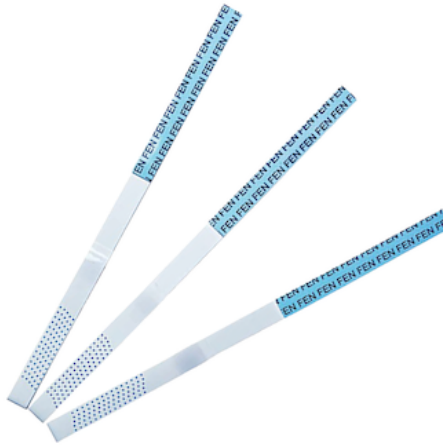


Fentanyl Test Strip Analytical Performance Evaluation

Lateral Flow Immunochromatographic Assay (*Harm Reduction Use*)

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SUMMARY

Fentanyl is a synthetic opioid 50 times more potent than heroin. Legally prescribed for severe pain management, it has become the dominant opioid on the illicit drug market. The proliferation of fentanyl has also resulted in the contamination of many non-opioid drug supplies (such as cocaine, methamphetamine, and ketamine) as well as a dramatic rise in counterfeit oxycodone and other pharmaceutical pills (a.k.a., "fentapills"). Together this has resulted in tens of thousands of accidental overdose deaths and fentanyl "poisonings" per year, many among people who were unaware there was fentanyl in their drugs.

W.H.P.M., Inc., a California-based immunoassay manufacturer, has developed a fentanyl test strip designed to facilitate the rapid, low-cost, and accurate detection of fentanyl in aqueous solutions (H_2O). As fentanyl is often mixed with other illicit substances and cutting agents, researchers have conducted the following studies to assess the fentanyl test strip's analytical sensitivity, its potential cross-reactivity in the presence of high concentrations of common cutting agents and illicit compounds, and its efficacy in detecting prevalent fentanyl analogs.

1. FENTANYL LIMIT OF DETECTION (ANALYTICAL SENSITIVITY STUDY)

MATERIALS AND METHODS FOR LIMIT OF DETECTION

To determine the limit of detection (LOD) for the W.H.P.M. test strips, pure fentanyl citrate was employed as the standard material and deionized (DI) water was the chosen diluent. The DI water yielded a negative result when tested on its own. To ascertain the fentanyl test strips' LOD, fentanyl citrate was introduced into the water at varying concentrations: 5 ng/mL, 10 ng/mL, 15 ng/mL, 20 ng/mL, 50 ng/mL, and 100 ng/mL. Each concentration was tested using five test strips. Notably, the fentanyl standard used was in the pure powdered form and was diluted in DI water at room temperature, without the addition of ethanol or methanol.

Each study device was tested in accordance with the instructions for use with the guidance of the following steps:

1. Dipping Fentanyl Test Strip in solution for 15 seconds
2. Laying Fentanyl Test Strip on a flat surface after dipping
3. Interpret results after 3 minutes

RESULTS FOR LIMIT OF DETECTION

Table 1. Fentanyl Citrate at Different Concentrations.

Test Strip #	5 ng/mL	10 ng/mL	15 ng/mL	20 ng/mL	50 ng/mL	100 ng/mL
1	NEG	POS	POS	POS	POS	POS
2	NEG	POS	POS	POS	POS	POS
3	POS	POS	POS	POS	POS	POS
4	NEG	POS	POS	POS	POS	POS
5	POS	POS	POS	POS	POS	POS

DISCUSSION AND CONCLUSION FOR LIMIT OF DETECTION

The Fentanyl Test Strip was determined to have a Limit of Detection (LOD) of 10 ng/mL of fentanyl citrate in water.

