510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k111560

B. Purpose for Submission:

New device

C. Measurand:

d-Amphetamine, Secobarbital, Oxazepam

D. Type of Test:

Qualitative lateral flow chromatographic immunoassay

E. Applicant:

Guangzhou Wondfo Biotech Co., Ltd.

F. Proprietary and Established Names:

Wondfo Amphetamine Urine Test
Wondfo Secobarbital Urine Test
Wondfo Oxazepam Urine Test

G. Regulatory Information:

1. Regulation section:

21 CFR §862.3100, Amphetamine Test System
21 CFR §862.3150, Barbiturate Test System
21 CFR §862.3170, Benzodiazepine Test System

2. Classification:

All are Class II

3. Product code:

DKZ, DIS, JXM

4. Panel:

Toxicology (91)

H. Intended Use:

1. Intended use(s):

See indications for use below.

2. Indications(s) for use:

Wondfo Amphetamine Urine Test:

Wondfo Amphetamine Urine Test is an immunochromatographic assay for the qualitative determination of d-amphetamine in human urine at a cutoff concentration of 1000 ng/mL. The test is available in a cassette format and a strip format. It is intended for prescription use and over the counter use.

The test provides only preliminary test results. A more specific alternative chemical method must be used in order to obtain a confirmed analytical result. GC/MS is the preferred confirmatory method. Clinical consideration and professional judgment should be exercised with any drug of abuse test result, particularly when the preliminary result is positive.

Wondfo Secobarbital Urine Test:

Wondfo Secobarbital Urine Test is an immunochromatographic assay for the qualitative determination of secobarbital in human urine at a cutoff concentration of 300 ng/mL. The test is available in a cassette format and a strip format. It is intended for prescription use and over the counter use.

The test provides only preliminary test results. A more specific alternative chemical method must be used in order to obtain a confirmed analytical result. GC/MS is the preferred confirmatory method. Clinical consideration and professional judgment should be exercised with any drug of abuse test result, particularly when the preliminary result is positive.

Wondfo Oxazepam Urine Test:

Wondfo Oxazepam Urine Test is an immunochromatographic assay for the qualitative determination of oxazepam in human urine at a cutoff concentration of 300 ng/mL. The test is available in a cassette format and a strip format. It is intended for prescription use and over the counter use.

The test provides only preliminary test results. A more specific alternative chemical method must be used in order to obtain a confirmed analytical result. GC/MS is the

preferred confirmatory method. Clinical consideration and professional judgment should be exercised with any drug of abuse test result, particularly when the preliminary result is positive.

3. Special conditions for use statement(s):

   For prescription and over-the counter use.

4. Special instrument requirements:

   Not Applicable

I. Device Description:

The tests are in different formats: strip, cassette.

The strip format is a urine test. It is a rapid test for the qualitative detection of the d-amphetamine, secobarbital, and oxazepam. It is a lateral flow chromatographic immunoassay. When the absorbent end of the test device is immersed into the urine sample, the urine is absorbed into the device by capillary action, mixes with the antibody-dye conjugate, and flows across the pre-coated membrane. When the amphetamine, secobarbital, and oxazepam levels are zero or below the target cut off (the detection sensitivity of the test), antibody-dye conjugate binds to the drug-protein conjugate immobilized in the Test Region (T) of the device. This produces a colored Test line, which regardless of its intensity, indicates a negative result.

When amphetamine, secobarbital, and oxazepam levels are at or above the target cutoff, the free drug in the binding sample binds to the antibody-dye conjugate preventing the antibody-dye conjugate from binding to the drug-protein conjugate immobilized in the Test region (T) of the device. This prevents the development of a distinct colored band in the test region, indicating a potentially positive result.

To serve as a procedure control, a colored line will appear at the Control Region (C), if the test has been performed properly because of the antibody-dye conjugate binding to anti-mouse IgG immobilized in the Control Region of the device.

The Cassette format comes as a test device for amphetamine, secobarbital, and oxazepam. The cassette format is the same function or performance specification of strip. It is composed of a functional strip and a plastic cover, which is a supporting container.

J. Substantial Equivalence Information:

1. Predicate device name(s):

ACON One Step Drug Screen Test Card

2. Predicate 510(k) number(s):

k020771

3. Comparison with predicate:

Reagent Similarities and Differences		
Feature	**Candidate Device: Wondfo Amphetamine Urine Test, Wondfo Secobarbital Urine Test, and Wondfo Oxazepam Urine Test (k111560)**	**Predicate Device: ACON One Step Drug Screen Card (k020771)**
Intended/Indications for Use	For the qualitative determination of d-Amphetamine, Secobarbital, or Oxazepam in human urine.	Same
Methodology	Competitive binding, lateral flow immunochromatographic assays based on the principle of antigen-antibody immunochemistry	Same
Type of Test	Immunoassay principles that rely on antigen-antibody interactions to indicate positive or negative result	Same
Results	Qualitative	Same
Specimen Type	Human Urine	Same
Cut Off Value	d-Amphetamine: 1000 ng/ml Secobarbital: 300 ng/ml Oxazepam: 300 ng/ml	Same
Configurations	Strip, Cassette	Card
Intended Use	OTC Use & Prescription Use	Prescription Use

