

CLIAwaived™ Inc. Rapid Drug Test Cup

FOR IN VITRO DIAGNOSTIC USE

INTENDED USE

CLIA CATEGORIZATION: WAIVED

The CLIAwaived Inc. "RDTc" is a one-step immunoassay for the qualitative detection of multiple drugs and drug metabolites in human urine at the following cutoff concentrations:

Test	Calibrator	Cut-off (ng/ml)
AMP	d-Amphetamine	1000
BAR	Secobarbital	300
BZO	Oxazepam	300
COC	Benzoyllecgonine	300
MDMA	3,4-methylenedioxyamphetamine	500
MET	d-Methamphetamine	1000
MTD	Methodone	300
OPI	Morphine	2000
OXY	Oxycodone	100
PCP	Phencyclidine	25
TCA	Nortriptyline	1000
THC	11-nor- Δ^9 -THC-9-COOH	50

The configurations of the CLIAwaived Inc. RDTc consist of any 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 CLIAwaived Inc. RDTc is used to obtain a visual, qualitative result and is intended for professional use only. A certificate of waiver is needed for your laboratory in order to run this test. Laboratories with a certificate of waiver must follow the manufacturer's instructions for performing the test or the test is considered high complexity and is no longer CLIA waived.

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.

SUMMARY AND EXPLANATION

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.

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

Methodone is a synthetic analgesic drug originally used for the treatment of narcotic addiction. The psychological effects induced by using methodone are analgesia, sedation, and respiratory depression. Overdose of methodone may cause coma or even death. Methodone 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.

Phencyclidine, commonly known as "angel dust" and "crystal cyclone", is an arylcyclohexylamine that is originally used as an anesthetic agent and a veterinary tranquilizer. The drug is abused by oral or nasal ingestion, smoking, or intravenous injection. It produces hallucinations, lethargy, disorientation, loss of coordination, trance-like ecstatic states, a sense of euphoria and visual distortions. It is well absorbed following all routes of administration. Unchanged PCP is excreted in urine in moderate amounts (10% of the dose).

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.

Tricyclic antidepressants (TCAs) have been prescribed for depression and compulsive disorders. Because of the possibility of causing serious cardiac complications, TCAs can be lethal if misused at high doses. TCAs are taken orally or sometimes by injection. TCAs are metabolized in the liver. TCAs and their metabolites are excreted in urine mostly in the form of metabolites for up to ten days.

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 drug, metabolic rate, excretion rate, drug half-life, and the drug user's age, weight, activity and diet.

Specimen Validity Tests

Information regarding Specimen Validity Tests does not require FDA review.

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 the 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.

TEST PRINCIPLE

The CLIAwaived Inc. RDTc 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 **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.

The Specimen Validity Tests (SVT) are included in certain versions of the CLIAwaived Inc. RDTc. 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 change 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.

REAGENTS & MATERIALS SUPPLIED

- 25 individually wrapped test cups. Each cup 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 adulterant pads when applicable.
- One instruction sheet
- One Adulteration Color Comparison Chart for interpretation of adulteration test result (when applicable)

MATERIAL REQUIRED BUT NOT PROVIDED

- Timer
- External positive and negative controls

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. Do not freeze.

STORAGE

The CLIAwaived Inc. RDTC 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.

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.

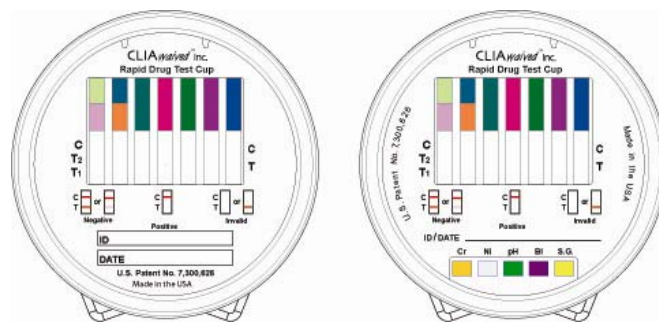
PROCEDURE

Preparation

1. If a specimen or test devices have been stored at refrigerated temperatures, allow them to warm to room temperature before testing.
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.
3. Place the cup on its side to activate test.
4. Read results of drugs of abuse tests in 5 minutes. Do not interpret the test results after 10 minutes.



Drug Test Cup Without Specimen Validity Test

Drug Test Cup With Specimen Validity Test

INTERPRETATION OF RESULTS

Specimen Validity Tests:

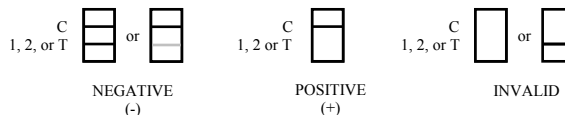
Specimen validity test results are obtained by directly comparing the color of each test pad with the color block of Adulteration Color Comparison Chart. Adulterated urine sample will produce abnormal color response. Unadulterated urine sample will produce normal color response within the time identified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



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.

