Buprenorphine and unconjugated norbuprenorphine in the urine samples collected were excreted in urine (27%). It was reported that urine samples taken from patients who were buprenorphine abusers, the ranges were 2.3 to 796 ng/ml for unconjugated buprenorphine and 5 to 2580 ng/mL for unconjugated norbuprenorphine. It was also found that the concentration of free buprenorphine and norbuprenorphine in urine may be relatively small (<1 ng/mL) if taken in clinically administered doses, but can reach up to 20 ng/mL if abused.

**SUMMARY AND EXAMPLEATION OF THE TEST**

Buprenorphine is an analgesic drug. It is also used in heroin substitution and detoxification treatment. With this increased medical use, it also occurs on the black market as an illicit drug, and fatalities have occurred when used in combination with other drugs.

Buprenorphine is administered clinically by intravenous, intramuscular or sublingual routes. Buprenorphine is metabolized by N-dealkylation to form the pharmacologically active Norbuprenorphine. Both buprenorphine and norbuprenorphine are also glucuronidated to the clinically inactive conjugates buprenorphine-3-beta-D-glucuronide and norbuprenorphine-3-beta-D-glucuronide.

Buprenorphine and its metabolite norbuprenorphine (along with the glucuronide forms) are both excreted in urine during the course of several days. Buprenorphine and its metabolites are eliminated mainly in the feces (68%), with a small proportion excreted in urine (27%). It was reported that urine samples taken from patients who had received treatment for 2 weeks with 4 mg of buprenorphine daily (sublingually) showed buprenorphine concentrations ranging from 54 to 260 ng/ml 24 hours after the dose. It was found in another study that the concentrations of the unconjugated buprenorphine and unconjugated norbuprenorphine in the urine samples collected 10 hours after a single dose intramuscular injection of 0.3 mg buprenorphine were 500 pg/ml and 2 ng/ml, respectively.

The concentration of the metabolite norbuprenorphine is usually higher than buprenorphine. The median ratio of buprenorphine to norbuprenorphine is dependent on the time between sampling and dose intake. It was reported that in suspected abusers, the ranges were 2.3 to 796 ng/ml for unconjugated buprenorphine and 5 to 2580 ng/mL for unconjugated norbuprenorphine. It was also found that the concentration of free buprenorphine and norbuprenorphine in urine may be relatively small (<1 ng/mL) if taken in clinically administered doses, but can reach up to 20 ng/mL if abused.

**PRINCIPLE OF THE PROCEDURE**

This test is a competitive binding immunoassay. The buprenorphine and norbuprenorphine are both detected in the urine sample by their respective antibody-antigen reactions. The test strip contains two distinct antibody-coated areas: one labeled “T” (Test Line) and one labeled “C” (Control Line). The “T” line is coated with a buprenorphine conjugate, while the “C” line is coated with a norbuprenorphine conjugate. Positive results indicate the presence of buprenorphine and norbuprenorphine in the urine sample.

**SPECIMEN COLLECTION**

1. Each urine specimen must be collected in a clean container. Do not combine specimens.
2. Specimens may be kept at 15-30°C (59-86°F) for 8 hours, at 2-8°C for up to 3 days and at -20°C or lower for long term storage.

**PRECAUTION**

1. The instructions must be followed exactly to obtain accurate results.
2. Do not open the sealed pouch, unless ready to conduct the assay.
3. Do not use expired devices.
4. Dispose of all specimens and used assay materials as potentially biohazardous.

**ASSAY PROCEDURE**

1. Refrigerated specimens and other test materials, including devices, must be equilibrated to room temperature before testing.
2. Remove the test device from its pouch and place it on a flat surface. Label the device with specimen identification.
3. Holding the dropper vertically, add four drops of the urine specimen to the sample well.
4. Read the test result between four (4) to seven (7) minutes after adding the specimen.

**INTERPRETATION OF RESULTS**

**IMPORTANT:** Do not read test results after seven (7) minutes. The T Line should always be interpreted independently of the C Line.