K. Standard/ Guidance Document Referenced (if applicable):

- In Vitro Diagnostic Devices; Guidance for the Preparation of 510(k) Submission
- Premarket Submission and Labeling Recommendations for Drugs of Abuse Screening Tests, Draft Guidance

L. Test Principle:

Immunochromatograph assay using a lateral flow, one step system for the qualitative detection of d-amphetamine, secobarbital and oxazepam in human urine. Each assay uses a mouse monoclonal antibody -dye conjugate against drug with gold chloride and fixed drug-protein conjugate and anti-mouse IgG polyclonal antibody in membrane.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
   a. Precision/Reproducibility:
   The precision study was performed by taking negative urine samples and spiking these with: 2000, 1750, 1500, 1250, 1000, 750, 500, 250 and 0 ng/ml of d-amphetamine, corresponding to ± 25, 50, 75, and 100% of the 1000 ng/ml cut-off value; 600, 525, 450, 375, 300, 225, 150, 750 and 0 ng/ml of secobarbital, corresponding to ± 25, 50, 75, and 100% of the 300 ng/ml cut-off value ; and 600, 525, 450, 375, 300, 225, 150, 750 and 0 ng/ml of oxazepam, corresponding to ± 25, 50, 75, and 100% of the 300 ng/ml cut-off value. Two sets of operators performed the testing (group A ran the strip format and group B ran the cassette format) and the samples were divided into 12 sets of 25 (one set per lot per run for each format). Three lots of the strip and three lots of the cassette formats of the Wondfo Amphetamine Urine Test, Wondfo Secobarbital Urine Test, and Wondfo Oxazepam Urine Test were used. Each of three operators tested 2 aliquot at each concentration for each lot per day (2 runs per day for 25 days), resulting in a total of 50 determinations by each operator at each concentration. The operators were blinded. A summary of the results is presented in the tables below.

   a. Strip Format

d-Amphetamine

Lot	**-100% Cut-off**	**-75% Cut-off**	**-50% Cut-off**	**-25% Cut-off**	**Cut-off**	**+25% Cut-off**	**+50% Cut-off**	**+75% Cut-off**	**+ 100% Cut-off**
	Neg/Pos	Neg/Pos	Neg/Pos	Neg/Pos	Neg/Pos	Ne/Pos	Neg/Pos	Neg/Pos	Neg/Pos
Lot 1	50/0	50/0	50/0	50/0	6/44	0/50	0/50	0/50	0/50
Lot 2	50/0	50/0	50/0	50/0	5/45	0/50	0/50	0/50	0/50
Lot 3	50-0	50/0	50/0	50/0	3/47	0/50	0/50	0/50	0/50

Secobarbital

Lot	**-100% Cut-off**	**-75% Cut-off**	**-50% Cut-off**	**-25% Cut-off**	**Cut-off**	**+25% Cut-off**	**+50% Cut-off**	**+75% Cut-off**	**+ 100% Cut-off**
	Neg/Pos	Neg/Pos	Neg/Pos	Neg/Pos	Neg/Pos	Ne/Pos	Neg/Pos	Neg/Pos	Neg/Pos

Lot 1	50/0	50/0	50/0	50/0	5/45	0/50	0/50	0/50	0/50
Lot 2	50/0	50/0	50/0	50/0	6/44	0/50	0/50	0/50	0/50
Lot 3	50/0	50/0	50/0	50/0	6/44	0/50	0/50	0/50	0/50

Oxazepam

Lot	**-100% Cut-off**	**-75% Cut-off**	**-50% Cut-off**	**-25% Cut-off**	**Cut-off**	**+25% Cut-off**	**+50% Cut-off**	**+75% Cut-off**	**+ 100% Cut-off**
	Neg/Pos	Neg/Pos	Neg/Pos	Neg/Pos	Neg/Pos	Ne/Pos	Neg/Pos	Neg/Pos	Neg/Pos
Lot 1	50/0	50/0	50/0	50/0	7/43	0/50	0/50	0/50	0/50
Lot 2	50/0	50/0	50/0	50/0	6/44	0/50	0/50	0/50	0/50
Lot 3	50/0	50/0	50/0	50/0	6/44	0/50	0/50	0/50	0/50

b. Cassette Format

d-Amphetamine

Lot	**-100% Cut-off**	**-75% Cut-off**	**-50% Cut-off**	**-25% Cut-off**	**Cut-off**	**+25% Cut-off**	**+50% Cut-off**	**+75% Cut-off**	**+ 100% Cut-off**
	Neg/Pos	Neg/Pos	Neg/Pos	Neg/Pos	Neg/Pos	Ne/Pos	Neg/Pos	Neg/Pos	Neg/Pos
Lot 1	50/0	50/0	50/0	50/0	6/44	0/50	0/50	0/50	0/50
Lot 2	50/0	50/0	50/0	50/0	6/44	0/50	0/50	0/50	0/50
Lot 3	50/0	50/0	50/0	50/0	5/45	0/50	0/50	0/50	0/50

