The One Step Fentanyl Test Dipcard is a lateral flow chromatographic immunoassay for the detection of Fentanyl in human urine. The test Dipcard contains mouse monoclonal anti-Fentanyl antibody-coupled particles and Fentanyl-protein conjugate. A goat antibody is employed in the control line system.

**INTERPRETATION OF RESULTS**

(Please refer to the illustration above)

**NEGATIVE:** Two lines appear. One red line should be in the control region (C), and another apparent red or pink line should be in the test region (T). This negative result indicates that the Fentanyl concentration is below the detectable level (200ng/mL).

**NOTE:** The shade of red in the test line region (T) may vary, but it should be considered negative whenever there is even a faint pink line.

**POSITIVE:** One red line appears in the control region (C). No line appears in the test region (T). This positive result indicates that the Fentanyl concentration exceeds the detectable level (200ng/mL).

**STORAGE AND STABILITY**

The kit can be stored at room temperature or refrigerated (2-30°C). The test Dipcard is stable through the expiration date printed on the sealed pouch. Do not use beyond the expiration date.

**SPECIMEN COLLECTION AND PREPARATION**

Urine specimens may be stored at 2-8°C for up to 48 hours prior to testing. For long-term storage, specimens may be frozen and stored below -20°C. Frozen specimens should thaw and be mixed before testing.

**SUMMARY**

Fentanyl, belongs to powerful narcotics analgesics, and is a μ special opiate receptor stimulant. Fentanyl is one of the varieties that been listed in management of United Nations “Single Convention of narcotic drug in 1961”. Among the opiates agents that under international control, fentanyl is one of the most commonly used to cure moderate to severe pain. After continuous injection of fentanyl, the sufferer will have the performance of protracted behavior and more lifelong medication overdose.

**PRINCIPLE**

The One Step Fentanyl Test Dipcard is a rapid chromatographic immunoassay based on the principle of competitive binding. Drugs which may be present in the urine specimen compete against the drug conjugate for binding sites on the antibody. During testing, a urine specimen migrates upward by capillary action. Norfentanyl, if present in the urine specimen below 20ng/mL, will not saturate the binding sites of the antibody coated particles in the test Dipcard. The antibody coated particles will then be captured by immobilized Fentanyl conjugate and a visible colored line will show up in the test line region. The colored line will not form in the test line region if the Norfentanyl level exceeds 20ng/mL because it will saturate all the binding sites of anti-Fentanyl antibodies.

A drug-positive urine specimen will not generate a colored line in the test line region, while a drug-negative urine specimen or a specimen containing a drug concentration less than the cut-off will generate a line in the test line region. 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**

For Forensic use only. Do not use after the expiration date. The test Dipcard should remain in the sealed pouch until use. All specimens should be considered potentially hazardous and handled in the same manner as an infectious agent. The used test Dipcard should be discarded according to federal, state and local regulations.

**MATERIALS**

Materials Provided

- Test strips
- Package insert
- Specimen collection container
- Timer

**PERFORMANCE CHARACTERISTICS**

**Reproducibility**

Reproducibility studies were carried out using commercially available stork solutions of the drug analytes listed. Dilutions were made from the stork solution of each drug to the concentrations specified in the following tables. The results are listed in the following tables.

<table>
<thead>
<tr>
<th>Fenconal conc. (mg/L)</th>
<th>Total number of Determinations</th>
<th>Result</th>
<th>Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td>No drug present</td>
<td>40</td>
<td>40 negative</td>
<td>&gt;99%</td>
</tr>
<tr>
<td>100</td>
<td>40</td>
<td>40 negative</td>
<td>&gt;99%</td>
</tr>
<tr>
<td>300</td>
<td>40</td>
<td>40 positive</td>
<td>&gt;99%</td>
</tr>
<tr>
<td>400</td>
<td>40</td>
<td>40 positive</td>
<td>&gt;99%</td>
</tr>
</tbody>
</table>

**Analytical Sensitivity**

A drug-free urine pool was spiked with drugs to the concentrations at ± 50% cut-off and ± 25% cut-off. The results are summarized below.

