

INTRODUCING A NEW AND IMPROVED FENTANYL TEST STRIP FOR THE HARM REDUCTION COMMUNITY

- No Known False Positives
- Higher Sensitivity to Fentanyl

Non-Profit Pricing

Full Transparency

DANCE AFE

- Available now at dancesafe.org/fentanyl
- Plain, white-label packaging will also be available. Call (888) 636-2411 Ext. 2 for more information.

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WHAT'S DIFFERENT?

NO KNOWN FALSE POSITIVES^{*}

DanceSafe is proud to introduce a new and improved fentanyl test strip for the harm reduction community. These new strips, manufactured by the USbased company WHPM, utilize a superior antibody with higher specificity to fentanyl and its analogs. This means that unlike the Rapid Response strips most of us have been using, our new strips do not produce false positives with methamphetamine, MDMA, or methadone (nor with cocaine that contains lidocaine or levamisole), if used according to the instructions. We have confirmed this through field testing, a commissioned study at Kara Lynch's lab at the University of California San Francisco (UCSF),¹ and a research collaboration with Marya Lieberman's lab at the University of Notre Dame (ND).²

HIGHER SENSITIVITY TO FENTANYL

Our UCSF study also determined that these new strips have a sensitivity to fentanyl that is 10 times greater than the Rapid Response strips. A newly published study by researchers from Johns Hopkins University similarly revealed that the Rapid Response strips, advertised to have a detection threshold of 20 ng/ml, actually have a detection threshold of 200 ng/ml.³

NON-PROFIT PRICING

We have contracted with WHPM to be the exclusive supplier of these new strips for drug checking purposes, and as a 501(c)(3) nonprofit we are committed to offering them to the harm reduction community at the lowest possible price. The strips are available to nonprofits and social service agencies right now for as low as \$0.45 each, and it is our commitment that over time, as our own purchasing costs are reduced, we will correspondingly reduce the costs to the harm reduction community.

FULL TRANSPARENCY

There has been a lot of confusion around how to use fentanyl test strips (originally designed for testing urine) for drug checking purposes, how they are manufactured, and what the differences are between the various strips on the market. We would like to set the record straight on all counts.

WHAT YOU NEED **TO KNOW**

NOT ALL FENTANYL TEST STRIPS WORK THE SAME

Fentanyl test strips utilize an immunoassay technology known as "competitive binding." The most important part of this process is the creation of an antibody that binds strongly to fentanyl (sensitivity) but does NOT bind to other drugs (specificity). There are many companies that produce such antibodies, but they all differ in sensitivity and specificity, and most of them will not work for harm reduction purposes.

Below is a summary of the science behind the manufacture and use of fentanyl test strips. A broader overview of fentanyl strip testing as a part of harm reduction is contained in the next section. "HOW THE INDUSTRY OPERATES."

HOW FENTANYL TEST STRIPS ARE MADE

STEP 1: CREATING THE ANTIBODIES

The first step in manufacturing a fentanyl test strip is to create an antibody with high sensitivity and specificity to fentanyl. To do this, scientists first take a fentanyl molecule and attach a protein to it so that it resembles a virus.



Adding a protein molecule to fentanyl makes it resemble a virus.

Next, they inject these molecules into mice.

B-cells inside the mice produce various antibodies against the molecule. These B-cells are extracted from the mice and then attached to myeloma cells in order to produce "hybridomas" that can continue to



produce antibodies outside the bodies of the mice.

Each of these hybridomas produce a 4 unique antibody that attaches to a different part of the fentanyl-protein molecule. The hybridomas are then screened, a process that separates them by type.

With the hybridomas separated, 5 the scientists then test to see which one produces an antibody that binds most strongly to fentanyl (high sensitivity) but does NOT bind to other drugs (high specificity).

They then clone the best performing 6 antibody. The final antibodies are called "monoclonal" antibodies, and are used to make the strips. (Note: All strips on the market were designed for urine testing only, an important fact we discuss more in the next section called "How the Industry Operates".)

STEP 2: CREATING THE STRIPS

The two most important parts of a competitive binding immunoassay strip are the test line and the conjugate pad. The monoclonal antibodies are painted onto the strip to form the test line. Inside the conjugate pad, colloidal gold is fused with a bunch of tiny molecules, which we'll call "fake fentanyl" (a non-technical term to refer to molecules that resemble the binding section of a fentanyl molecule).



If the strip is dipped into plain water (or urine) containing no fentanyl, the antibodies in the test line bind to the fake fentanyl and the colloidal gold produces a red-colored line.



If there is no fentanyl, the antibodies in the test line bind with the fake fentanyl and the colloidal gold, creating a red/pink line. The control line always turns red.

