LOUISIANA STATE UNIVERSITY HEALTH SCIENCES CENTER

SHREVEPORT, LA

POINT-OF-CARE TESTING PROCEDURE

iCUP10 URINE DRUG SCREEN

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REPLACES: N/A

Alere iCUP10

A rapid, one step screening test for the simultaneous, qualitative detection of multiple drugs and drug metabolites in human urine.

AMP/BAR/BZO/COC/MDMA/MET/MTD/OPI/OXY/ THC

Multi Drug Screening Test iCup10 (Urine)

Alere Catalog No. IDXA110714

The iCup®10 Dx Drug Screen Cup10 is a one-step immunoassay for the qualitative detection of multiple drugs and drug metabolites in human urine at the following cutoff concentrations:

Detects presence of Amphetamine/Methamphetamine , Benzodiazepines, Cocaine, Methadone, Opiates, Tetrahydrocannabinol, Oxycodone, MDMA, and Barbiturates at the following SAMSHA cutoff levels:

AMP	1000 ng/ml	MTD	300 ng/ml	MDMA	500 ng/ml
MET	1000 ng/ml	OPI	2000 ng/ml	BAR	300 ng/ml
BZO	300 ng/ml	THC	50 ng/ml		
COC	300 ng/ml	OXY	100 ng/ml		

The configurations of the iCup® Dx Drug Screen Cup consists of the combination of the drugs listed above with or without specimen validity test. The specimen validity test provides information regarding the integrity of urine sample in the drugs of abuse test by the semi-quantitative determination of Creatinine, nitrite, pH, bleach/oxidant, and specific gravity in human urine. The iCup® Dx Drug Screen Cup is used to obtain a visual, qualitative result and is intended for professional use only. The iCUP10 with SVT is used at LSUHSC where approved.

This assay provides only a preliminary result. Clinical consideration and professional judgment must be applied to any drug of abuse test result, particularly in evaluating a preliminary positive result. In order to obtain a confirmed analytical result, a more specific alternate chemical method is needed. Gas Chromatography/Mass Spectroscopy (GC/MS) is the preferred confirmation method.

BACKGROUND

Summary and Explanation of Test

Amphetamine/Methamphetamine, and metabolites are potent central nervous system stimulants. Acute higher doses induce euphoria, alertness, and sense of increased energy and power. More acute responses produce anxiety, paranoia, psychotic behavior, and cardiac dysrhythmias. Methamphetamine is excreted in urine as amphetamine and oxidized as deaminated and hydroxylated derivatives. However, methamphetamine is also excreted to some extent unchanged. Thus the presence of the parent compound and metabolite in the urine indicates the use of methamphetamine.

Barbiturates are classified as central nervous system depressants. These products produce a state of intoxication that is similar to alcohol intoxication. Symptoms include slurred speech, loss of motor coordination, and impaired judgment. Depending on the dose, frequency, and duration of use, one can rapidly develop tolerance, physical dependence and psychological dependence on barbiturates. Barbiturates are taken orally, or by intravenous and intramuscular injections. They are excreted in urine as parent compound as well as metabolites.

Benzodiazepines are central nervous system (CNS) depressants commonly prescribed for the short-term treatment of anxiety and insomnia. In general, benzodiazepines act as hypnotics in high doses, as anxiolytics in moderate doses and as sedatives in low doses. The use of benzodiazepines can result in drowsiness and confusion. Psychological and physical dependence on benzodiazepines can develop if high doses of the drug are given over a prolonged period. Benzodiazepines are taken orally or by intramuscular or intravenous injection, and are extensively oxidized in the liver to metabolites. Parent compounds, as well as metabolites are excreted in the urine.

Cocaine is a potent central nervous system stimulant and a local anesthetic found in the leaves of the coca plant. The psychological effects induced by using cocaine are euphoria, confidence and sense of increased energy. These psychological effects are accompanied by increased heart rate, dilation of the pupils, fever, tremors and sweating. Cocaine is excreted in the urine primarily as benzoylecgonine 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 hour), and can be generally detected for 24 to 60 hours after cocaine use or exposure.

Methadone is a synthetic analgesic drug originally used for the treatment of narcotic addiction. The psychological effects induced by using methadone are analgesia, sedation, and respiratory depression. Overdose of methadone may

cause coma or even death. Methadone is taken orally or intravenously and is metabolized in the liver and has a biological half-life of 15-60 hours.

