One Step Fentanyl Drug of Abuse Test

For Forensic Use Only

INTENDED USE

The One Step Fentanyl Drug of Abuse Test is a lateral flow chromatographic immunoassay for the qualitative detection of Fentanyl in substances or urine at the following cut-off concentration:

TEST	CALIBRATOR	CUT-OFF
Fentanyl (FEN)	Fentanyl	10 ng/mL

This assay provides only a preliminary qualitative test result. Use a more specific alternate quantitative analytical method to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) or Liquid chromatography/mass spectrometry (LC/MS) is the preferred confirmatory method.[1] Apply clinical and professional judgment to Fentanyl test result, particularly when a preliminary positive result is obtained.

SUMMARY AND EXPLANATION OF THE TEST

The One Step Fentanyl Drug of Abuse Test is a competitive immunoassay utilizing highly specific reactions between antibodies and antigens for the detection of Fentanyl in substances or urine without the use of an instrument.

FENTANYL (FEN)

Fentanyl is a potent, synthetic opioid analgesic with a rapid onset and short duration of action.[7] It is a strong agonist at the μ-opioid receptors. Historically, it has been used to treat breakthrough pain and is commonly used in pre-procedures as a pain reliever as well as an anesthetic in combination with a benzodiazepine. Fentanyl is approximately 80 to 100 times more potent than morphine and roughly 15 to 20 times more potent than heroin.[8,9] Fentanyl and its derivatives are used recreationally. Deaths have resulted from both recreational and improper medical use.[10]

The FEN assay contained within the One Step Fentanyl Drug of Abuse Test yields a positive result when the concentration of Fentanyl in substances or urine exceeds **10 ng/mL.**

PRINCIPLE

The One Step Fentanyl Drug of Abuse Test is an immunoassay based on the principle of competitive binding. A drug which may be present in the substances or urine specimen competes against its respective drug conjugate for binding sites on its specific antibody. During testing, a substances or urine specimen migrates upward by capillary action. A drug, if present in the substances or urine specimen below its cut-off concentration, will not saturate the binding sites of its specific antibody. The antibody will then react with the drug-protein conjugate and a visible colored line will show up in the test line region of the specific drug strip. The presence of drug above the cut-off concentration will saturate all the binding sites of the antibody. Therefore, the colored line will not form in the test line region. A drug-positive substances or urine specimen will not generate a colored line in the specific test line region of the strip because of drug competition, whereas a drug-negative substances or urine specimen will generate a line in the test line region because of the absence of drug competition. To serve as a procedural control, a colored line will always appear at the control line region, indicating that proper volume of specimen has been added and membrane wicking has occurred.

REAGENTS

The test contains a membrane strip coated with drug-protein conjugates (purified bovine albumin) on the test line, a goat polyclonal antibody against gold-protein conjugate at the control line, and a dye pad which contains colloidal gold particles coated with mouse monoclonal antibody specific to Fentanyl.

PRECAUTIONS

- For Forensic Use Only.
- Do not use after the expiration date.
- The test device should remain in the sealed pouch until use.
- The test is for single use.
- While substances or urine is not classified by OSHA or the CDC as a biological hazard unless visibly contaminated with blood,[5,6] the use of gloves is recommended to avoid unnecessary contact with the specimen.
- The used test device and substances or urine specimen should be discarded according to federal, state and local regulations.

STORAGE AND STABILITY

Store as packaged in the sealed pouch at 4-30°C (39-86° F). The test is stable through the expiration date printed on the sealed pouch. The test device must remain in the sealed pouch until use. DO NOT FREEZE. Do not use beyond the expiration date.

SPECIMEN COLLECTION AND PREPARATION

Substances or Urine Assay

If the substance you are testing is in liquid form, or if you are testing substances or urine, proceed to the respective Step 1 (see directions below) which corresponds to your device. If the substance you are testing is in powder form, place substance in a container and add 10 drops of water to the substance and mix well. Proceed to the respective Step 1 (see directions below) which corresponds to your device. If the substance you are testing is in pill format, crush or scrape some of the pill into a container. Add 10 drops of water to the substance and mix well. Proceed to the respective Step 1 (see directions below) which corresponds to your device.

The urine specimen must be collected in a clean and dry container. Urine collected at any time of the day may be used. Urine specimens exhibiting visible precipitates should be allowed to settle to obtain a clear specimen for testing.

MATERIALS

Materials Provided

- Test device · Desiccants · Package insert
- Disposable specimen droppers (for test cassette use only)

Materials Required But Not Provided

- Specimen collection container · Timer · Disposable gloves

DIRECTIONS FOR USE

Allow the test device substance, or urine specimen to come to room temperature [15-30°C (59-86°F)] prior to testing.