2. ANALYTICAL SPECIFICITY STUDY (CROSS-REACTIVITY)

MATERIALS AND METHODS TO TEST FOR CROSS REACTIVITY

A study was conducted to evaluate the potential cross-reactivity of fentanyl test strips in the presence of increased concentrations of cutting agents and illicit substance compounds in aqueous solutions. The rationale behind this investigation stemmed from the observed occurrence of fentanyl in conjunction with various illicit substances and cutting agents. The primary objective of the study was to assess the performance characteristics of the test strips when subjected to such conditions. The substances, commonly co-detected with fentanyl and included in this study were MDMA, Methamphetamine, Methadone, Lidocaine HCL, Levamisole HCL, Procaine HCL, and Diphenhydramine HCL. Each substance was tested at a range of concentrations: 5 µg/mL, 2 mg/mL, 5 mg/mL, and 10 mg/mL, all substances were diluted in DI water.

Each concentration was tested with 5 fentanyl test strips in accordance with the instructions for use with the guidance of the following steps:

1. Dipping Fentanyl Test Strip in solution for 15 seconds

2. Laying Fentanyl Test Strip on a flat surface after dipping
3. Interpret results after 3 minutes

ANALYTICAL SPECIFICITY STUDY TEST RESULTS

Table 2. Analytical Specificity with Potentially Cross-reacting Substances (*Test results are displayed as Positive Results/Total Results*)

Substance	500 µg/mL	1 mg/mL	5 mg/mL	10 mg/mL
MDMA	0/5	0/5	0/5	0/5
Methamphetamine	0/5	0/5	0/5	0/5
Methadone	0/5	0/5	0/5	0/5
Lidocaine HCL	0/5	0/5	0/5	0/5
Levamisole HCL	0/5	0/5	0/5	0/5
Procaine HCL	0/5	0/5	0/5	0/5
Diphenhydramine HCL	0/5	2/5	5/5	5/5

DISCUSSION AND CONCLUSION TO TEST FOR CROSS REACTIVITY

The study results indicate that Fentanyl Test Strips when testing Methamphetamine, MDMA, Methadone, Lidocaine HCL, Levamisole HCL, and Procaine HCL returns negative results at concentrations of up to 10 mg/mL in aqueous solution. Cross-reactivity with Diphenhydramine HCL was observed at concentrations of 10 mg/mL, 5 mg/mL, and 1 mg/mL, indicating a high likelihood of false-positive results in samples containing relatively high concentrations of a Diphenhydramine HCL cutting agent. Due to this observed cross-reactivity it is vital that sample preparation procedures be followed, and proper sample dilution is ensured prior to testing to avoid the possibility of false-positive results.

3. ANALYTICAL INCLUSIVITY STUDY (ANALOG CROSS-REACTIVITY)

MATERIALS AND METHODS FOR ANALOG TESTING

In the study 51 fentanyl analogs were tested using the Fentanyl Test Strip. The cross-reactivity % indicated in the table 3 below is defined by the following equation: $(\text{LOD}_{\text{Fentanyl}} / \text{LOD}_{\text{Compound}}) \times 100\%$. The analogs were tested in order to determine the Fentanyl Test Strip's ability to detect analogs of fentanyl that may potentially be found in illicit substances. It should be noted that there is little evidence to support that the majority of the analog substances listed below have been discovered in the illicit drug supply. Further information regarding the common fentanyl analogs detected in the current illicit drug supply can be found at: www.drugsdata.org.

Each study device was tested in accordance with the instructions for use with the guidance of the following steps:

1. Dipping Fentanyl Test Strip in solution for 15 seconds
2. Laying Fentanyl Test Strip on a flat surface after dipping
3. Interpret results after 3 minutes

RESULTS FOR ANALOG TESTING

The following outlined in the tables show the results of each respective analog compound on the fentanyl test strips.

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Table 3. Fentanyl Analog Compounds showing various degrees of cross-reactivity.

