A visual one-step immunoassay for the simultaneous qualitative detection of amphetamine, benzoylcgonine (cocaine), methamphetamine, MDMA (Ecstasy), methadone, morphine (opiates), and 11-nor-\(\text{\AA}\)-caboxylic acid (cannabinoids). For professional in-vitro-diagnostic use only.

INTENDED USE

The SERATEC® Drug Screen Multi-7 is an immunochromatographic test for the rapid and qualitative detection of 7 drug types and/or their major metabolites. The following drug types can be detected with the test:

<table>
<thead>
<tr>
<th>Drug type</th>
<th>Cut-off</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMP</td>
<td>Amphetamine</td>
</tr>
<tr>
<td>MCC</td>
<td>Benzylecgonine/Cocaine</td>
</tr>
<tr>
<td>MET</td>
<td>Methamphetamine</td>
</tr>
<tr>
<td>MDMA</td>
<td>Ecstasy</td>
</tr>
<tr>
<td>MTD</td>
<td>Methadone</td>
</tr>
<tr>
<td>MOR</td>
<td>Morphine</td>
</tr>
<tr>
<td>THC</td>
<td>11-Nor-(\text{\AA})-THC-9-COOH</td>
</tr>
</tbody>
</table>

RELEVANT INFORMATION

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 on Drug Abuse (NIDA). Clinical consideration and professional judgement should be applied to any drug of abuse test result, particularly when preliminary positive results are indicated. The assay should not be used without proper supervision and is not intended for over the counter sales to lay persons. It is only for professional use.

BACKGROUND

The SERATEC Drug Screen Multi-7 Device Test detects the most frequently used drugs simultaneously. In this connection, the cut-off is adjusted to the demands of the American National Institute on Drug Abuse (NIDA). Urine based screening tests 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 Multi-7 Device Test is based on the principle of the highly specific immunochemical reactions of antigens and antibodies which are used to detect drugs resp. its metabolites in human urine.

The following drugs are detected with this test:

Amphetamine

Amphetamine is a sympathomimetic phenethylamine derivative that prominently stimulates the central nervous system. The compound has been used in the treatment of obesity, narcolepsy and hypotension. D-amphetamine is 3-4 times more potent than the L-form. Moderate doses of amphetamine may result in euphoria, a feeling of increased energy and alertness, and insomnia. This is generally accompanied by a suppression of the appetite and an increase of the heart rate and the blood pressure. Some individuals become anxious, irritable and aggressive. Few may experience drowsiness. Higher doses may cause visual, auditory and tactile hallucinations that are sometimes accompanied by a paranoid psychosis that resembles a schizophrenic reaction. Cardiac dysrhythmias, hypertension, hyperpyrexia, convulsions and shock symptoms that might be followed by death due to respiratory and cardiac failure has been observed. Some studies indicate that heavy abuse may result in permanent damage to certain essential nerve structures in the brain. Amphetamine is excreted with the urine either unchanged or after deactivation in the liver. The half-life is around 12 hours. As metamphetamine is metabolized partly to amphetamine, the detection of amphetamine in the urine indicates the consumption of amphetamine/metamphetamine within the previous 1-2 days. The SERATEC Drug Screen AMP test is a rapid, visual, competitive immunoassay that can be used for the qualitative detection of benzoylcgonine in human urine at a cut-off concentration of 500 ng/ml.

Benzoylcgonine/Cocaine

Cocaine is derived from the leaves of the coca plant. It is a potent stimulant of the central nervous system and a local anesthetic. Among the psychological effects induced by using cocaine are euphoria, confidence, and a sense of increased energy. They can be accompanied by an increased heart rate, dilation of the pupils, fever, tremors and sweating. Cocaine is excreted with the urine primarily as benzoylcgonine in a short period of time. Benzoylecgonine has a biological half-life of 5 to 8 hours, which is much longer than that of cocaine (0.5 to 1.5 hours), and can be generally detected for 24 to 60 hours after cocaine use. The SERATEC Drug Screen COC test is a rapid, visual, competitive immunoassay that can be used for the qualitative detection of benzoylcgonine in human urine at a cut-off concentration of 300 ng/ml.

