

**OneStep
 Amphetamine
 RapiCard™ InstaTest**

REF 121020-1-21

IVD  See external Label  2-30°C  Σ=1 Test

Sensitivity	1000 ng/ml
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INTENDED USE

The Cortez Diagnostics, Inc. OneStep AMP RapiCard™ InstaTest is an immunochromatography based one step in vitro test. It is designed for qualitative determination of amphetamine in human urine specimens above a cut-off level of 1000 ng/ml. This assay may be used in the point of care setting.

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/MC) has been established as the preferred confirmatory method by the Substance Abuse Mental Health Services Administration (SAMHSA). Clinica consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are indicated.

SUMMARY AND EXPLANATION

Amphetamines are a class of potent sympathomimetic agents with therapeutic applications. The most common amphetamines are d-amphetamine and d/l-amphetamine. Amphetamines are central nervous stimulants that cause the neurotransmitters epinephrine, norepinephrine and dopamine to be released into the brain and body giving users feelings of euphoria, alertness, and increased energy. Chronic abuse of amphetamine leads to tolerance and drug reinforcement effect. Cardiovascular responses to amphetamine include increased blood pressure and cardiac arrhythmias. More

acute responses produce anxiety, paranoia, hallucinations and psychotic behavior. Amphetamine is metabolized by a number of pathways. In general, acid urine promotes excretion whereas alkaline urine retards it. In 24 hours, approximately 79% of the amphetamine dose is excreted in acid urine and about 45% in alkaline urine. Typically, about 20% are excreted as unchanged amphetamine. Unchanged amphetamine can be detected up to 1 –2 days after use. However, The length of time following drug use for 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.

TEST PRINCIPLE

The Cortez Diagnostics, Inc. OneStep AMP RapiCard™ InstaTest is based on the principle of specific immunochemical reaction between antibodies and antigens to analyze particular compounds in human urine specimen. The assay relies on the competition for binding antibody between drug conjugate and free drug which may be present in the urine specimen being tested.

When drug is present in the urine specimen, it competes with drug conjugate for the limited amount of antibody-dye conjugate. When the amount of drug is equal or more than the cut-off, 1000 ng/ml, it will prevent the binding of drug conjugate to the antibody. Therefore, a positive urine specimen will not show a colored band on the test line zone, indicating a positive result, while the presence of a colored band indicates a negative result.

A control line is present in the test window to work as procedural control. This colored band should always appear on the control line zone if the test device is stored in good condition and the test is performed appropriately.

SPECIMEN COLLECTION AND PREPARATION

It is required that approximately 150 µl of sample for each test. Fresh urine specimens do not need any special handling or treatment. Specimens should be collected in a clean, dry, plastic or glass container. If the assay is not performed immediately, urine specimen may be refrigerated at 2-8°C or frozen up to 7 days. Specimens should be thawed and brought to room temperature

before testing. Urine specimens exhibiting a large amount of precipitate or turbidity should be centrifuged or allowed to settle before testing. Avoid contact with skin by wearing gloves and proper laboratory attire.

MATERIALS AND COMPONENTS

Materials provided with the test kits

1. Instructions for use.
2. Cortez AMP test card. The amount of *each* coated antigen and/or antibody on the strip is less than 1.0mg for antigen conjugate and is less than 1.0 mg for Goat anti-mouse IgG antibody.

Test zone: contains Amphetamine bovine protein antigen conjugates.

Control zone: contains Goat anti-mouse IgG antibody.

Conjugate Pad: contains mouse monoclonal anti-Amphetamine.

Materials required but not provided

1. Urine collection container.
2. Timer or clock.

STORAGE

The test device should be stored at 2 to 30°C and will be effective until the expiration date stated on the package. The product is humidity-sensitive should be used immediately after being open. Any improperly sealed product should be discarded.

PRECAUTION

1. For in vitro diagnostic and forensic use only.
2. Do not use the product beyond the expiration date.
3. Handle all specimens as potentially infectious.
4. Humidity sensitive product, do not open foil pouch until it is ready to be tested.
5. Use a new urine specimen cup for each sample to avoid cross contamination.

ASSAY PROCEDURE

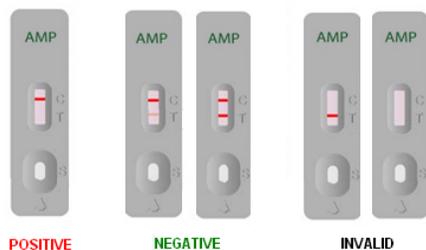
1. Bring all materials and specimens to room temperature.
2. Remove the test strip from the sealed foil pouch.
3. Place the transfer pipette in the specimen and depress the bulb to withdraw a sample.

4. Hold the pipette in a vertical position over the sample well of the test card and deliver 3 drops (120-150 µl) of sample in to the sample well.
5. Read the results at 5 minutes after adding the sample.
Do not interpret the result after 10 minutes.

RESULTS

- **Negative:** Two colored bands form. The appearance of two colored bands, one in test line zone and the other in control line zone, indicates negative results. The negative result indicates that the amphetamine concentration in the specimen is either zero or less than cut-off level.
- **Positive:** One colored band forms. One colored band appears in control line zone. No colored band is found in test line zone. This is an indication that the amphetamine level in the specimen is above the cut-off level.
- **Invalid:** If there are no colored bands in control line zone, the test result is invalid. Retest the sample with a new device.

Note: A borderline (+/-) in test line zone should be considered negative result.