Secobarbital

Lot	**-100% Cut-off**	**-75% Cut-off**	**-50% Cut-off**	**-25% Cut-off**	**Cut-off**	**+25% Cut-off**	**+50% Cut-off**	**+75% Cut-off**	**+ 100% Cut-off**
	Neg/Pos	Neg/Pos	Neg/Pos	Neg/Pos	Neg/Pos	Ne/Pos	Neg/Pos	Neg/Pos	Neg/Pos
Lot 1	50/0	50/0	50/0	50/0	7/43	0/50	0/50	0/50	0/50
Lot 2	50/0	50/0	50/0	50/0	6/44	0/50	0/50	0/50	0/50
Lot 3	50/0	50/0	50/0	50/0	5/45	0/50	0/50	0/50	0/50

Oxazepam

Lot	-100% Cut-off	-75% Cut-off	-50% Cut-off	-25% Cut-off	Cut-off	+25% Cut-off	+50% Cut-off	+75% Cut-off	+ 100% Cut-off
	Neg/Pos	Neg/Pos	Neg/Pos	Neg/Pos	Neg/Pos	Ne/Pos	Neg/Pos	Neg/Pos	Neg/Pos
Lot 1	50/0	50/0	50/0	50/0	7/43	0/50	0/50	0/50	0/50
Lot 2	50/0	50/0	50/0	50/0	6/44	0/50	0/50	0/50	0/50
Lot 3	50/0	50/0	50/0	50/0	5/45	0/50	0/50	0/50	0/50

b. Linearity/assay reportable range:

Not Applicable, the assay is intended for qualitative use

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Control standards are not supplied with this device; however, this device has internal process controls. A colored line appearing in the control region confirms sufficient sample volume and adequate membrane wicking. Users are informed that the test is invalid if a line fails to appear in the control region.

Stability

Accelerated stability and real time stability tests were performed on three batches of strip and cassette Wondfo Amphetamine Urine Tests, Wondfo Secobarbital Urine Tests, and Wondfo Oxazepam Urine Tests. Accelerated stability was performed at 50°C for 1 month to support a one year shelf life and for 2-4 months to support a two year shelf life; the data supports an 18 month shelf life. Real time studies were performed by storing three lots of strips and cassettes at 4° and 30°C. Performance tests were completed at defined intervals and the results support a 23 month shelf life at 4°C for the Wondfo Secobarbital Urine Test and 24 months for the Wondfo Amphetamine and Wondfo Oxazepam Urine Tests; and 24 month shelf life at 30°C for the Wondfo Secobarbital Urine Test and 23 months for the Wondfo Amphetamine and Wondfo Oxazepam Urine Tests. Shipping studies were performed on three lots of each strip and cassette formats for the Wondfo Amphetamine Urine Tests, Wondfo Secobarbital Urine Tests, and Wondfo Oxazepam Urine Tests to simulate the coldest (12°C average temp) and hottest (36.8°C average temp)months of the year. These studies showed that the devices can be used for the international transport environment.

d. Detection limit:

Analytical performance of the device around the cutoff is described in Section f. (Assay cut-off) below.

e. Analytical specificity:

Specificity and cross-reactivity was performed by using three batches of both the strip and cassette formats of the Wondfo Amphetamine Urine Test, Wondfo Secobarbital Urine Tests, and Wondfo Oxazepam Urine Tests. The samples were split into two aliquots and one group of operators ran the strip format on one set of aliquots and a separate group of operators ran the cassette format on the other set of aliquots. Thirty drug-free urine samples were tested and all were found to be negative. Cross-reactivity was determined by spiking the target drug, drug metabolites and the same class compounds that may cross-react with the target drugs into drug-free urine at three different concentrations and measuring the samples in triplicate using three batches of strips and three batches of cassettes.

d-Amphetamine

AMP (Amphetamine) (d-Amphetamine, Cutoff = 1000 ng/ml)	**Result** (Positive at 1000 ng/ml**)**	**% Cross Reactivity** 100%
1-Amphetamine	Positive at 50000 ng/ml	2%
dl-Amphetamine	Positive at 3000 ng/mL	33%
(+/-) 3,4-methylenedioxyamphtamine (MDA)	Positive at 5,000 ng/mL	20%
Phentermine	Positive at 3000 ng/mL	33%

Secobarbital

Secobarbital, Cutoff= 300 ng/ml)	**Result** (Positive at 300 ng/ml)	**% Cross Reactivity** 100%
Amobarbital	Positive at 300 ng/mL	100%
Alphenol	Positive at 150 ng/mL	200%
Aprobarbital	Positive at 200 ng/mL	150%
Butabarbital	Positive at 75 ng/mL	400%
Butathal	Positive at 100 ng/mL	300%