<table>
<thead>
<tr>
<th>Fenconal Concentration (mg/L)</th>
<th>Percent of Cut-off</th>
<th>n</th>
<th>Visual Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>-50%</td>
<td>30</td>
<td>30 0</td>
</tr>
<tr>
<td>150</td>
<td>-25%</td>
<td>30</td>
<td>29 1</td>
</tr>
<tr>
<td>200</td>
<td>Cut-off</td>
<td>30</td>
<td>16 14</td>
</tr>
<tr>
<td>250</td>
<td>+25%</td>
<td>30</td>
<td>3 27</td>
</tr>
<tr>
<td>300</td>
<td>+50%</td>
<td>30</td>
<td>0 30</td>
</tr>
</tbody>
</table>
Analytical Specificity

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

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norfentanyl</td>
<td>40</td>
</tr>
<tr>
<td>Fentanyl</td>
<td>200</td>
</tr>
<tr>
<td>Buspirone</td>
<td>30,000</td>
</tr>
<tr>
<td>Sufentanyl</td>
<td>50,000</td>
</tr>
<tr>
<td>Fenfuramine</td>
<td>50,000</td>
</tr>
</tbody>
</table>

Effect of Urinary Specific Gravity

Fifteen (15) urine samples of normal, high, and low specific gravity ranges (1.005, 1.015, 1.030) were spiked with drugs at 50% below and 50% above cut-off levels respectively. The One Step Fentanyl Test Dipcard was tested in duplicate using ten drug-free urine and spiked urine samples. The results demonstrate that varying ranges of urinary specific gravity do not affect the test results.

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 drugs at 50% below and 50% above cut-off levels. The spiked, pH-adjusted urine was tested with The One Step Fentanyl Test Dipcard. The results demonstrate that varying ranges of pH do not interfere with the performance of the test.

Cross-Reactivity

A study was conducted to determine the cross-reactivity of the test with compounds in either drug-free urine or Fentanyl positive urine. The following compounds show no cross-reactivity when tested with The One Step Fentanyl Test Dipcard (Urine) at a concentration of 100 µg/mL.

Non Cross Reacting Compounds

- Acetophenetidin
- l-Cotinine
- Cortisone
- d-Pseudoephedrine
- N-Acetylprocainamide
- Creatinine
- Ketoprofen
- Quinidine
- Acetylsalicylic acid
- Deoxycorticosterone
- Labetalol
- Quinine
- Aminopryine
- Dexamethasone
- Loperamide
- Salicylic acid
- Amoxicillin
- Diclofenac
- Meprobamate
- Serotonin
- Ampicillin
- Diflunisal
- Methoxyphenamine
- Sulfamethazine
- I-Ascorbic acid
- D-Glisoxin
- Methylphenidate
- Sulindac
- Asparine
- Diphenhydramine
- Naproxen
- Tetracycline
- Atropine
- l-Estradiol
- Niacinamide
- 3-Acetyl
- Benzilic acid
- Estrone-3-sulfate
- Nifedipine
- Tetracycloxicortone
- Benzoic acid
- Erythromycin
- Norethindrone
- Tetrahydrozoline
- Bilubin
- Fenoprofen
- Noscapine
- Thiamine
- d,l-Brompheniramine
- Furosemide
- d,l-Octopamine
- Thioridazine
- Caffeine
- Gentisic acid
- Oxalic acid
- d,l-Tyrosine
- Cannabis
- Hemoglobin
- Oxolinic acid
- Tobilutamide
- Chlorhydrate
- Hydroalazine
- Oxymetazoline
- Trimaterene
- Chloramphenicol
- Hydrocortisone
- Papaverine
- Trifluoperazine
- Chlorothiazide
- Hydrocortisone
- Penicillin-G
- Trimethoprim
- d,l-Chlorpheniramine
- d,l-Hydroxyhypricum acid
- Perphenzine
- d,l-Tryptophan
- Chlorpromazine
- 3-Hydroxytryramine
- Phenelzine
- Uric acid
- Cholesterol
- d,l-Isoproterenol
- Prednisone
- Verapamil
- Clonidine
- Isoxsuprime
- d,l-Propanol
- Zomepirac

BIBLIOGRAPHY