PERFORMANCE CHARACTERISTICS

A. Accuracy

The accuracy of the Cortez AMP test was evaluated in comparison to GC/MS method at a cut-off of 1000 ng/ml. One hundred urine specimens with GC/MS confirmed d-Amphetamine concentration were evaluated in this study. The results are summarized and presented below:

Cortez AMP Test	(-)			(+) GC/MS		Percent agreement with GC/MS
	GC/MS Negative (Less than -25% cut off)	Near cutoff negative (between -25% and c/o)	Near cutoff positive (between c/o and +25%)	GC/MS Positive (greater than +25% cut off)		
Positive	1	4	5	33	88.4	
Negative	47	6	2	2	93.0	
Total	48	10	7	35		

Positive % agreement: 88.4, Negative % agreement: 93.0
Nine specimens were found discrepant between the new screening method and the GC/MS method. When compared those data, 67% (6 out of 9) of the discrepancy specimens were found between +25% to -25% of cutoff concentration (750-1250 ng/ml).

B. Sensitivity

The cut-off concentration (sensitivity level) of Cortez AMP test is determined to be 1000ng/ml.

C. Precision

The precision study was performed by three individuals observing the test result to determine the random error of visual interpretation. The test result were found to have no significant differences between the three observers.

Device	Control Con. ng/ml	No. of Tested	No. of positive			No. of borderline #			No. of negative		
			1*	2*	3*	1*	2*	3*	1*	2*	3*
	500	40							40	40	40
	750	40				2	3	2	38	37	38
AMP	1000	40	34	34	33	6	7	7			
	1250	40	39	38	39	1	2	1			
	1500	40	40	40	40						

D. Specificity

The specificity for Cortez AMP 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.

1. Interference Testing

The Cortez AMP test performance at negative and positive (cutoff) levels may not be affected when pH and Specific Gravity ranges of urine specimen are at 4.0 to 9.0 and 1.005 to 1.035.

Each listed substance that commonly found in the urine was evaluated and indicated negative result at the concentration listed below (by adding the compound in the negative urine):

Glucose	2000 mg/dl,
Human albumin	2000 mg/dl
Human hemoglobin	10 mg/dl,
Urea	4000 mg/dl
Uric acid	10 mg/dl

2. Specificity

The following table lists compounds that are detected by Cortez AMP test, producing positive results when tested at levels equals to or greater than the concentrations listed below:

Compounds	Cut-off (ng/ml)
Amphetamine	1,000
D/L-Amphetamine	2,000
(±)3,4Methylenedioxyamphetamine	2,500
L-Amphetamine	30,000
(+) Methamphetamine	> 100 µg/ml
(±)3,4Methylenedioxymethamphetamine	> 100 µg/ml

Each listed substance that commonly found in the urine was evaluated and indicated negative result at concentration of 100 µg/ml (by adding the compound in the negative urine):

Acetaminophen	4-Acetamidophenol	Acetylsalicylic acid
Amitriptyline	Amobarbital	Arterenol
Ascorbic Acid	Chlopheniramine	Caffeine
Chloroquine	Dextromethorphan	Cocaine
Deoxyephedrine	Ecgonine	Digitoxin
Epinephrine	Gentisic Acid	Ecgonine methylester
Hydrochlorothiazide	Homatrophine	Guaiaicol glycerester
Isoproterenol	Ketamine	Imipramine
Methadone	Methaqualone	Lidocaine
Neomycin	Niacinamide	Methylphenidate
Penicillin G	Phentermine	Oxazepam
Promethazine	Pseudoephedrine	Phenylethylamine-α
Tetracycline	Tetrahydrozoline	Quinine antidine
11-nor-Δ ⁹ -THC-9-COOH(10 µg/ml)		Theophylline
Tryptophan	Tyramine	Thioridazine
Amikacin	Perphenazine	Ephedrine
Aspartame	Phenylpropanolamine	Histamine
Camphor	Salicylic acid	Ibuprofen
Cortisone	11-nor-Δ ⁹ -THC-9-COOH(10 µg/ml)	
Digoxin	Trifluoperazine	Meperidine
Morphine		

QUALITY CONTROL

Good Laboratory practice recommends the daily use of control materials to validate the reliability of device. Control materials should be assayed as clinical specimen and challenging to the assay cutoff concentration, e.g., 25% above and below cutoff concentration. If control values do not fall within establish range, assay results are invalid. Control materials which is not provided with this test kit are commercially available.

The Cortez Drugs of Abuse Test provides a built-in process control with a different antigen/antibody reaction at the control region (C). This control line should always appear regardless the presence of drug or metabolite. If the control line does not appear, the test device should be discarded and the obtained result is invalid. The presence of this control band in the control region serve as 1) verification that sufficient volume is added, 2) that proper flow is obtained.

LIMITATIONS OF PROCEDURE

The assay is designed for use with human urine only. A positive result with any of the tests indicates only the presence of a drug/metabolite and does not indicate or measure intoxication. There is a possibility that technical or procedural error as well other substances in certain foods and medicines may interfere with the test and cause false results. Please refer “SPECIFICITY” section for lists of substances that will produce either positive results, or that do not interfere with test performance. If a drug/metabolite is found present in the urine specimen, the assay does not indicate frequency of drug use or distinguish between drug of abuse and certain foods and medicines.

EXPECTED VALUES

The Cortez Diagnostics, Inc. OneStep AMP RapiCard™ InstaTest is a qualitative assay. It identifies d-amphetamine in human urine at a concentration of 1000 ng/ml or higher. The concentration of the d-amphetamine can not be determined by this assay. The test is intended to distinguish negative result from presumptive positive result. All positive results must be confirmed using an alternate method, preferably GC/MS.

<p>ISO 13485 ISO 9001</p>  <p> Diagnostic Automation/ Cortez Diagnostics, Inc. 21250 Califa St, Suite 102 and 116, Woodland Hills, California 91367 USA</p>	
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