[For Strip]

1. Remove strip from its foil pouch or the desiccated container (bring the container to the room temperature before opening to avoid condensation of moisture in container). Label the strip with patient or control identifications.
2. Insert the test strip into the substance or urine sample for 15 seconds with the arrow end pointing towards the substance or urine. Do not let the substance or urine sample touch the MAX (maximum) line on the test strip, this could cause an inconclusive result. After 15 seconds, place the test strip on a flat surface.
3. Read result at 5 minutes. ***DO NOT READ RESULT AFTER 10 MINUTES. (Fig. 1)***

(Fig. 1)

INTERPRETATION OF RESULTS

NEGATIVE: Two lines appear. *One color line should be in the control region (C)and another apparent color line adjacent should be in the test region (T). This negative result indicates that the drug concentration is below the detectable level.
***NOTE:** The shade of color in the test line region (T) will vary, but it should be considered negative when there is even a faint distinguishable color line.
POSITIVE: One color line appears in the control region (C). No line appears in the test region (T). This positive result indicates that the drug concentration is above the detectable level.
INVALID: Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test using a new test device. If the problem persists, discontinue using the lot immediately and contact your supplier.

QUALITY CONTROL

A procedural control is included in the test. A color line appearing in the control region (C) is considered an internal procedural control. It confirms sufficient specimen volume, adequate membrane wicking and correct procedural technique.

LIMITATIONS

1. The One Step Fentanyl Drug of Abuse Test provides only a qualitative, preliminary analytical result. A secondary analytical method must be used to obtain a confirmed result. Gas chromatography/mass spectrometry (GC/MS) or Liquid chromatography–mass spectrometry (LC/MS) is the preferred confirmatory method.
2. There is a possibility that technical or procedural errors, as well as other interfering substances in the substances or urine specimen may cause erroneous results.
3. A positive result does not indicate intoxication of the donor, the concentration of drug in the substances or urine, or the route of drug administration.
4. A negative result may not necessarily indicate drug-free substances or urine. Negative results can be obtained when drug is present but below the cut-off level of the test.
5. Test does not distinguish between drugs of abuse and certain medications.
6. A positive test result may be obtained from certain foods or food supplements.

PERFORMANCE CHARACTERISTICS

Reproducibility

Reproducibility studies were carried out using commercially available stock solutions of the drug analytes listed. The results are listed in the following table.

FENTANYL CONCENTRATION (ng/mL)	TOTAL NUMBER OF DETERMINATIONS	RESULT	PRECISION
No Drug Present	60	60 negative	>99%
5	60	60 negative	>99%
15	60	60 positive	>99%

Analytical Sensitivity

A drug-free substances or urine pool was spiked with drug at concentrations listed. The results are summarized below.

DRUG CONCENTRATION CUT-OFF RANGE	n	FEN	
		-	+
0% Cut-Off	30	30	0
-50% Cut-Off	30	30	0
-25% Cut-Off	30	30	0
Cut-Off	30	3	27
+25% Cut-Off	30	0	30
+50% Cut-Off	30	0	30

Analytical Specificity

The following table lists the concentration of compounds (ng/mL) that were detected positive in substances or urine by the One Step Fentanyl Drug of Abuse Test at a read time of 5 minutes.

DRUG	CONCENTRATION (ng/mL)
Fentanyl	10
Valeryl fentanyl HCl	5,000
Butyryl fentanyl	50
Furanyl fentanyl HCl	250
Norfentanyl oxalate	25
Ocfentanil	5,000
Para-Fluorofentanyl	25
(±)-cis-3-Methylfentanyl HCL	250
Acetyl fentanyl	1,000

EFFECT OF URINARY SPECIFIC GRAVITY

Urine samples of normal, high, and low specific gravity ranges from 1.000 - 1.025 were spiked with drug at 50% below and 50% above cut-off levels respectively and tested using One Step Fentanyl Drug of Abuse Test. The results demonstrate that varying ranges of specimen specific gravity do not interfere with the performance of the test.

EFFECT OF URINARY PH

The pH of an aliquoted negative urine pool was adjusted to pH ranges of 4.0, 4.5, 5.0, 6.0 and 9.0, and spiked with drug at 50% below and 50% above cut-off levels. The spiked, pH-adjusted urine was tested with the One Step Fentanyl Drug of Abuse Test. The results demonstrate that varying ranges of pH do not interfere with the performance of the test.

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