However, if there is enough real fentanyl in the water or urine it will overwhelm the fake fentanyl and react with the antibodies first. In this case, the colloidal gold will move through the test line area and no red line will appear.



first, preventing the colloidal gold from binding there.

This is why the technology is called "competitive binding." If enough real fentanyl is in the liquid (or a fentanyl analog that resembles fentanyl closely enough), it will out-compete the fake fentanyl. The colloidal gold won't bind to the test line area. (The control line contains a different antibody that will react with the colloidal gold regardless of whether there is real fentanyl in the liquid.)



HOW THE INDUSTRY OPERATES

The previous summary describes the basic science of fentanyl testing strips, but not all strips work the same. The quality of the antibody itself is the most important factor, but the ratio and quantity of ingredients embedded in the conjugate pad—as well as the amount of antibody painted on the test linework together to determine the sensitivity and specificity of any given strip. These things dictate 1) the concentration at which fentanyl can be detected and 2) whether or not the strip produces false positives with other drugs at various concentrations.

ASSESSING THE STRIPS

Given that all the fentanyl strips on the market today were originally designed to detect fentanyl in urine, they were only tested to see if they produced false positives with other drugs at the very small concentrations that other drugs are typically found in urine (about 100 micrograms per milliliter). They were never tested for false positives using the far higher concentrations of drugs dissolved in water during a dilution process (milligrams per milliliter), as would be needed for harm reduction purposes, because they were never intended to be used in that way.

As such, we needed to do those false positive tests ourselves - and we did. Back in 2016 DanceSafe commissioned a lab study at UCSF that revealed that the Rapid Response strips caused false positives with meth and MDMA at a concentration of 10 mg/ml, the concentration best able to detect fentanyl and most of its analogs in commonly used drugs.¹ To avoid false positives with meth and MDMA, five times the standard dilution needed to be used, down to 2 mg/ml. This was not ideal, but the Rapid Response strips performed better than any of the others we tested at the time, so we began distributing them with two sets of instructions that differed based on what drugs were being tested.

More recently, our field tests revealed that the Rapid Response strips can also produce false positives with cocaine that contains levamisole and/or lidocaine. This was confirmed by Marya Lieberman's lab at the University of Notre Dame, where it was also learned that the strips produce false positives with methadone at 10 mg/ml.²

A NEW, SUPERIOR ANTIBODY

Around this same time last fall, we were contacted by WHPM, who had recently manufactured new fentanyl strips using a brand new antibody. Our field testing of these new strips demonstrated that they did not produce false positives with meth and MDMA, even at the higher, optimal harm reduction concentration of 10 mg/ml. We then confirmed our observations with a second lab study at UCSF,¹ as well as a research collaboration with Dr. Marya Lieberman at the University of Notre Dame, where we also verified that these new strips did not produce false positives with methadone or with cocaine that contained levamisole or lidocaine.²

Why do the Rapid Response strips produce false positives at practical drug checking concentrations, while the new WHPM strips do not? The answer is probably luck. As previously explained, immunoassay antibodies are produced through a natural process inside the bodies of mice. In all likelihood, a mouse in the WHPM lab just happened to produce an antibody with a higher specificity to fentanyl than the mice in the lab (or labs) that created the antibodies used in the Rapid Response strips.

QUALITY CONTROL AND LOT-TO-LOT VARIABILITY

Strip manufacturers are supposed to perform quality control (QC) tests on a certain number of strips each time they produce a new batch, in order to ensure that the strips' sensitivity to fentanyl matches their advertised claim (being able to detect nanograms per milliliter in urine). Unfortunately, there are many reasons why these QC tests fall short for harm reduction purposes.

First, QC testing for potential false positives are never performed. Strip manufacturers simply rely on the original tests done by the antibody manufacturers, which are conducted using the small concentrations found in urine. Furthermore, strip manufacturers sometimes use totally different antibodies in different batches of strips. They purchase antibodies from multiple sources (whichever is cheapest or available at the time). Because each antibody has a different specificity, the binding affinity for non-fentanyl related drugs might be completely different with each lot.

What does this mean for the harm reduction community?

The most important fact about fentanyl test strips (largely unknown within the harm reduction community until recently) is that any given lot of any given brand of strip may not be made the same way, and might not even contain the same antibody. There are hundreds of companies that produce monoclonal antibodies, and dozens of companies who buy antibodies and use them to manufacture strips (let's call these "partial manufacturers"), as well as thousands of distributors who then buy and resell all these strips.

Because distributors are usually the companies who own the brand, and because distributors often buy their strips from multiple manufacturers-including "partial manufacturers" who, in turn, may buy antibodies from multiple antibody manufacturers—there is no way to know whether any particular lot of any particular brand works the same way. Each could be completely different. Most distributors, for their part, do not disclose the manufacturer (or multiple manufacturers) of their strips, as they are trying preserve their market share and do not want their customers circumventing them.