Marijuana/THC

Marijuana is a hallucinogenic agent derived from the flowering portion of the hemp plant. Smoking is the primary method of use of marijuana/cannabis. Higher doses used by abusers produce central nervous system effects, altered mood and sensory perceptions, loss of co-ordination, impaired short-term memory, anxiety, paranoia, depression, confusion, hallucinations and increased heart rate. A tolerance to the cardiac and psychotropic effects can occur, and withdrawal syndrome produces restlessness, insomnia, anorexia and nausea. When marijuana is ingested, the drug is metabolised by the liver. The primary urinary metabolite of marijuana is 11-nor-\(\text{\AA}\)-THC-9-carboxylic acid, and its glucuronide. This means that the presence of these compounds in the urine indicates marijuana/cannabis use. The detection is possible around 1-5 days after consumption. The SERATEC Drug Screen THC test is a rapid, visual, competitive immunoassay that can be used for the qualitative detection of 11-nor-\(\text{\AA}\)-THC-9-carboxylic acid in human urine at a cut-off concentration of 50 ng/ml.

MDMA

(±)-3,4-methylenedioxymethamphetamine (MDMA) is the main component of ecstasy. Ecstasy influences the central nervous system as a stimulant. In addition to psychological addiction, taking ecstasy also causes general unrest, a reduced feeling of hunger and overall feeling of well being. Ecstasy appears in the urine within three hours after administration (any type) and be present for 24-48 hours after the last dose. Overdose and extended usage of Ecstasy may lead to substance abuse, which may cause severe and/or permanent damage to the human nervous system. A relatively frequent outcome is physical overexertion resulting in death, due to the elimination of the body's warning signals. The SERATEC Drug Screen MDMA test is a rapid, visual, competitive immunoassay that can be used for the qualitative detection of methadone in human urine at a cut-off concentration of 500 ng/ml.

Methadone

Methadone is a synthetic analgesic drug that was originally used in the treatment of narcotic addicts. Among the effects induced by using methadone are analgesia, sedation and respiratory depression. Overdose of methadone may cause coma or even death. It is administered orally or intravenously.
Membrane strips which are pre-coated with drug-protein antibody binding sites. The test device contains 6 individual competitors with the drug that may be present in urine for limited antibody binding sites and prevent the attachment of the drug conjugate to the test band region. When the drug/metabolite is present in the urine, it competes with the drug conjugate for the limited antibody binding sites. The SERATEC Drug Screen MOR test is a rapid, visual, competitive immunoassay that can be used for the qualitative detection of metamphetamine in human urine at a cut-off concentration of 300 ng/ml.

Methamphetamine
Methamphetamine, amphetamine, and metabolites are potent sympathomimetic agents. Acute higher doses lead to enhanced stimulation of the central nervous system and include euphoria, alertness, and a sense of increased energy and power. A prolonged abuse of methamphetamine or amphetamine may lead to hallucinations and psychotic behaviour. After intake, methamphetamine is partly metabolized to amphetamine and its derivatives. However, around 40% of the metamphetamine are excreted unchanged in the urine (neutral pH). The rate of excretion and the fraction of unchanged drug are influenced by the pH of the urine, increasing in acidic urine and decreasing under alkaline conditions. The SERATEC Drug Screen MET test is a rapid, visual, competitive immunoassay that can be used for the qualitative detection of methamphetamine in human urine at a cut-off concentration of 500 ng/ml.

Opiumates/Morphine
The opiates such as heroine, morphine, and codeine are derived from the resin of opium poppy. In the body, heroine and codeine are quickly metabolized to morphine with a half-life of about 3 hours. Thus, the presence of morphine and/or morphine glucuronide in the urine of a person indicates the use of heroine, morphine, and/or codeine. The SERATEC Drug Screen MOR test is a rapid, visual, competitive immunoassay that can be used for the qualitative detection of morphine in human urine at a cut-off concentration of 300 ng/ml.

PRINCIPLE OF THE TEST
The SERATEC Drug Screen Multi Test is a one-step immunoassay in which a protein bound drug (drug conjugate) competes with the drug that may be present in urine for limited antibody binding sites. The test device contains 6 individual membrane strips which are pre-coated with drugprotein conjugate (AMP; COC, MET, MDMA, MTD, THC) or antibodies (MOR), respectively, at the test result regions. The corresponding antibodies (or drug conjugate) colloidal gold conjugates are placed at the end of the membrane strips on pads. In the absence of drug in the urine, the solution of colored colloidal gold conjugates and urine moves chromatographically by the capillary action across the membrane of the strips. This solution then migrates to the test band regions and forms visible lines, as the gold conjugates complex with their respective drug conjugates or antibodies. Therefore, the formation of a visible line at the test result zone of a strip occurs when the test urine is negative for the drug. When the drug/metabolite is present in the urine, it competes with the drug conjugate for the limited antibody binding sites. When a sufficient concentration of drug is present, it will fill the limited antibody binding sites and prevent the attachment of the colloidal gold conjugate to the test band region. Therefore, the absence of a colored band on the test result region of a membrane strip indicates a positive result for the respective drug. A control band that is based on a different antigen/antibody reaction is found on each 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 or metabolite (control for proper capillary flow). If the urine comes into direct contact with the MAX mark. If the urine comes into direct contact with the open test result window, the test is destroyed and gets invalid.