It is recommended that external positive and negative controls be tested with each new lot or shipment of product, with each new operator (i.e. one who has not performed the test recently), when problems (storage, operator, instrument, or other) are suspected or identified, and as otherwise required by your laboratory's internal quality system procedures. Depending on storage conditions, operators may also test controls monthly as a check on continued storage conditions. Control specimens should be performed the same as patient specimens (refer to Directions for Use and Interpretation of Results). If unexpected results are seen when running the external positive or negative controls, review the Directions for Use, Interpretation of Results and Limitations sections and repeat the test with another cup. If the problem persists, discontinue use of the test kit immediately and call (1-888-882-7739).

LIMITATIONS OF PROCEDURE

1. The CLIAwaived Inc. RDTC 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.

PERFORMANCE CHARACTERISTICS

Accuracy

The accuracy of the CLIAwaived Inc. RDTC was evaluated in comparison to commercially available drug screen tests. Sixty (60) negative urine samples collected from presumed non-user volunteers were tested by both CLIAwaived Inc. RDTC and commercially available drug screen tests. Of these negative urine samples tested, all were found negative by both methods. In a separate study, positive urine samples, obtained from clinical laboratories where the drug concentrations were determined by GC/MS (TCA concentrations were determined by HPLC), were tested by CLIAwaived Inc. RDTC and commercial drug screen tests. The results of accuracy study are presented below:

Drug Test		GC/MS (<-50% C/O)	GC/MS (-50% C/O to C/O)	GC/MS (C/O to +50% C/O)	GC/MS (>+50% C/O)	% Agreement with GC/MS
AMP	(+)	0	0	10	55	98.5
	(-)	15	9	1	0	100
BAR	(+)	0	1	5	83	97.8
	(-)	15	7	2	0	95.7
BZO	(+)	0	2	13	37	100
	(-)	18	18	0	0	94.7
COC	(+)	0	0	8	71	98.8
	(-)	15	8	1	0	100
MDMA	(+)	0	1	6	37	100
	(-)	24	6	0	0	96.8
MET	(+)	0	0	5	58	98.4
	(-)	20	8	1	0	100
MTD	(+)	0	0	6	65	98.6
	(-)	15	5	1	0	100
OPI	(+)	0	2	9	45	100
	(-)	15	6	0	0	91.3
OXY	(+)	0	1	6	47	100
	(-)	15	7	0	0	95.7
PCP	(+)	0	0	4	56	96.8
	(-)	15	4	2	0	100
TCA	(+)	0	1	12	9	100
	(-)	23	11	0	0	97.1
THC	(+)	0	1	24	32	100
	(-)	15	12	0	0	96.4

Precision

The precision of the CLIAwaived Inc. RDTC was evaluated by testing three lots of the test devices at four study sites with spiked drug sample solutions on three consecutive days. Sample concentrations were confirmed by GC/MS.

AMP (ng/ml)	0	500	750	1000	1250	1500
(+/-)	0/135	0/135	34/101	75/60	110/25	135/0
BAR (ng/ml)	0	150	225	300	375	450
(+/-)	0/135	0/135	34/101	74/61	102/33	135/0
BZO (ng/ml)	0	150	225	300	375	450
(+/-)	0/135	0/135	29/106	75/60	107/28	135/0
COC (ng/ml)	0	150	225	300	375	450
(+/-)	0/135	0/135	30/105	65/70	96/36	135/0
MDMA (ng/ml)	0	250	375	500	625	750
(+/-)	0/135	0/135	35/100	75/60	95/40	135/0
MET (ng/ml)	0	500	750	1000	1250	1500
(+/-)	0/135	0/135	31/104	77/58	98/37	135/0
MTD (ng/ml)	0	150	225	300	375	450
(+/-)	0/135	0/135	31/104	69/66	95/40	135/0
OPI (ng/ml)	0	1000	1500	2000	2500	3000
(+/-)	0/135	0/135	37/98	76/59	104/31	135/0
OXY (ng/ml)	0	50	75	100	125	150
(+/-)	0/135	0/135	50/85	86/49	111/24	135/0
PCP (ng/ml)	0	12.5	18.75	25	31.25	37.5
(+/-)	0/135	0/135	26/109	62/73	99/36	135/0
TCA (ng/ml)	0	500	750	1000	1250	1500
(+/-)	0/135	0/135	24/111	60/75	99/36	135/0
THC (ng/ml)	0	25	37.5	50	62.5	75
(+/-)	0/135	0/135	27/108	58/77	91/44	135/0

Specificity

The specificity for the CLIAwaived Inc. RDTC was determined by testing 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 compounds produce positive results when tested at levels greater than the concentrations listed below.

