A visual one-step immunoassay for the qualitative detection of Buprenorphine in human urine. For professional in vitro diagnostic use only

INTENDED USE
The SERATEC® Drug Screen BUP is a lateral flow, one-step immunoassay for the qualitative detection of Buprenorphine in human urine at a cut-off concentration of 10 ng/ml. This product is used to obtain a qualitative result and is intended for professional use. The assay should not be used without proper supervision and is not intended for use outside the counter safe for lay persons.

This assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) has been established as the preferred confirmatory method by the National Institute of Drug Abuse (NIDA). Clinical considerations and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are indicated.

BACKGROUND
Buprenorphine (BUP) is available under the trade name Subutex® as tablet for the drug substitution. The active agent is already known for 30 years as pain reliever. In comparison to Methadone, Buprenorphine acts in high doses less harmful.

Depending on the application form, buprenorphine is indicated for the treatment of moderate to severe chronic pain or for peri-operative analgesia. Like full agonist opiates, buprenorphine can cause drowsiness, vomiting and respiratory depression. Taking buprenorphine in conjunction with central nervous system (CNS) depressants such as sedatives, tranquilizers, alcohol, and especially benzodiazepines can be particularly dangerous. Falling asleep while abusing this drug, especially while combining it with other central nervous system depressants, can be extremely dangerous and thus greatly increases the chance of serious complications or death.

Buprenorphine is excreted with the urine either unchanged or after deactivation in the liver. The rate of excretion and the fraction of unchanged drug are dependent on the pH of the urine, increasing if the urine is acidic. The half-life is around 8–15 hours. The detection of Buprenorphine in the urine indicates the consumption of within the previous 2-6 days.

Urinalysis for drugs of abuse range from simple immunoassay tests to complex analytical procedures. The speed and sensitivity of immunoassays have made them the most widely accepted method for screening urine for drugs of abuse. The SERATEC® Drug Screen BUP is based on the principle of the highly specific immunochromatographic reactions of antigens and antibodies which are used for the analysis of specific compounds in biological fluids. This test is a rapid, visual, competitive immunoassay that can be used for the qualitative detection of Buprenorphine in human urine at 10 ng/ml cut-off concentration.

PRINCIPLE
The SERATEC® Drug Screen BUP is a one-step immunoassay in which a chemically labeled drug (drug conjugate) competes with the drug which may be present in urine for limited antibody binding sites. The test device contains a membrane strip which was pre-coated with monoclonal antibody-colloidal gold conjugate. When a sufficient concentration of drug is present, it will fill the limited antibody binding sites. This will prevent attachment of the colored antibody-colloidal gold conjugate to the drug conjugate zone on the test band region. Therefore, absence of the colored band on the test region indicates a positive result.

A control band with a different antigen/antibody reaction is also added to the immunochromatographic membrane strip at the control region (C) to indicate that the test has performed properly. This control line should always appear, regardless of the presence of drug and metabolite. This means that negative urine will produce two colored bands, and positive urine will produce only one band. The presence of this colored band in the control region also serves as 1) verification that sufficient volume has been added, and 2) that proper flow was obtained.

STORAGE AND STABILITY
The test kit is to be stored refrigerated or at room temperature +4 – +30 °C (38-86 °F) in the sealed pouch for the duration of the shelf life.

PRECAUTIONS
- For single in-vitro diagnostic use.
- For professional use only
- Urine specimens may be potentially infectious. Proper handling and disposal methods should be established.
- Avoid cross-contamination of urine samples by using a new specimen collection container and specimen pipette for each urine sample.
- Do not use test device if the pouch is damaged
- The components of the test of animal origin (e.g. antibodies) do not cause any danger if the test is used according to the instructions.

MATERIALS SUPPLIED IN THE KIT
- Test devices with disposable pipettes
- One instruction sheet

MATERIALS REQUIRED
- Specimen collection container
- Timer

SPECIMEN COLLECTION AND HANDLING
The SERATEC® Drug Screen BUP is formulated for use with urine specimens. Fresh urine does not require any special handling or pre-treatment. Urine samples should be collected such that testing can be performed as soon as possible after the specimen collection, preferably during the same day. The specimen may be refrigerated at +2-8°C for 2 days, or frozen at -20°C for a longer period of time. Specimens that have been refrigerated must be equilibrated to room temperature prior to testing. Specimens previously frozen must be thawed, equilibrated to room temperature, and mixed thoroughly prior to testing.

Note: Urine specimens and all materials coming in contact with them should be handled and disposed of as if capable of transmitting infection. Avoid contact with skin by wearing gloves and proper laboratory attire.

TEST PROCEDURE
Review “Specimen Collection” instructions. Test device, patient’s samples, and controls should be brought to room temperature (20–30 °C) prior to testing. Do not open pouches until ready to perform the assay.