INTERPRETATION OF RESULTS

Positive result:

The SERATEC Drug Screen Multi-7 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. Label the device with patient or control identification.
2. Remove the protective cap form the test device and hold the colloidal gold conjugates and urine moves chromatographically by the capillary action across the membrane of the strips. This solution then migrates to the test band regions and forms visible lines, as the gold conjugates complex with their respective drug conjugates or antibodies. Therefore, the formation of a visible line at the test result zone of a strip occurs when the test urine is negative for the drug. When the drug/metabolite is present in the urine, it competes with the drug conjugate for the limited antibody binding sites. When a sufficient concentration of drug is present, it will fill the limited antibody binding sites and prevent the attachment of the colloidal gold conjugate to the test band region. Therefore, the absence of a colored band on the test result region of a membrane strip indicates a positive result for the respective drug. A control band that is based on a different antigen/antibody reaction is found on each 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 or metabolite (control for proper capillary flow). This means that negative urine will produce two colored bands, and positive urine will produce only one colored band at the individual membrane strip.

STORAGE AND STABILITY

The test kit is to be stored refrigerated or at room temperature +2 – +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 for each urine sample.

• Do not use test device if the pouch is damaged.

• The chemicals and potentially infectious components of animal origin (e.g. antibodies) used in the test do not cause any danger if the test is used according to the instructions.

MATERIALS SUPPLIED IN THE KIT

• Individually wrapped test devices

• One instruction sheet

MATERIALS REQUIRED

• Specimen collection containers

• Timer

SPECIMEN COLLECTION AND HANDLING

The SERATEC Drug Screen Multi-7 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.
Invalid:
No line appears in the control region. Under no circumstances should a positive sample be identified until the control line forms in the viewing area. If the control line does not form, the test result is inconclusive and should be repeated.

Note:
A very faint line in the test region indicates that the respective drug in the sample is near the cut-off level of the test. In this case the test should be repeated or the urine sample should be tested with a more specific method (e.g. GC-MS). If only one parameter (e.g. AMP) does not show a control line, you only have to retest the respective parameter with a single test.

Scheme of the test

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 mislabeled 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 OF THE TEST
A. Sensitivity
For every single parameter about 60 positive urine samples were obtained from clinical laboratories. The exact concentrations of the respective drugs were detected in advance via GC/MS or HPLC. Furthermore, 100 negative urine samples have been tested. The following results have been obtained:

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (ng/ml)</th>
<th>Number of Samples</th>
<th>Positive/Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>COC</td>
<td>&lt;300</td>
<td>100</td>
<td>0/100</td>
</tr>
<tr>
<td></td>
<td>&gt;300</td>
<td>100</td>
<td>100/0</td>
</tr>
<tr>
<td>MET</td>
<td>&lt;500</td>
<td>100</td>
<td>0/100</td>
</tr>
<tr>
<td></td>
<td>&gt;500</td>
<td>100</td>
<td>100/0</td>
</tr>
</tbody>
</table>

B. Reproducibility
The accuracy of the SERATEC Drug Screen Multi-7 test was determined by spiking drug-free urine with drug standards purchased at SIGMA. Samples with drug concentrations, that were 50 % below the respective cut-offs, were in all cases correctly determined as negative. Samples with drug concentration that were twice as high as the respective cutoffs consistently showed positive results.