Opiates, such as heroin, morphine, and codeine, are central nervous system (CNS) depressants. Opiates are prescribed primarily as analgesics. The use of opiates at high doses produces euphoria and release from anxiety. Physical dependence is apparent in users and leads to depressed coordination, disrupted decision making, decreased respiration, hypothermia and coma. Heroin is quickly metabolized to morphine, morphine glucuronide and 6-acetylmorphine. Thus, the presence of morphine (or the metabolite, morphine glucuronide) in the urine indicates heroin, morphine, and/or codeine use.

Oxycodone is a semi-synthetic opioid with a structural similarity to codeine. It produces potent euphoria, analgesic and sedative effects, and has a dependence liability similar to morphine. Oxycodone is most often administered orally and is metabolized by demethylation to noroxycodone and oxymorphone followed by glucuronidation, all of which are excreted in urine. The window of detection for oxycodone in urine is expected to be similar to that of other opioids such as morphine.

Tetrahydrocannabinol is generally accepted to be the principle active component in marijuana. When ingested or smoked, it produces euphoric effects. Abusers exhibit central nervous system effects, altered mood and sensory perceptions, loss of coordination, impaired short term memory, anxiety, paranoia, depression, confusion, hallucinations and increased heart rate. When marijuana is ingested, the drug is metabolized by the liver, the primary metabolite of marijuana excreted in the urine is 11-nor-Δ-9-tetrahydrocannabinol-9-carboxylic acid. Therefore, the presence of detected cannabinoids, including the primary carboxyl metabolite, in the urine indicate marijuana/cannabis use.

3,4-methylenedioxymethamphetamine is classified as both a stimulant and a hallucinogen, and is commonly known as Ecstasy. Like methamphetamine, adverse effects of 3,4-methylenedioxymethamphetamine use include jaw clenching, teeth grinding, dilated pupils, perspiring, anxiety, blurred vision, vomiting, and increased blood pressure and heart rate. Overdose of 3,4-methylenedioxymethamphetamine may cause heart failure or extreme heat stroke. 3,4-methylenedioxymethamphetamine is taken orally in tablets or capsules and excreted in urine as parent compound as well as metabolite.

For all drugs, the length of time following drug use of which a positive result may occur is dependent upon several factors, including the frequency and amount of weight, activity and diet.

Principle of the Test

The iCup® Dx Drug Screen Cup is based on the principle of competitive immunochemical reaction between a chemically labeled drug (drug-protein conjugate) and the drug or drug metabolites which may be present in the urine sample for the limited antibody binding sites. The test contains a nitrocellulose membrane strip pre-coated with drug-protein conjugate in the test region and a pad containing colored antibody-colloidal gold conjugate. During the test, the urine sample migrates upward and re-hydrates the antibody-colloidal gold conjugate. The mixture then migrates along the membrane chromatographically by the capillary action to the immobilized drug-protein band on the test region. When drug is absent in the urine, the colored antibody-colloidal gold conjugate and immobilized drug-protein bind specifically to form a visible line in the test region as the antibody complexes with the drug-protein. When drug is present in the urine, it will compete with drug-protein for the limited antibody sites. The line on the test region will become less intense with increasing drug concentration.

When a sufficient concentration of drug is present in the urine, it will fill the limited antibody binding sites. This will prevent attachment of the colored antibody colloidal gold conjugate to the drug-protein on the test region. Therefore, the presence of the line on the test region indicates a **negative** result for the drug and the absence of the test line on the test region indicates a **presumptive positive** result for the drug.

A visible line generated by a different antigen/antibody reaction is also present at the control region of the test strip. This line should always appear, regardless of the presence of drugs or metabolites in the urine sample. This means that a negative urine sample will produce both a test line and a control line, and a positive urine sample will generate only a control line. The presence of a control line serves as a built-in control, which demonstrates that the test is performed properly.

Adulteration of urine samples may cause erroneous results in drugs of abuse tests by either interfering with the drug screening test and/or destroying the drugs in the urine. Dilution of urine with water is probably the simplest urine adulteration method. Bleach, vinegar, Visine, sodium bicarbonate, sodium nitrite, Drano, soft drinks and hydrogen peroxide are examples of adulterants used to adulterate the urine sample. It is important to insure the integrity of urine samples when performing drugs of abuse testing. The Specimen Validity Tests (SVT) are included in the iCup®10 Dx Drug Screen Cup. Each test will generate a color response of chemical indicators. Creatinine and Specific Gravity are used to determine if a sample has been diluted, which can occur either by increased fluid intake or by adding liquid to a urine sample. The Nitrite,

Bleach/Oxidant and pH tests will determine if an adulterant has been added to the sample. **The results of all SVTs are used to determine overall sample integrity**.