Compound	Conc. (ng/ml)	Compound	Conc. (ng/ml)
Amphetamine			
d-Amphetamine	1,000	d-Methamphetamine	50,000
dl-Amphetamine	2,500	(+/-)3,4-MDMA	50,000
(+/-)3,4-MDA	1,250		
Barbiturates			
Secobarbital	300	Butabarbital	400
Allobarbital	600	Butalbital	300
Alphenal	200	Butethal	450
Amobarbital	1500	Pentobarbital	400
Aprobarbital	300	Phenobarbital	450
Barbital	1500		
Benzodiazepines			
Oxazepam	300	Flunitrazepam	300
Alprazolam	400	Flurazepam	300
Bromazepam	250	Lorazepam	500
Chlordiazepoxide	300	Medazepam	300
Clobazam	1000	Nitrazepam	250
Clonazepam	500	Nordiazepam	150
Clorazepate	150	Prazepam	500
Desalkylflurazepam	200	Temazepam	200
Diazepam	450	Triazolam	450
Estazolam	300		
Cocaine			
Benzoyllecgonine		Cocaine	300
Methamphetamine			
d-Methamphetamine	1000	(+/-)3,4-MDMA	3,000
l-Amphetamine	50,000	l-Methamphetamine	10,000
l-Amphetamine	>100,000	Ephedrine	>100,000
(+/-)3,4-MDEA	50,000	Mephentermine	75,000
(+/-)3,4-MDA	100,000		
MDMA			
(+/-)3,4-MDMA	500	(+/-)3,4-MDA	4,000
(+/-)3,4-MDEA	450		
Methadone			
(+/-) Methadone	300	Methadol	1,500
Opiates			
Morphine	2,000	Hydrocodone	4,000
Codeine	2,000	Hydromorphone	5,000
Ethylmorphine	1,000	Morphine-3-glucuronide	2,500
Heroin	5,000	Nalorphine	5,000
Oxycodone			
Oxycodone	100	Morphine	>100,000
Hydrocodone	5000	Codeine	50,000
Hydromorphone	50,000	Heroin	>100,000
PCP			
Phencyclidine	25	Tenocyclidine	2,000
THC			
11-nor- Δ^9 -THC-9-COOH	50	Δ^9 -tetrahydrocannabinol	5,000
11-hydroxy- Δ^9 -THC	1,000	Cannabinol	10,000
Δ^8 -tetrahydrocannabinol	5,000	Cannabidiol	>100,000
Tricyclic Antidepressant			
Nortriptyline	1,000	Promazine	1,500
Nordoxepin	2,000	Desipramine	400
Trimipramine	2,000	Doxepin	3,000
Amtriptyline	1,500	Maprotiline	2,000

Interference

Two pools of drug-free urine were spiked with drug standards to 50% below and 50% above cutoff concentrations. The drug concentrations were confirmed by GC/MS. The following compounds were evaluated for potential positive and/or negative interference with the CLIAwaived Inc. RDTC. All compounds were dissolved in the spiked sample solutions and tested with CLIAwaived Inc. RDTC. An unaltered sample was used as a control. No positive interference or negative interference was found for the following compounds when tested at concentrations up to 100 µg/ml.

Acetaminophen	Diphenhydramine	(+/-)-Norephedrine
Acetone	Dopamine	Oxalic Acid
Albumin	(+/-)-Epinephrine	Penicillin-G
Ampicillin	Erythromycin	Pheniramine
Ascorbic Acid	Ethanol	Phenothiazine
Aspartame	Furosemide	l-Phenylephrine
Aspirin	Glucose	β-Phenylethylamine
Atropine	Guaiacol Glyceryl Ether	Procaine
Benzocaine	Hemoglobin	Quinidine
Bilirubin	Ibuprofen	Ranitidine
Caffeine	(+/-)-Isoproterenol	Riboflavin
Chloroquine	Ketamine	Sodium Chloride
(+)-Chlorpheniramine	Levorphanol	Sulindac
(+/-)-Chlorpheniramine	Lidocaine	Theophylline
Creatine	(+)-Naproxen	Tyramine
Dexbrompheniramine	Niacinamide	4-Dimethylaminoantipyrine
Dextromethorphan	Nicotine	(1R,2S)-(-)-N-Methyl-Ephedrine

Effect of Specimen pH

Drug sample solutions with 50% below and 50% above cutoff concentrations were adjusted to pH 4-9 and tested using CLIAwaived Inc. RDTC. An unaltered sample was used as a control. The results demonstrate that varying ranges of specimen pH do not interfere with the performance of the test.

Effect of Specimen Specific Gravity

Drug sample solutions with 50% below and 50% above cutoff concentrations were adjusted to specific gravity 1.003-1.04 and tested using CLIAwaived Inc. RDTC. An unaltered sample was used as a control. The results demonstrate that varying ranges of specimen specific gravity do not interfere with the performance of the test.

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Distributed by:
CLIAwaived™ Inc.
San Diego, CA 92130

42141-Ic-W Revision 0 May, 2009

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