1. Remove the test device from its protective pouch (bring the device to room temperature before opening the pouch to avoid condensation of moisture on the membrane). Label the device with patient or control identification.
2. Draw the urine sample to the line marked on the pipette (approximately 0.2 ml). Dispense the entire contents into the sample well. Use a separate pipette and device for each sample or control.
3. Read result between 3 to 8 minutes after the addition of sample. Do not read result after 8 minutes.
**INTERPRETATION OF RESULTS**

**Negative result:**
Two colored lines appear in the viewing window. The line in the test region (T) is the drug probe line; the line in the control region (C) is the control line, which indicates proper performance of the device. The color intensity of the test line may be weaker or stronger than that of the control line.

Note: A weak test line indicates that the Buprenorphine concentration is close to the cut-off level. In this case the test should be repeated or the urine sample should be tested with a more specific method.

**Positive result**
Only one colored line appears in the control region (C). The absence of a test line indicates a positive result.

**Invalid:**
If no line appears in the control region the test is invalid and should be repeated

**LIMITATIONS OF PROCEDURE**
- • The assay is designed for use with human urine only.
- • A positive result with the test indicates the presence of a drug/metabolite only and does not indicate or measure intoxication.
- • There is a possibility that technical or procedural errors as well as other substances and factors not listed (see SPECIFICITY) may interfere with the test and cause false results.
- • If it is suspected that the samples have been mislabelled or tampered with, a new specimen should be collected.

**QUALITY CONTROL**
Good laboratory practice recommends the use of control materials to ensure proper kit performance. Quality control specimens are available from commercial sources. When testing the positive and negative controls, use the same assay procedure as with a urine specimen.

**PERFORMANCE CHARACTERISTICS***

*To adjust the concentration of Buprenorphine in the non-clinical samples the Sigma Drug Standard A 3278 was diluted into drug-free human urine.

**A. Accuracy**
The accuracy of the SERATEC® Drug Screen BUP was evaluated in comparison to a commercially available immunoassay at a cut-off of 10 ng/ml. 120 urine samples, collected from presumed non-user volunteers, have been tested as negative by both procedures with 100% agreement.

In a separate study, 70 urine samples, obtained from a clinical laboratory where they were screened and confirmed as positive by the commercially available immunoassay and by GC/MS, were tested with the SERATEC® Drug Screen BUP. The concentration of Buprenorphine in the urine samples ranged from 4.5 ng/ml to 42 ng/ml. 48 samples with Buprenorphine concentrations ≥15 ng/ml, were found to be positive with the SERATEC® test (100% agreement). 13 samples with Buprenorphine concentrations from 4.5 to 7.5 ng/ml were identified as negative by the SERATEC® Drug Screen BUP. With the data obtained from the clinical specimens the performance characteristics of the test were calculated:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buprenorphine</td>
<td>20</td>
</tr>
<tr>
<td>Buprenorphine-3-β-d-glucuronide</td>
<td>20</td>
</tr>
<tr>
<td>Nor-Buprenorphine</td>
<td>4000</td>
</tr>
<tr>
<td>Nor-Buprenorphine-3-β-d-glucuronide</td>
<td>4000</td>
</tr>
</tbody>
</table>

The following compounds were found not to cross-react when tested at concentrations up to 100 µg/ml.


**B. Reproducibility**
The reproducibility of the SERATEC® Drug Screen BUP test was evaluated at four different sites using blind controls. 60 of the samples containing 500 ng/ml Buprenorphine showed negative results. 60 samples with Buprenorphine concentrations of 2000 ng/ml were determined as positive. Of the 60 samples containing Buprenorphine at the cut-off level of 1000 ng/ml 5% tested positive, 5% tested negative and 90% were determined as (+/−), showing a very faint test line.

**C. Precision**
The precision of the test was determined with blind controls of the following Buprenorphine concentrations: 500; 750; 1250; 1500 ng/ml, respectively.

<table>
<thead>
<tr>
<th>Conc. (ng/mL)</th>
<th># samples</th>
<th>correct results</th>
<th>in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>50</td>
<td>50 (−)</td>
<td>100</td>
</tr>
<tr>
<td>750</td>
<td>50</td>
<td>50 (−)</td>
<td>100</td>
</tr>
<tr>
<td>1250</td>
<td>50</td>
<td>32 (+)*</td>
<td>64</td>
</tr>
<tr>
<td>1500</td>
<td>50</td>
<td>50 (+)</td>
<td>100</td>
</tr>
</tbody>
</table>

1: including 19 (+/−) results 2: the remaining 18 tests showed (+/−) results

**D. Specificity**
The specificity for the SERATEC® Drug Screen BUP Test was tested by adding various drugs, drug metabolites, and other compounds that are likely to be present in urine. All compounds were prepared in drug-free normal human urine.

The following structurally related compounds produced positive results when tested at levels equal to or greater than the concentrations listed below.

**SUGGESTED READING**
1. Baselt, R.C. Disposition of Toxic Drugs and Chemicals in Man, Biomedical Publications, 1982

**Symbols**

- For single use only
- Expiry date
- Store at room temperature
- For in-vitro diagnostic use only
- Lot number

---

June 2009