Butalbital	Positive at 2,500 ng/mL	12%
Cyclopentobarbital	Positive at 600 ng/mL	50%
Pentobarbital	Positive at 300 ng/mL	100%
Phenobarbital	Positive at 150 ng/mL	200%

Oxazepam

Oxazepam, Cutoff= 300 ng/ml	**Result** Positive at 300 ng/ml	**% Cross Reactivity** 100%
Alprazolam	Positive at 200 ng/mL	150%
a-hydroxyalprazolam	Positive at 1,500 ng/mL	20%
Bromazepam	Positive at 1,500 ng/mL	20%
Chlordiazepoxide	Positive at 1,500 ng/mL	20%
Clonazepam HCl	Positive at 800 ng/mL	37.5%
Clobazam	Positive at 100 ng/mL	300%
Clonazepam	Positive at 800 ng/mL	37.5%
Clorazepate dipotassium	Positive at 200 ng/mL	150%
Delorazepam	Positive at 1,500 ng/mL	20%
Desalkylflurazepam	Positive at 400 ng/mL	75%
Diazepam	Positive at 200 ng/mL	150%
Estazolam	Positive at 2,500 ng/mL	12%
Fluintrazepam	Positive at 400 ng/mL	75%
D,L-Lorazepam	Positive at 1,500 ng/mL	20%
Midazolam	Positive at 12,500 ng/mL	2.4%
Nitrazepam	Positive at 100 ng/mL	300%

Norchlordiazepoxide	Positive at 200 ng/mL	150%
Nordiazepam	Positive at 400 ng/mL	75%
Temazepam	Positive at 100 ng/mL	300%
Trazolam	Positive at 2,500 ng/mL	12%

The following unrelated compounds were found not to cross-react when tested spiked (100 μg/mL) into drug-free urine, as well as into urine spiked with ± 25% of the cut-off concentration of d-amphetamine, secobarbital, or oxazepam individually:

d-Amphetamine:

4-Acetamindophenol
Acetophenetidin
N-acetylprocainamide
Acetylsalicyclic Acid
Aminopyrine
Amitryptyline
Amobarbital
Amoxicillin
Ampicillin
Ascorbic Acid
Apomorphine
Aspartame
Atropine
Benzilic Acid
Benzoic Acid
Benzoylecgonine
Bilirubin
Brompheniramine
Caffeine
Cannabidiol
Cannabinol
Chloralhydrate
Chloramphenicol
Chlordiazepoxide
Chlorothiazide
(+/-) Chlorpheniramine
Chlorpromazine
Chlorquine
Cholesterol
Clomipramine
Clonidine
Cocaine hydrochloride
Codeine
Cortisone
(-) Cotinine
Creatinine
Deoxycorticosterone
Dextromethorphan
Diazepam
Diclofenac
Diflunisal
Digoxin
Diphenydramine
Doxylamine
Ecgonine hydrochloride
Ecgonine methylester
(IR,2S(-(-)-Ephedrine
L-Ephedrine
(-) Y Ephedrine
Erythromycin
b- Estradiol
Estrone-3-sulfate
Ethyl-p-aminobenzoate
Fenfluramine
Fenoprofen
Furosemide
Gentisic acid
Hemoglobin
Hydralazine
Hydrochlorthiazide
Hydrocodone
Hydrocortisone
O-Hydroxyhippuric acid
3-Hydroxytyramine
Ibuprofen
Imipramine
(-) Isoproterenol
Isoxsuprine
Ketamine
Ketoprofen
Labetalol
Levorphanol
Loperamide
Maprotiline
Meperidine
Meprobamate
Methadone
Methylphenidate
Morphine-3-D-glucoronide
Nalidixic acid
Naloxone
Naltrexone
Naproxen
Niacinamide
Nifedipine
Norcodein
Norethindrone
D-Norpropoxyphene
Noscapine
D,L-Octopamine
Oxalic Acid
Oxazepam
Oxolinic Acid
Oxycodone
Oxymetazoline
Papaverine
Penicillin-G
Pentazocaine
Pentobarbital
Perphenazine
Phencyclidine
Phenelzine
Phendimetrazine
Phenobarbitol
Phetoin
L-Phenylephrine
b-Phenylethlamine
Phenylpropanolamine
Prednisolone
Prednisone
Procaine
Promazine
Promethazine
D,L-Propranolol
Propiomazine
D-Propoxyphene
Quinidine
Quinine
Ranitidine
Salicylic Acid
Secobarbital
Serotonin
Sulfamethazine
Sulindac
Temazepam
Tetracycline
Tetrahydrocortisone
Tetrahydrozoline
d9-THC-COOh
Thebaine
Thiamine
Thioridazine
D,L-Thyroxine
Tolbutamine
Triamterene
Trifluoperazine
Trimethoprim
Trimipramine
Tryptamine
D,L- Tyrosine
Uric Acid
Verapamil
Zomepirac

Secobarbital:

Acetaminophen
Acetophenetidin
N-acetylprocainamide
Acetylsalicyclic Acid
Aminopyrine
Amitryptyline
Amoxicillin
Ampicillin
Ascorbic Acid
Apomorphine
Aspartame
Benzilic Acid
Benzoic Acid
Benzoylecgonine
Bilirubin
Brompheniramine
Caffeine
Cannabidiol
Cannabinol
Chloralhydrate
Chloramphenicol
Chlordiazepoxide
Chlorothiazide
(+/-) Chlorpheniramine
Chlorpromazine
Chlorquine
Cholesterol
Clomipramine
Clonidine
Cocaine hydrochloride
Codeine
Cortisone
(-) Cotinine
Creatinine
Deoxycorticosterone
Dextromethorphan
Diazepam
Diclofenac
Diflunisal
Digoxin
Diphenydramine
Doxylamine
Ecgonine hydrochloride
Ecgonine methylester
(IR,2S(-(-)-Ephedrine
L-Ephedrine
Erythromycin
b- Estradiol
Estrone-3-sulfate
Ethyl-p-aminobenzoate
Fenfluramine
Fenoprofen
Furosemide
Gentisic acid
Hemoglobin
Hydralazine
Hydrochlorthiazide
Hydrocodone
Hydrocortisone
O-Hydroxyhippuric acid
3-Hydroxytyramine
Ibuprofen
Imipramine
(-) Isoproterenol
Isoxsuprine
Ketamine
Ketoprofen
Labetalol
Levorphanol
Loperamide
Maprotiline
Meperidine
Meprobamate
Methadone
Methylphenidate
Morphine-3-D-glucoronide
Nalidixic acid
Naloxone
Naltrexone
Naproxen
Niacinamide
Nifedipine
Norcodein
Norethindrone
D-Norpropoxyphene
Noscapine
D,L-Octopamine
Oxalic Acid
Oxazepam
Oxolinic Acid
Oxycodone
Oxymetazoline
Papaverine
Penicillin-G
Pentazocaine
Perphenazine
Phencyclidine
Phenelzine
Phendimetrazine
Phetoin
L-Phenylephrine
b-Phenylethlamine
Phenylpropanolamine
Prednisolone
Prednisone
Procaine
Promazine
Promethazine
D,L-Propranolol
Propiomazine
D-Propoxyphene
Quinidine
Quinine
Ranitidine
Salicylic Acid
Serotonin
Sulfamethazine
Sulindac
Temazepam
Tetracycline
Tetrahydrocortisone
Tetrahydrozoline
Thiamine
Thioridazine
D,L-Thyroxine
Tolbutamine
Triamterene
Trifluoperazine
Trimethoprim
Trimipramine
Tryptamine
D,L- Tyrosine
Uric Acid
Verapamil
Zomepirac

Oxazepam:

4-Acetamindophenol	Diflunisal	Phencyclidine
Acetophenetidin	(-) Y Ephedrine	Phenelzine
N-acetylprocainamide	Fenoprofen	Phenobarbitol
Acetylsalicyclic Acid	Furosemide	Phenteramine
Aminopyrine	Gentisic acid	L-Phenylephrine
Amitryptyline	Hemoglobin	b-Phenylethlamine
Amorbarbital	Hydrocortisone	Phenylpropanolamine
Amoxicillin	O-Hydroxyhippuric acid	Prednisone
Ampicillin	p-Hydroxy-methamphetamine	D,L-Propranolol
l-Ascorbic Acid	3-Hydroxytyramine	D-Propoxyphene
Apormorphine	Ibuprofen	D-Pseudoephedrine
Asppartame	Imipramine	Quinine
Atropine	Iproniazid	Ranitidine
Benzilic Acid	(-) Isoproterenol	Salicylic Acid
Benzoic Acid	Isoxsuprine	Secobarbital
Benzoylecgonine	Ketamine	Serotonin (5-hydroxytyramine)
Bilirubin	Ketoprofen	Sertraline
Caffeine	Loperamide	Sulframethazine
Cannabidiol	Maprotiline	Sulindac
Chloralhydrate	Meperidine	Tetrahydrocortisone, 3 Acetate
Chloramphenicol	Meprobamate	Tetrahydrocortisone, (b-D glucuronide)
Chlordiazepoxide	Methadone	Tetrahydrozoline
Chlorothiazide	Methylphenamine	Thiamine
(+/-) Chlorpheniramine	(+) 3,4-Methylenedioxyamphetamine	Thioridazine
Chlorpromazine	(+) 3,4-Methylenedioxymethamphetamine	D,L-Thyroxine
Chlorquine	Nalidixic acid	Tolbutamine
Cholesterol	Nalorphine	Triamterene
Clomipramine	Naloxone	Trifluoperazine
Clonidine	Naltrexone	Trimethoprim
Cocaine hydrochloride	Naproxen	Triyptamine
Cortisone	Niacinamide	D,L-Tryptophan
(-) Cotinine	Nifedipine	Tyramine
Creatinine	Norethindrone	Uric Acid
Dextromethlorphan	D-Norpropoxyphene	Verapamil
Diazepam	Noscapine	Zomepirac
Diclorenac		

The pH of a drug-free urine pool was adjusted to pH 4, pH 5, pH 6, pH 7, pH 8 or pH 9; the urine was spiked with ± 25% of the cut-off concentration of d-amphetamine, secobarbital, or oxazepam, individually and tested using three batches of strips and three batches of cassettes of the Wondfo Amphetamine Urine Test, Wondfo Secobarbital Urine Tests, and Wondfo Oxazepam Urine Tests, respectively. The spiked, pH-adjusted urine was tested in triplicate. Altering the pH of the urine sample did not affect the accuracy of any of the test results.

