Diagnostic Automation / Cortez Diagnostics, Inc.
IMMUNO DIAGNOSTICS

CE

OneStep Buprenorphine RapiCard™ InstaTest

REF 121003-1-21

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

Specificity	**100 µg/ml**
Sensitivity	**10 ng/ml**

INTENDED USE

The Cortez Diagnostics, Inc. OneStep BUP RapiCard™ InstaTest is an immunochromatography based one step in vitro test.

SUMMARY AND EXPLANATION

Buprenorphine, a derivative of thebaine, is an opioid that has been marketed in the United States as the Schedule V parenteral analgesic Buprenex. In 2003, based on a reevaluation of available evidence regarding the potential for abuse, addiction, and side effect, DEA reclassified buprenorphine from a Schedule V to a Schedule III narcotic.

Buprenorphine resembles morphine structurally but has a longer duration of action than morphine and can be administrated sublingually as an analgesic. In October 2002, FDA approved the use of a buprenorphine monotherapy product, Subutex, and a buprenorphine/naloxone combination product, Suboxone, for the treatment of opioid addiction. Subutex and Suboxone are the first narcotic drugs available under the US Drug Act (DATA) of 2003 for the treatment of opiate dependence that can be prescribed in the US in a physician's work place. It has also been shown that buprenorphine has abuse potential and may itself cause dependency. In addition, a number of deaths have been recorded as a result of overdose with intravenously injected buprenorphine in conjunction with other psychotropic drugs such as benzodiazepines. Buprenorphine is metabolized primarily by n-dealkylation to form glucuronide-buprenorphine and glucuronide-norbuprenorphine.

TEST PRINCIPLE

The Cortez Diagnostics, Inc. OneStep BUP 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, 10 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 2-8ºC 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 BUP test device. 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.
3. Test zone: contains buprenorphine bovine protein antigen conjugates.
4. Control zone: contains Goat anti-mouse IgG antibody.
5. Conjugate Pad: contains mice monoclonal anti-buprenorphine antibody.

Materials required but not provided

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

ASSAY PROCEDURE

1. Bring all materials and specimens to room temperature.
2. Remove the test card from the sealed foil pouch.
3. Place the transfer pipette in the specimen and depress the bulb to to withdraw a sample.
4. Hold the pipette in a vertical positon over the sample well of the test card and deliver 3 drops (120-150 µl) os sample in to the sample well.
5. Read the results at 5 minutes after adding the sample.

Do not interpret the result after 5 minute

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 buprenorphine 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 buprenorphine 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.*

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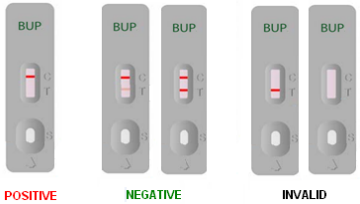

PERFORMANE CHARACTERISTICS

A. Accuracy

The accuracy of the Cortez BUP test was evaluated in comparison to GC/MS at a cut-off of 10 ng/ml of buprenorphine-3-β-d-glucoronide. One hundred and one urine specimens with confirmed buprenorphine-3-β-d-glucoronide concentrations were evaluated in this study. Borderline readings were recorded as negative. The results are summarized and presented below:

	(-)		(+)		
Cortez BUP Test	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)	Percent agreement with GC/MS
Positive	1	1	10	39	96
Negative	41	9	0	0	100
Total	42	10	10	39	

Positive % agreement: 96, Negative % agreement: 100.
Two specimens were found discrepant between the Cortez BUP and GC/MS method. When compared those data, 50% (1out of 2) of the discrepancy specimens were found between cut-off and +25% cut-off concentration (7.5 – 12.5 ng/ml).

B. Sensitivity

The cut-off concentration (sensitivity level) of Cortez BUP test is determined to be 10 ng/ml of buprenorphine-3-β-d-glucoronide or 10 ng/ml of buprenorphine.

C. Precision

The precision study was performed by three individuals observing the test result to determine the random error of visual interpretation.

The test results 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*
	5	42							42	42	42
	7.5	42				2		2	40	42	40
BUP	10	42	29	27	24	13	15	18			
	12.5	42	42	42	42						
	15	42	42	42	42						

D. Specificity

The specificity for Cortez 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.

1. Interference Testing

The Cortez BUP test performance at cut-off level is not affected when pH and Specific Gravity ranges of urine specimen are at 4.0 to 8.0 and 1.005 to 1.035.

The following substances were tested and confirmed not to interfere with Cortez BUP test at the listed concentrations.

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 BUP test, producing positive results when tested at levels equals to or greater than the concentrations listed below:

Compounds	Concentration
Buprenorphine-3-β-d-glucoronide	10 ng/ml 200 µg/ml
Buprenorphine	100 µg/ml
Norbuprenorphine	100 µg/ml
Norbuprenorphine-3-β-d-glucoronide	

Each listed substance that commonly found in the urine was evaluated and indicated negative result at concentration of 100 µg/ml.

Acetaminophen	4-Acetamidophenol	Acetylsalicylic acid
Amitriptyline	Amobarbital	Amphetamine
Aspartame	Ascorbic Acid	Atrophine
Camphor	Chloroquine	Chlopheniramine
Cortisone	Deoxyephedrine	Dextromethorphan
Digoxin	Diphenhydramine	Ecgonine
Enalapril maleate salt	Ephedrine	Epinephrine
Guaiacol glycer ester	Histamine	Homatrophine
Ibuprofen	Imipramine	Isoproterenol
Lidocaine	Meperidine	Metformin
Methamphetamione	3,4 ± MDMA	Methaqualone
Mycophenolic acid	Neomycin	Niacinamide
Penicillin G	Perphenazine	Phencyclidine
Phenylpropanolamine	Prednisolone	Promethazine
Quinine antidine	Salicylic acid	Tracrolimus
Tetrahydrozoline	Theophyline	11-nor-Δ^8–THC-9-COOH (10 µg/ml)
11-nor-Δ^9-THC-9-COOH	(10 µg/ml)	Thioridazine
Trifluoperazine	Tryptophan	Tyramine

Amikacin	Methadone
Arterenol	Methylphenidate
Caffeine	Oxazepam
Citalopram	Phenylethylamine-α
Digitoxin	Pseudoephedrine
Ecgonine methyl ester	Tetracycline
Gentisic	Trazodone
Hydrochlorothiazide	
Ketamine	

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 concentrtion, e.g., 25% above and below cutoff concentrtion. 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.

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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 BUP Test is a qualitative assay. It identifies buprenorphine's major metabolite, Buprenorphine-3-β-d-glucoronide in human urine at a concentration of 10 ng/ml or higher. The concentration of the Buprenorphine-3-β-d-glucoronide cannot 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.

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

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