. The screening protocol is next repeated with a negative sample (water) and ten additional strips. If the

lot passes both of these screening procedures, we know it meets a minimal level of effectiveness.

(2) Lot quality assurance testing: If fewer than three errors are observed in either of the fast screens, it does not adequately establish the quality of the lot. An additional 15 strips from the brand/batch must be tested. This requirement is based on lot quality assurance sampling (LQAS) methodology [12]. LQAS provides a statistically rigorous test of whether the performance of a suspect lot is significantly worse than the expected 95% correct performance of a "good" lot. In the LQAS method, a fixed number n of samples is tested, and if more than a critical value d fail the tests, the whole lot fails. For our lot checking process, n is 25 strips (the 10 used in the fast screen, plus 15 additional strips). If four or more of these 25 strips fail, the lot fails. The assumptions used in calculating n and d, using equations described in Lemeshow and Taber [13], are explained in Table 2. The same methodology is followed for true negative results with water. If the lot passes both of these assessments, it is acceptable. The total number of strips that must be tested for lot acceptance is 50.

(3) The testing sites are asked to check the results of common interferences at concentrations of 2.0 mg/mL, 0.7 mg/mL, and 0.20 mg/mL on single test strips, although some sites may not be able to perform all these tests. If an interference gives a false positive at 2.0 or 0.7 mg/mL, it is not necessary to test lower concentrations. A statistical model for lot acceptance is not suitable here because different drug checking sites focus on different types of drugs (eg, pills used at music festivals vs. injectable drugs) and employ different sample preparation methods. However, the results obtained at multiple sites will help harm reduction organizations judge whether a given brand/lot might be suitable for their anticipated use case. Up to 21 additional strips are required for interference testing. Additional interferences may be tested and results reported in the "lab notes" section.

(4) In order to rule out site-specific errors, such as problems with a reference standard, it is preferable that strip failures for a particular lot of FTS be observed by at least two testing sites before the lot is flagged as a problem.

At the time of publication, nine lots of FTS and one lot of a dual FTS/XTS strip have undergone the fast screen, and five brand/batch combinations have undergone the full LQAS testing (Table 3). All the strips gave correct responses with water. With 200 ng/mL fentanyl (FEN), two lots showed some false negatives in the fast screening stage, although the number of failures did not rise to the level where the brand/batch would be rejected. All of these failures were false negatives due to very faint test lines (visual score card reading of 1). Faint test lines are difficult to interpret; some people might read these strips as positives, particularly in poor lighting conditions. However, since manufacturer instructions stress that any faint line should be interpreted as a negative result, we scored the faint lines as false negative results.

Two lots of FTS failed the lot quality assurance sampling, one with 9 errors out of 25 FEN samples, the other with 7 errors out of 25 FEN samples. All of these failures were false negatives due to very faint test lines (visual score card reading of 1).

Five of the lots showed false positives from diphenhydramine. Two lots showed very faint test lines (visual score card reading of 1 or 2) with other interferants; technically, these are correct negative results, but some people might read these strips as false positives, particularly in poor lighting.

Table 2 LQAS lot performance criteria

We here define the lot performance thresholds for FTS as outlined in Lemeshow and Taber [13]. The sample sizes n and values for d were calculated using the equations on page 130 of this reference

Po is the proportion of strips that fail to give a true positive result for 200 ng/mL fentanyl (or that fail to give a true negative result for a water blank)

Pa is the "acceptable" failure rate of 0.05

α is the chance of accepting a bad batch of strips

β is the chance of rejecting a good batch of strips

n is the sample size

d is the maximum number of failed strips that will not result in rejection of the lot

Po is set as 0.30 and Pa as 0.05, which means it is unacceptable for more than 30% of strips in a lot to fail, but acceptable for 5% or fewer of strips in a lot to fail. We accept a 3% risk that a bad quality lot will be accepted, and a 3% risk that a good-quality lot will be rejected. The number of samples needed for testing is 25, and d = 3. If four or more errors are observed after testing 25 strips from a lot, the lot should be rejected

Table 3 Initial lot checking results

Brand/Batch number	Fast screen errors	LQAS errors	Interferences
BTNX Rapid Response D805010, tested 4 years post expiration	1*	9*(fail)	Diphenhydramine at 2 mg/mL
BTNX Rapid Response DOAA2309301	2*	7*(fail)	Diphenhydramine at 0.7 mg/mL
BTNX Rapid Response DOA2211008	0	1*(pass)	None
DanceSafe D3180516	0	0(pass)	Diphenhydramine at 0.2 mg/mL
DoseTest FYL24030005	0	0(pass)	weak test lines (score card reading of 1-2) from 2 mg/mL meth or levamisole
WiseBatch W2308010	0	1*(pass)	Diphenhydramine at 0.7 mg/mL
BTNX Rapid Response DOA2210012	0	–	Diphenhydramine at 2 mg/mL
Bunk Police K3081344	0	–	Diphenhydramine at 0.2 mg/mL
DSG Fentanyl/Xylazine Dual Test D2306281	0#	–	None#
STAT Fentanyl TestH0206240018	2	–	Diphenhydramine at 1 mg/mL

*Error due to very faint test line (visual score card reading 1)

Only the fentanyl test line was evaluated here

Three caveats should be noted. First, fentanyl test strips have a limited product lifetime. The Rapid Response D805010 lot, which failed LQAS testing, was 4 years past expiration at the time it was tested, so degradation of the performance is not surprising. Second, all of the LQAS errors seen with the Rapid Response products consisted of very faint test lines (visual score card reading of 1), which as previously mentioned are difficult to interpret and might not have been visible in a poorly lit field setting. Finally, the results represent testing in one lab; participation by other academic labs and harm reduction sites will increase the robustness of these findings [14].

Conclusions

A simple protocol is proposed to evaluate the quality of fentanyl test strips being sold for drug checking applications. The results will provide feedback to manufacturers to improve their products, help harm reduction organizations avoid test strips that are not suitable for drug checking, and serve as an early warning system for ineffective products.

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Availability of data and materials
Data from lot checking is provided at https://tinyurl.com/LotResults.

Declarations

Ethics approval and consent to participate
N/a.

Consent for publication
N/a.

Competing interests
ML serves on the advisory board for the Massachusetts Drug Supply Data Stream (MADDS).

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