Cr: Creatinine reacts with a creatinine indicator in an alkaline medium to form a purplish-brown color complex. The color intensity is directly proportional to the concentration of creatinine. A urine sample with a creatinine concentration of less than 20 mg/ml may indicate that the sample has been diluted.
Ni: Nitrite reacts with the reagent's aromatic amine to form a diazonium salt which couples with an indicator to yield a pink-red/purple color complex. Urine sample containing nitrite at level greater than 15 mg/dl is considered adulterated.
pH: pH determination of urine sample is based on color change of indicator in different acidic or basic medium. The normal urine pH ranges from 4 to 9. Urine pH below 4 or above 9 indicates adulteration with an acidic or basic compound.
Bl: Bleach or other oxidizing agents react with an oxidant indicator to form a color complex. Observation of a blue-green, brown, or orange color indicates adulteration with bleach or other oxidizing agents.
S.G.: The **Specific Gravity** test is based on the pKa change of certain pretreated polyelectrolytes in relation to the ionic concentration. In the presence of an indicator, the colors changes from dark blue to blue-green in urine of low ionic concentration to green and yellow-green in urine of higher ionic concentration. Urine specific gravity below 1.005 or above 1.025 is considered abnormal.

MATERIALS

Reagents and Materials provided

• 25 individually wrapped test devices. Each device consists of different test strips in a plastic test strip holder. The test strip contains a colloidal gold pad coated with antibody and rabbit antibody. It also contains a membrane coated with drug-bovine protein conjugate in the test band and goat anti-rabbit antibody in the control band.
• One instruction sheet
• One Adulteration Color Comparison Chart for interpretation of adulteration test result (when applicable)

S.V.T. REAGENTS

• One Step Multi-Drug Screen Test Card with the Integrated iCup®/iCup® A.D.
[Note: A Fahrenheit temperature strip is affixed to aid in the determination of specimen validity. Please use this temperature strip in conjunction with your Drug Free Policy (if applicable)].
• Adulteration color chart (if applicable)

• Package insert
• Procedure card

Materials Required but not Provided
• Timer
• External positive and negative controls

Storage
The iCup® 10 Dx Drug Screen Cup should be stored at 2-30°C (36-86°F) in the original sealed pouch. Do not freeze. Do not store or expose reagent kits at temperature greater than 30°C.

Warnings and Precautions:
• For professional *in vitro* diagnostic use only
• Urine specimens may be potentially infectious. Proper handling and disposal methods should be established.
• Test device should remain sealed until ready for use.
• Do not use the test kit after the expiration date.
• Color blindness may affect interpretation of results.
• Do not store or expose reagent kits at temperature greater than 30°C (86°F). Do not freeze.

Specimen Collection and Handling
Fresh urine does not require any special handling or pretreatment. A fresh urine sample should be collected in the container provided. Alternately, a clean, dry plastic or glass container may be used for specimen collection. The temperature strip (affixed to the provided container) can be used to determine that the specimen whether the specimen temperature falls in the normal range of 90-100° F within four (4) minutes of collection. If a specimen temperature falls outside of this range, it is likely that either water or some other substance has been added. If the specimen will not be tested immediately after collection, the specimen may be refrigerated at 2-8°C up to 2 days or frozen at -20°C for longer period of time. Specimens that have been refrigerated must be equilibrated to room temperature prior to testing. Specimens previously frozen must be thawed 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

PROCEDURE

Preparation

1. Collect the specimen. Sample must meet minimum urine level as indicated on the cup.
2. Do not open test device pouch until ready to perform the test.

Testing

1. Remove test device lid from the sealed pouch.
2. Secure test device lid to the filled specimen cup. NOTE: Screw the cap tightly to ensure adequate seal. Cup lid must be secured tightly by twisting lid a quarter turn AFTER lid is snug.
3. Set timer for 5 minutes. Place the cup on its side to activate test. Place on a flat surface.
4. Read adulteration results by visually comparing color of reagent pads to corresponding blocks on the Color Chart at time indicated for each test.
5. Read results of drugs of abuse tests in 5 minutes. Do not interpret the test results after 10 minutes.

INTERPRETATION OF RESULTS

Specimen Validity Tests:

Specimen validity test results are obtained by directly comparing the color of each test pad with the color block of Adulteration Color Comparison Chart. Adulterated urine sample will produce abnormal color response. Unadulterated urine sample will produce normal color response within the time identified on the color chart.

Drugs of Abuse Tests:

Negative (-): Colored lines appear in both Control Region (C) and Test Region (1, 2, or T). The line in the control region is the control line, which is used to indicate proper performance of the device. The line in the test region is the drug line. The test line may have varying intensity either weaker or stronger in color than that of the control line. A negative result for a drug indicates that the concentration of that drug in urine is below the cutoff level.