C. Specificity
The specificity for the SERATEC Drug Screen Multi-7 was tested by adding various drugs, drug metabolites, and other compounds that might be present in urine. All compounds were prepared in drug-free normal human urine. The following compounds showed positive results when tested with the SERATEC Drug Screen Multi-7 test:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamine</td>
<td>500</td>
</tr>
<tr>
<td>D-Amphetamine</td>
<td>5,000</td>
</tr>
<tr>
<td>(+/-)-3,4-Methylenedioxymphetamine</td>
<td>2,500</td>
</tr>
<tr>
<td>Cocaine</td>
<td>300</td>
</tr>
<tr>
<td>Benzoylcgonin</td>
<td>300</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>500</td>
</tr>
<tr>
<td>(+/-)-Methamphetamine</td>
<td>25,000</td>
</tr>
<tr>
<td>Chloroquin</td>
<td>25,000</td>
</tr>
<tr>
<td>(+/-)-Ephedrin</td>
<td>25,000</td>
</tr>
<tr>
<td>(+/-)-Methamphetamine</td>
<td>12,500</td>
</tr>
<tr>
<td>Mephenetermine</td>
<td>25,000</td>
</tr>
<tr>
<td>(+/-)-3,4-methylenedioxy-</td>
<td></td>
</tr>
</tbody>
</table>

With the data obtained with the clinical specimens, the performance characteristics of the test were calculated. They are summarized in the following table:

<table>
<thead>
<tr>
<th>Drug</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive Prediction</th>
<th>Negative Prediction</th>
<th>Reproducibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>COC</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>MET</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>MOR</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>THC</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>AMP</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>MTD</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>MDMA</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
amphetamin (MDMA) 1.000
β-Phenylethylamin 25.000
Ranitidin 25.000
Trimethobenzamid 5.000

**MDMA**

(+/-)3,4-Methylendioxy-methamphetamine (MDMA) 500
(+/-)3,4-Methylendioxy-amphetamine (MDA) 1000
(+/-)3,4-Methylendioxyethylamphetamine (MDEA) 300
D-Amphetamin >50.000
D-Methamphetamine 50.000
L-Methamphetamine >50.000
Paramethoxyamphetamine (PMA) 2.500

**Methadone & derivates**

Methadone 300
Doxylamine 50.000
2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine 50.000
Methadol 25.000

**Morphine (opiates)**

Morphine 300
Codeine 300
Ethyl Morphine 300
Hydrocodone 5.000
Hydromorphone 5.000
Morphine-3-β-d-glucuronide 1.000
Thebaine 30.000

**THC & derivates**

11-nor-Δ^9^-THC-9-COOH 50
11-nor-Δ^9^-THC-9-COOH 50
11-hydroxy-Δ^9^-Tetrahydrocannabinol 2.500
Δ^9^-Tetrahydrocannabinol 7.500
Δ^9^-Tetrahydrocannabinol 10.000
Cannabinol 10.000
Cannabidiol 100.000

With exception of the above, for the respective parameter listed positive-reacting drugs/drug metabolites, the following listed compounds reacted negative up to a concentration of 100 mg/ml:

Acetaminophen, Acetone, Albumin, Amoxaoine, Ampicilline, Aspartame, Aspirine, Atropine, Baclofene, Benzocaine, Benzalfrate, Billirubine, (+)Brompheniramine, Caffeine, Dexamethasone, Dextrbrompheniramine, Dextromethorphan, 4-Dimethylaminoantipyrine, Diphenhydramine, 5,5-Diphenylhydantoine, Dopamine, Ecgonine, Ecgonin Methyl Ester, (-)-y-Ephedrine, (+)-y-Ephedrine, (+/-)-Epinephrine, Erythromycin, Ethanol, Fenofibrate, Fentanyl, Fluoxetine, Gemfibrozil, Glucose, Guaicol, Glyceryl Ether, L-Homatropine, Hemoglobin, Hydrochlorothizid, Ibuprofen, Isoproterenol, Ketamine, Lidocaine, Maprotiline, Methanol, 2-IN-morpholino-1athanesaltonic acid, Methaqualone, 1R,2S-(+)-N-Methyl-Ephedrine, Methylphenidat, Naltrexone, Acetyl-Naphtaline, 3-Naproxen, (-)-Nicotine, Nicotine acid, Noscapine, Hydrochloride, (+-)-Norephedrine, Orphenadrine, Oxalic acid, Penterazocine, Penicillin-G, Phenothiazine, Phenelzine, Pheniraminate, L-Phenylephrine, Primidone, Procaine, Promethazine, 2-Propylpantan acid, Pseudoephedrine, Proposphyrine, Quinidine, Quinine, Riboflavine, Salicylic acid, Sodium chloride, Sulindac, Tenocyclidine, Theophylline, Thioridazine, cis-Thiohexin, D(−)-Trehalen, Trifluoperazine, Vitamin C

### Literature