**Positive:**
If only the C line appears, the result indicates that the buprenorphine and/or norbuprenorphine level in the sample is at a cutoff of 10 ng/ml or higher.

**Negative:**
If both C line and T line appear, the result indicates that the buprenorphine and/or norbuprenorphine level is below 10 ng/mL.

**Invalid:**
If no C line develops within 4 minutes, the result is invalid. Repeat the assay with a new test device.

**QUALITY CONTROL**

- **Built-in Control Features**
This test contains a built-in control feature, the C line. The appearance of the burgundy C line indicates an adequate volume of specimen has been applied to the sample well.

- **External Quality Control**
Users should always follow the appropriate federal, state, and local guidelines concerning the running of external quality controls. SAMHSA


**INSTANT-VIEW® Buprenorphine/Norbuprenorphine Urine Test (Cassette) **

**Buprenorphine**

- 10 ng/ml

**Norbuprenorphine**

- 10 ng/ml

**Buprenorphine-3-O-glucuronide**

- 750 ng/ml

**Norbuprenorphine-3-O-glucuronide**

- 30,000 ng/ml

**Nalorphine**

- 100,000 ng/ml

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**LIMITATIONS**

1. This test is for professional in-vitro diagnostic use only.

2. Results obtained by this device provide only a preliminary qualitative result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result.

3. This product is designed to detect only buprenorphine and/or norbuprenorphine in human urine.

4. Adulterants such as bleach or other strong oxidizing agents may produce erroneous test results. When adulteration is suspected, collect a fresh specimen and repeat the test with a new device.

5. Samples in which bacterial contamination is suspected should not be used. These contaminants may interfere with the test and cause false results.

**EXPECTED VALUES**

This device is designed to detect the combination of buprenorphine and norbuprenorphine at a cutoff of 10 ng/ml.

**PERFORMANCE CHARACTERISTICS**

1. **Accuracy**

   The accuracy of this device was evaluated using 54 clinical urine specimens and 40 drug-free urine samples with varying un-hydrorolized concentrations of both buprenorphine and norbuprenorphine in different ratios, each blind-labeled. The results from this test device agreed 100% with the HPLC/MS and GC/MS on the non-hydrorolized specimens at levels below 75% of the cutoff (negative) and above 125% of the cutoff (positive). Three (3) discrepancies were observed on the specimens of buprenorphine/norbuprenorphine at the level between 75% and 125% of the cutoff. The overall agreement was 96.8% (3 discrepancies/94 specimens).

   ![HPLC/MS Table]

   *Indicates discrepancy.

2. **Reproducibility**

   Reproducibility was evaluated at three POL locations. Personnel with diverse educational backgrounds and work experience performed the tasks. Eighty-pooled drug-free human urine specimens were spiked with buprenorphine at different levels. All specimens were blind labeled and tested. Results are summarized in the table below.

   ![Buprenorphine/Norbuprenorphine Table]

   The results indicated 97.1% and 98.4% separately concordance with the expected results.

3. **Cross-Reactivity**

   To evaluate the cross-reactivity of the device, compounds structurally related to buprenorphine were studied. The lowest concentration of the drugs producing a positive response equivalent to the cutoff level of the device was listed in the table below.

   ![Compounds Table]

**4. Interfering Substances**

The following substances, often found in urine, were spiked and evaluated in urine pools containing 0, 15 ng/ml buprenorphine and norbuprenorphine, separately, with the device. No interference was observed from those analytes at the concentration listed in the following tables.

![Substances Table]

The following substances were tested and confirmed not to interfere with BUP/NBUP test device at the concentration listed below.

![Biological Analytes Table]

There is a possibility that other substances and/or factors not listed above may interfere with the test and cause false results.

**REFERENCES**


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**Temperature limitation**

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**In vitro diagnostic medical device**

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**For IVD performance evaluation only**

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**Caution, consult accompanying documents**

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