Positive (+): Colored line appears in the control region. **No line appears in the test region.** The complete absence of a test line indicates a positive result for that drug. A preliminary positive result for a drug indicates that the concentration of that drug in urine is at or above the cutoff level.

Invalid: No colored line appears in the control region. If the control line does not form, the test result is invalid and should be repeated.

LIMITATIONS OF PROCEDURE

1. The iCup® Dx Drug Screen Cup 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) is the preferred confirmatory method.
2. There is a possibility that technical or procedural errors, as well as interfering substances in the urine specimen may cause erroneous results.
3. Adulterants, such as bleach and/or alum, in urine specimens may produce erroneous results regardless of the analytical method used. If adulteration is suspected, the test should be repeated with another urine specimen.
4. A positive result does not indicate level or intoxication, administration route or concentration in urine.
5. A negative result may not necessarily indicate drug-free urine. Negative results can be obtained when drug is present but below the cut-off level of the test.
6. This test does not distinguish between drugs of abuse and certain medications.
7. A positive test result may be obtained from certain foods or food supplements.

QUALITY CONTROL

A procedural control is included in the test. A colored 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.

Manufacturer's recommendations are that external positive and negative controls be tested:

1) **with each new lot or shipment of product,**
2) **when problems (storage, operator, instrument, or other) are suspected or identified, or**
3) **at least once monthly as a check on continued storage conditions.**

Control specimens should be performed the same as patient specimens using the same safety precautions you would use for processing any "unknown" urine sample containing potentially infectious biological material. Protect product from exposure to direct sunlight. Contains sodium azide: To prevent formation of explosive metal azides,

dispose of waste by flushing with copious amounts of water or according to local governing regulations. *Do not use beyond the expiration date*.

If unexpected results are obtained when running the external positive or negative controls, repeat the test with another cup. If the problem persists, discontinue use of the test kit immediately and contact POC personnel at ext. 57951. Patient results may not interpreted until the problem is resolved satisfactorily per POC personnel.

iScreen Urine Drug External Control: Negative, High Positive

Intended Use: The iScreen Urine Drug Controls are designed to monitor and validate the performance of drugs of abuse detection methods at levels esteablished by SAMHSA, CAP/AACC and many state programs. The iScreen Drug Control product line of controls is manufactured using a human based matrix that has been stabilized to insure that the product will be viable until the date of expiration. Positive controls are spiked with reference drug standards and/or appropriate metabolites that have been obtained from ISO certified manufacturers. Standards are certified by the manufacturers to be at lease 98% minimum purity. Specific gravity, pH, and creatinine fall within the limits of normal human urine.

The Controls are stable until the expiration date when stored at -20 to 110 degrees C and protected from light. The controls are stable until the expiration date when stored at -20 to 8 degrees C, however no stability claims can be made for Oxazepam as it may deteriorate over time when stored refrigerated. After Opening: (Controls can be aliquoted and frozen). The Controls are stable for six months or until the expiration date, whichever comes first, when stored at -20 to -10 degrees C. The Controls are stable for 31 days or until the expiration date, whichever comes first, when stored tightly capped at -20 to 8 degrees C. Thaw controls as needed; allow Controls to come to room temperature followed by gentle swirling or inversion before use. DO NOT SHAKE. Expected Results: The positive control must test positive on the drugs of abuse test device or screening method. The negative control must test negative. Limitations of Procedure: *This product is not meant to be used as a standard or calibrator.*

LIMITATIONS OF PROCEDURE

1. The iCup®10 Dx Drug Screen Cup 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) is the preferred confirmatory method.
2. There is a possibility that technical or procedural errors, as well as interfering substances in the urine specimen may cause erroneous results.
3. Adulterants, such as bleach and/or alum, in urine specimens may produce

erroneous results regardless of the analytical method used. If adulteration is suspected, the test should be repeated with another urine specimen.
4. A positive result does not indicate level or intoxication, administration route or concentration in urine.
5. A negative result may not necessarily indicate drug-free urine. Negative results can be obtained when drug is present but below the cut-off level of the test.
6. This test does not distinguish between drugs of abuse and certain medications.
7. A positive test result may be obtained from certain foods or food supplements.

Reference:

iCUP10 Dx Drug Screen Cup Package Insert, Alere (previously Inverness Medical)
iScreen Urine Drug Controls Package Insert, Alere (previously Inverness Medical)

Distribution:

This procedure is on file in the Point-of-Care section of the Clinical Laboratory and in the LSUHSC-Shreveport website.