This phenomenon could account for the lot-to-lot variability seen in other brands of strips. When we spoke to one of these brands they told us the lot-to-lot variability occurred because they buy and resell strips from multiple manufacturers. The lot-to-lot variability was the result of poor manufacturing processes at the factory from which they purchase strips.

Regardless of who is telling the truth here, our recent UCSF study,² as well as a recently published Johns Hopkins study,³ found that some of these other brand strips tested were ten times less sensitive to fentanyl than advertised on the package, and all had different sensitivities and specificities to fentanyl and its analogs than the strips tested in earlier studies.

GUARANTEED LOT-TO-LOT UNIFORMITY

The good news is that WHPM is one of the few companies in the world who produces, exclusively, their own antibodies as well as their own strips. They are what you might call a "full manufacturer" rather than a "partial manufacturer." This means that DanceSafe brand fentanyl test strips will always contain the same antibody, and they will always be manufactured by the same company using the same formula. Additionally, our exclusive distribution contract with WHPM includes an agreement that they will perform harm reduction-based quality control testing on every batch of strips they produce for us, doubly ensuring that every strip meets the higher quality control standards required for drug checking purposes.

PAY ATTENTION TO THE COLOR OF THE STRIP

In order to help clearly identify our new strips, we made them yellow. If the strips you have are blue like the one shown below, or any other color, you have no idea 1) what antibody they contain, 2) the formula that was used to make them, or 3) who manufactures them. The blue strips may be from many different distributors using different formulas.



SETTING STANDARDS BASED ON SCIENCE

Because there are no government agencies regulating fentanyl test strips for harm reduction purposes, we are currently the primary organization performing this function. Without regulations, many manufacturers do not utilize proper quality control standards needed for drug checking. Distributors, as well, often provide no harm reduction instructions with their strips, or they disregard the need for proper dilutions in order to avoid false positives and false negatives. For some, this could be because they do not understand the important differences between urine testing and drug checking. Others may be solely motivated by selling as many strips as possible. Our goal is to bring honesty and transparency to the fentanyl test strip industry, to improve the regulatory standards in manufacturing and distribution throughout the supply chain, and to provide the harm reduction community with more reliable and more affordable fentanyl test strips.

Written by Emanuel Sferios, edited by Rachel Clark, designed by Favorly Agency.

(1) https://dancesafe.org/ucsf-fentanyl-study (2) https://padproject.nd.edu/partners/dancesafe (3) https://www.sciencedirect.com/science/article/abs/pii/S0376871622003477

^{*} Very high concentrations of some drugs can produce false positives with any immunoassay strip, including ours. Although we tested our strips on the most commonly used illicit drugs, as well as known counterfeited pharmaceuticals, there are tens of thousands of other drugs we did not test. When used according to the instructions, our strips do not produce false positives with commonly used drugs and drugs known to sometimes be adulterated with fentanyl.

HOW TO USE OUR NEW FENTANYL TEST STRIPS

BEFORE USING THE STRIPS, READ ALL THE INSTRUCTIONS TWICE.

IMPORTANT

When fentanyl is mixed with other drugs, it is never mixed evenly. Powder from one side of a baggie may contain no fentanyl at all, yet powder from the other side could contain a fatal dose. This is called the "chocolate chip cookie effect" and it's why it is best to test everything you intend to consume.

BEST METHOD

TESTING EVERYTHING YOU INTEND TO CONSUME

This requires dissolving your entire dose in water. For drugs consumed orally, you can simply drink the water after testing. The strips do not contaminate the water. For drugs like cocaine or meth that people like to insufflate (snort), you can get the powder back by evaporating the water.

BACKUP METHOD

TESTING ONLY A PORTION OF YOUR DRUGS

If you choose this method. first crush any crystals, shards, or rocks into a fine powder and mix it up as thoroughly as possible by stirring or shaking the baggie. This will help distribute any fentanyl that might be clumped up inside due to the chocolate chip cookie effect. There's no quarantee, but this increases the likelihood that if fentanyl is inside the baggie, some will be present in your smaller sample. Always have Narcan on hand and never use alone

STEP 1

PREPARING AND DILUTING YOUR DRUGS

Whether you are testing everything you intend to consume or a smaller portion of your drugs, you need to dilute the powder in the correct amount of water. Follow the instructions below for each form of drug **carefully.**

FOR CRYSTALS OR POWDERED DRUGS**

Weigh your drugs. Use a milligram scale to get the weight of the crystals or powder you are going to test. Write it down so you don't forget. If you don't have a milligram scale, you can use one of our 10 mg micro scoops.