#	Compound Name	LoD Concentration	Cross-reactivity %
1	Acetyl- α -methyl fentanyl	50ng/mL	20%
2	Acryl fentanyl	40ng/mL	25%
3	α -methyl fentanyl	10ng/mL	100%
4	Benzyl fentanyl	25ng/mL	40%
5	β -hydroxythio fentanyl	10ng/mL	100%
6	Cyclopropyl fentanyl	10ng/mL	100%
7	4-Fluoroisobutyryl Fentanyl	10000ng/mL	0.1%
8	Methoxyacetyl fentanyl	125ng/mL	8%
9	4-methoxybutyryl fentanyl (para)	4000ng/mL	0.25%
10	4'-methyl acetyl fentanyl	250ng/mL	4%
11	3'-methyl Fentanyl	10ng/mL	100%
12	N-methyl norfentanyl	15ng/mL	66.7%
13	o-Fluorofentanyl	25ng/mL	40%
14	p-Fluorobutyryl fentanyl	20ng/mL	50%
15	Tetrahydrofuran fentanyl	5000ng/mL	0.2%
16	2-Thiofuranyl fentanyl	500ng/mL	2%
17	4-Piperidone	25000ng/ml	0.04%
18	2',4'-dimethoxy Fentanyl	25ng/mL	40%
19	3',4'-dimethoxy Fentanyl	5ng/mL	200%
20	meta-fluoro Acrylfentanyl	25ng/mL	40%
21	para-chloro Furanyl fentanyl 3-furancarboxamide	50ng/mL	20%
22	Thiophene fentanyl 3-thiophenecarboxamide	250ng/mL	4%
23	3'-Fluorofentanyl	12.5ng/mL	80%
24	ortho-fluoro Valeryl fentanyl	5000ng/mL	0.2%
25	4-methyl Fentanyl	50ng/mL	20%
26	Cyclopropaneacetyl fentanyl	25ng/mL	40%
27	para-Chloroacetyl fentanyl	50ng/mL	20%
28	para-hydroxy Butyryl fentanyl	15ng/mL	66.7%
29	2'-Fluoro ortho-Fluorofentanyl	100ng/mL	10%
30	meta-methoxy Furanyl fentanyl	250ng/mL	4%
31	3'-fluoro ortho-Fluorofentanyl	50ng/mL	20%
32	2',3'-dimethoxy Fentanyl	10ng/mL	100%
33	2',6'-dimethoxy Fentanyl	25ng/mL	40%
34	3',5'-dimethoxy Fentanyl	2.5ng/mL	400%
35	Acetyl norfentanyl	1000ng/mL	1%

Table 4. Fentanyl Analog Compounds showing no cross-reactivity.

#	Compound Name
1	Isotonitazene
2	O-Desmethyl-cis-tramadol
3	Benzoyl fentanyl (Phenyl fentanyl)
4	Despropionyl para-Fluoro fentanyl
5	N-Phenethyl-4-piperidone(NPP)
6	4-ANPP
7	Despropionyl ortho-Fluorofentanyl
8	AP-237
9	2-methyl AP-237

10	Tianeptine
11	Piperidylthiambutene
12	4-Anilino-1-Boc-piperidine
13	Norcarfentanil
14	2-fluoro Viminol
15	4-Anilino-1-benzylpiperidine
16	Etonitazene
17	AP-238
18	2,3-Benzodioxole fentanyl
19	N-Benzyl-4-piperidone
20	Brorphine
21	4-Anilinopiperidine

DISCUSSION AND CONCLUSION FOR ANALOG TESTING

Within the study there were 35 compounds that demonstrated cross-reactivity to various degrees (Table 3) and are able to be detected by the Fentanyl Test Strip. The other remaining 21 analog compounds did not cross react with the Fentanyl Test Strips (Table 2) at concentrations of up to 100 µg/mL (100,000ng/mL). Fentanyl analogs are commonly identified in illicit substances in the presence of Fentanyl. There is little evidence indicating that fentanyl analogs alone, without the presence of fentanyl, exist in the illicit drug supply of the United States. Given the current prevalence of analogs in combination with Fentanyl, the Fentanyl Test Strip may be considered an effective solution to identify the presence fentanyl, as well as the most common fentanyl analogs, in the current illicit drug supply.

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