The specific gravity of a drug-free urine pool was measured by obtaining urine samples with specific gravities of 1.000-1.035 and spiking with ± 25% of the cut-off concentration of d-amphetamine, secobarbital, or oxazepam, individually using three batches of strips and three batches of cassettes of the Wondfo Amphetamine Urine Test, Wondfo Secobarbital Urine Tests, and Wondfo Oxazepam Urine Tests, respectively. The spiked urines of different specific gravities were tested in triplicate. The results indicate that there is stable performance of the Wondfo Amphetamine Urine Test, Wondfo Secobarbital Urine Tests, and Wondfo Oxazepam Urine Tests when urine has a specific gravity between 1.00-1.035.

f. Assay cut-off:

The assay cut off was investigated by using three batches of both the strip and cassette formats of the Wondfo Amphetamine Urine Test, Wondfo Secobarbital Urine Tests, and Wondfo Oxazepam Urine Test to test 150 urine samples each. These samples came from the Shezhen Drug Addiction Recovery Center (25 Drug urine samples each for amphetamine, barbiturate, and benzodiazepine) and from drug-free urine samples (125 samples) spiked with d-amphetamine, secobarbital, or oxazepam (125 samples for each drug) diluted from the International Drug Standard (Sigma) to concentrations that are plus and minus 25% and 50 % of the cutoff concentrations. Drug concentrations were confirmed by GC/MS. Results were read by three laboratory assistants with relevant experience. The cutoff studies were performed by two separate groups of operators (one for the strip format and one for the cassette format). Three operators in each group performed the readings and they were blinded to the sample. Each result was confirmed by at least two assistants.

a. Strip

d-Amphetamine

[Drug] (ng/ml)	Cut-off range	n	Batch 1		Batch 2		Batch 3		Total	
			-	+	-	+	-	+	-	+
500	-50% Cutoff	30	30	0	30	0	30	0	90	0
750	-25% Cutoff	30	30	0	30	0	30	0	90	0
1000	Cut-off	30	4	26	2	28	3	27	9	81
1250	+25% Cut-off	30	0	30	0	30	0	30	0	90

1500	+50% Cut-off	30	0	30	0	30	0	30	0	90

Secobarbital

[Drug] (ng/ml)	Cut-off range	n	Batch 1		Batch 2		Batch 3		Total	
			-	+	-	+	-	+	-	+
150	-50% Cutoff	30	30	0	30	0	30	0	90	0
225	-25% Cutoff	30	30	0	30	0	30	0	90	0
300	Cut-off	30	2	28	3	27	2	28	7	83
375	+25% Cut-off	30	0	30	0	30	0	30	0	90
450	+50% Cut-off	30	0	30	0	30	0	30	0	90

Oxazepam

[Drug] (ng/ml)	Cut-off range	n	Batch 1		Batch 2		Batch 3		Total	
			-	+	-	+	-	+	-	+
150	-50% Cutoff	30	30	0	30	0	30	0	90	0
225	-25% Cutoff	30	30	0	30	0	30	0	90	0
300	Cut-off	30	3	27	4	26	4	26	11	79
375	+25% Cut-off	30	0	30	0	30	0	30	0	90
400	+50% Cut-off	30	0	30	0	30	0	30	0	90

b. Cassette:

d- Amphetamine

[Drug] (ng/ml)	Cut-off range	n	Batch 1		Batch 2		Batch 3		Total	
			-	+	-	+	-	+	-	+
500	-50% Cutoff	30	30	0	30	0	30	0	90	0
750	-25% Cutoff	30	30	0	30	0	30	0	90	0
1000	Cut-off	30	2	28	4	26	3	27	9	81
1250	+25% Cut-off	30	0	30	0	30	0	30	0	90
1500	+50% Cut-off	30	0	30	0	30	0	30	0	90

Secobarbital

[Drug] (ng/ml)	Cut-off range	n	Batch 1		Batch 2		Batch 3		Total	
			-	+	-	+	-	+	-	+
150	-50% Cutoff	30	30	0	30	0	30	0	90	0
225	-25% Cutoff	30	30	0	30	0	30	0	90	0
300	Cut-off	30	3	27	4	26	4	26	17	79
375	+25% Cut-off	30	0	30	0	30	0	30	0	90
400	+50% Cut-off	30	0	30	0	30	0	30	0	90