A level scoop of finely crushed powder (not rounded) is approximately ten milligrams (available at <u>dancesafe.org/product/micro-scoop</u>).

Place your drugs into a small container. If you are testing 50 mg or less, a standard bottle cap from any 20 oz soda bottle works great. If you are testing more than 50 mg, use a small glass or ceramic cup.

Add one teaspoon of water (5 ml) for every 50 mg of powder.



This equals five level micro scoops (50 mg total) into a standard bottle cap of water (one teaspoon or 5 ml).

You can also use a 5 ml pipette to measure the water (available at <u>dancesafe.org/product/pipette)</u> Whatever you use to measure, make sure the ratio of powder to water is 10 mg/ml.

100 mg into 10 ml 1 gram into 100 ml

20 mg into 2 ml 50 mg into 5 ml

Stir the mixture until completely dissolved. Proceed to STEP 2.

WARNING: If you have the older, blue strips they can give false positives with meth, MDMA and methadone, as well as cocaine that contains levamisole or lidocaine.

FOR PHARMACEUTICAL PILLS

Counterfeit pharmaceutical tablets that contain fentanyl kill thousands of people every year. They can look exactly like the real ones, and there is no way to know whether illicitly-purchased pharmaceuticals contain fentanyl without testing them first.

- **1** Crush the entire tablet into a fine powder.
- 2 Pour the powder into a small cup.
- Add approximately 4 tablespoons or a quarter cup of water.

Unless you have your own prescription, always test pharmaceutical pills. As of 2022, opiolds like Oxycontin and Vicodin are the ones most likely to be counterfeit and contain fentanyl.

4 Stir the mixture well. (Binder material may not completely dissolve. That's ok.) **Proceed to STEP 2.**

FOR PRESSED MDMA ("ECSTASY") TABLETS

1 Use the same steps above for testing pharmaceutical pills.

WARNING: Only the DanceSafe-branded yellow fentanyl test strips work on pressed ecstasy tablets. The older, blue strips will give false positives.

ALWAYS TEST YOUR DRUGS • NEVER USE ALONE CARRY NALOXONE WHENEVER POSSIBLE

FOR BLOTTER LSD

- Cut off a small corner of the blotter.
- Soak it in a teaspoon of water for 10 minutes. Proceed to STEP 2.

FOR IV DRUG USERS

If you inject heroin or other drugs, you should test every time you inject. The easiest method is to test the residue from your spoon or cooker.

- **1** After preparing your shot, set the needle aside and wait to inject.
- 2 Add about 1 ml (1/5th of a teaspoon, or 1 cc) of clean water into the spoon or cooker. Proceed to STEP 2.

STEP 2

USING THE STRIPS

After following STEP 1 to dilute your drugs, it's time to use the strips.

- **1** Hold the yellow end of the test strip and insert the other end into the liquid, no higher than the top of the dotted section.
- 2 Allow the liquid to travel up the strip into the test area. This should take about 15 seconds.
 - Remove the strip and set it down on a flat surface. The results should appear within three minutes. Proceed to STEP 3.

STEP 3

3

INTERPRETING THE RESULTS

One red line on top *after waiting three minutes* is a **POSITIVE** result for the presence of fentanyl.

Two red lines is a **NEGATIVE** result. The lower red line may be significantly lighter than the upper red line. **If you can see it at all after** waiting three minutes, no matter how faint, it is still as negative result.

No red lines (or one red line on the bottom) means the test is invalid.

1 RED LINE = POSITIVE FOR FENTANYL 2 RED LINES = NEGATIVE FOR FENTANYL Do not insert above Capillary action pulls the liquid into the test area IMPORTANT: Sometimes a very faint red line will initially appear in the

IMPORTANT: Sometimes a very faint red line will initially appear in the lower area, then quickly fade away. Do **NOT** confuse this with a negative result. Always wait three minutes before interpreting the results.

** Diphenhydramine will produce false positives with all known fentanyl test strips, even at low concentrations of around 1 mg/ml. Because diphenhydramine is a common cut in illicit opioids, a different method of testing is needed to prevent false positives. This is why we recommend that IV drug users test the residue inside the spoon or cooker they used to prepare their shot. After drawing all the dissolved liquid from the cooker back into the syringe, there should never be an entire milligram of powder residue left over. Therefore, adding one milliliter of clean water back into the cooker will never create a mixture containing 1 mg/ml of diphenhydramine (even if the original powder was 100% diphenhydramine). If there was fentanyl in the powder, on the other hand, enough should remain for the strips to detect, since they react to fentanyl at concentrations 10,000 times lower than diphenhydramine (nanograms per milliliter).