Oxazepam

[Drug] (ng/ml)	Cut-off range	n	Batch 1		Batch 2		Batch 3		Total	
			-	+	-	+	-	+	-	+
150	-50% Cutoff	30	30	0	30	0	30	0	90	0
225	-25% Cutoff	30	30	0	30	0	30	0	90	0
300	Cut-off	30	4	26	3	27	2	28	9	81
375	+25% Cut-off	30	0	30	0	30	0	30	0	90
400	+50% Cut-off	30	0	30	0	30	0	30	0	90

The cut-off values were determined to be:

Test	Calibrator	Cut-off (ng/ml)
Amphetamine	D-Amphetamine	1000
Secobarbital	Secobarbital	300
Oxazepam	Oxazepam	300

2. Comparison studies:
   a. Method comparison with predicate device:

      1. Performance of the Wondfo Amphetamine Urine Test, Wondfo Secobarbital Urine Tests, and Wondfo Oxazepam Urine Test (strip and cassette formats) was established by comparing 80 samples of each analyte against GC/MS. These samples came from drug-free urine (10 samples each), the Shezhen Drug Addiction Recovery Center (45 samples for each analyte) and from clinical

samples with concentrations ranges as follows: drug free urine, < -50% of the cut-off, -50% of the cut-off to the cut-off, cut-off to +50% of the cut-off, and > +50% of the Cut-off. There were two groups of operators (one for the strip format and one for the cassette format) and they were blinded to the samples. Each result was read by three laboratory assistants with relevant experience and a lay person with no experience other than reading the instructions for use.

a. Strip

d-Amphetamine:

Wondfo Device Result		**Drug-free urine**	**< -50% of the cut-off**	**-50% of the cut-off to the cut-off**	**cut-off to +50% of the cut-off**	**> +50% of the Cut-off**
Viewer A	+	0	0	2	11	29
	-	10	18	10	0	0
Viewer B	+	0	0	2	11	29
	-	10	18	10	0	0
Viewer C	+	0	0	2	11	29
	-	10	18	10	0	0
Lay Person	+	0	0	3	11	29
	-	10	18	9	0	0

Secobarbital:

Wondfo Device Result		**Drug-free urine**	**< -50% of the cut-off**	**-50% of the cut-off to the cut-off**	**cut-off to +50% of the cut-off**	**> +50% of the Cut-off**
Viewer A	+	0	0	2	20	20
	-	10	10	18	0	0
Viewer B	+	0	0	1	20	20
	-	10	10	19	0	0
Viewer C	+	0	0	2	20	20
	-	10	10	18	0	0
Lay Person	+	0	0	3	20	20
	-	10	10	17	0	0

Oxazepam:

Wondfo Device Result		Drug-free urine	< -50% of the cut-off	-50% of the cut-off to the cut-off	cut-off to +50% of the cut-off	> +50% of the Cut-off
Viewer A	+	0	0	2	20	20
	-	10	10	18	0	0
Viewer B	+	0	0	2	20	20
	-	10	10	18	0	0
Viewer C	+	0	0	1	20	20
	-	10	10	19	0	0
Lay Person	+	0	0	3	20	20
	-	10	10	17	0	0

b. Cassette

d-Amphetamine:

Wondfo Device Result		Drug-free urine	< -50% of the cut-off	-50% of the cut-off to the cut-off	cut-off to +50% of the cut-off	> +50% of the Cut-off
Viewer A	+	0	0	1	11	29
	-	10	18	11	0	0
Viewer B	+	0	0	2	11	29
	-	10	18	10	0	0
Viewer C	+	0	0	2	11	29
	-	10	18	10	0	0
Lay Person	+	0	0	3	11	29
	-	10	18	9	0	0

Secobarbital:

Wondfo Device Result		Drug-free urine	< -50% of the cut-off	-50% of the cut-off to the cut-off	cut-off to +50% of the cut-off	> +50% of the Cut-off
Viewer A	+	0	0	1	20	20
	-	10	10	19	0	0
Viewer B	+	0	0	2	20	20
	-	10	10	18	0	0

Viewer C	+	0	0	1	20	20
	-	10	10	19	0	0
Lay Person	+	0	0	2	20	20
	-	10	10	18	0	0

Oxazepam:

Wondfo Device Result		**Drug-free urine**	**< -50% of the cut-off**	**-50% of the cut-off to the cut-off**	**cut-off to +50% of the cut-off**	**> +50% of the Cut-off**
Viewer A	+	0	0	1	20	20
	-	10	10	19	0	0
Viewer B	+	0	0	1	20	20
	-	10	10	19	0	0
Viewer C	+	0	0	2	20	20
	-	10	10	18	0	0
Lay Person	+	0	0	2	20	20
	-	10	10	18	0	0

The discordant results are listed in the table below.

a. Strip:

Viewer	Sample Number	GC/MS result	Viewer Result
Viewer A	AMPC62	921	positive
Viewer A	AMPC65	993	positive
Viewer B	AMPC31	818	positive
Viewer B	AMPC65	993	positive
Viewer C	APMC31	818	positive
Viewer C	AMPC62	921	positive
Lay person	AMPC31	818	positive
Lay person	AMPC35	797	positive
Lay person	APMC65	993	positive
Viewer A	BARC34	243	positive
Viewer A	BAR216	280	positive
Viewer B	BARC34	243	positive
Viewer C	BARC35	237	positive
Viewer C	BAR216	280	positive
Lay person	BARC34	243	positive
Lay person	BARC35	237	positive
Lay person	BAR216	280	positive
Viewer A	BZOC34	243	positive
Viewer A	BZOC65	277	positive
Viewer B	BZOC32	226	positive
Viewer B	BZOC65	277	positive
Viewer C	BZOC34	243	positive
Lay person	BZOC32	226	positive
Lay person	BZOC34	243	positive
Lay person	BZOC65	277	positive

The results indicate that similar positive, negative and overall agreement rates for all three tests using the strip format.

b. Cassette:

Viewer	Sample Number	GC/MS result	Viewer Result
Viewer A	AMPC31	818	positive
Viewer B	APMC31	818	positive
Viewer B	AMPC62	921	positive
Viewer C	AMPC35	797	positive
Viewer C	APMC65	993	positive
Lay person	AMPC31	818	positive
Lay person	AMPC35	797	positive
Lay person	APMC65	993	positive
Viewer A	BARC35	237	positive
Viewer B	BARC34	243	positive
Viewer B	BAR216	280	positive
Viewer C	BAR216	280	positive
Lay person	BARC35	237	positive
Lay person	BAR216	280	positive
Viewer A	BZOC65	277	positive
Viewer B	BZOC34	243	positive
Viewer C	BZOC32	226	positive
Viewer C	BZOC65	277	positive
Lay person	BZOC34	243	positive
Lay person	BZOC65	277	positive

The results indicate that similar positive, negative and overall agreement rates for all three tests using the cassette format.

The overall agreement between the Wondfo devices and GC/MS is presented in the table below:

% Agreement	AMP (Strip)	AMP (Cassette)	BAR (Strip)	BAR (Cassette)	BZO (Strip)	BZO (Cassette)
Positive	100%	100%	100%	100%	100%	100%
Negative	95%	95.8%	95.8%	96.7%	95.8%	96.7%
Overall	97.5%	97.9%	97.9%	98.4%	97.9%	98.4%

The overall agreement between the lay viewer and experienced viewer is presented in the table below:

% Agreement	AMP (Strip)	AMP (Cassette)	BAR (Strip)	BAR (Cassette)	BZO (Strip)	BZO (Cassette)
Positive	100%	100%	100%	100%	100%	100%
Negative	97.4%	96.6%	96.6%	98.2%	96.6%	98.2%
Overall	98.7%	98.3%	98.3%	99.1%	98.3%	99.1%

B. A lay-user study was performed to assess the suitability of the device for home use. Six drug-free urine sample pools were spiked to ± 25%, ± 50%, ± 75% of the cutoff of target drug, additionally a negative urine pool with no drug was tested. These concentrations were confirmed by GC/MS and aliquoted into 40 individual containers per concentration (20 aliquots per concentration for each strip format). The testing was performed at three sites by 140 blinded consumers divided between three sites (140 users for strip, 140 for cassette for each of the Wondfo Amphetamine Urine Test, Wondfo Secobarbital Urine Tests, and Wondfo Oxazepam Urine Test). The lay users were chosen from likely intended users at the Shenzhen Drug Addiction Recovery Center, The First Affiliated Hospital of Jinan University, and the No. 177 Hospital of the People's Liberation Army. Each participant received the package inset, 1 blinded sample, and either a test strip or cassette. The lay persons test result was compared to the GC/MS result to demonstrate accuracy by lay-users. The following are the results of the lay-user study pooled together from all three sites for the strip format:

d-Amphetamine:

% of Cutoff	Number of Samples	d-Amphetamine GS/MS Concentration (ng/mL)	Lay person results: Pos/Neg	Percentage of Correct Results
-100% of the Cut-off	20	0	0/20	100
-75 % Cutoff	20	240.1	0/20	100
-50 % Cutoff	20	523.4	0/20	100
-25 % Cutoff	20	721.2	2/18	90.0
+25% Cutoff	20	1320.5	18/2	90.0
+50 % Cutoff	20	1465.5	20/0	100
+75 % Cutoff	20	1830.2	20/0	100

Secobarbital:

% of Cutoff	Number of Samples	Secobarbital GS/MS Concentration (ng/mL)	Lay person results: Pos/Neg	Percentage of Correct Results
-100% of the Cut-off	20	0	0/20	100
-75 % Cutoff	20	86.2	0/20	100
-50 % Cutoff	20	173.5	0/20	100
-25 % Cutoff	20	203.4	3/17	85.0
+25% Cutoff	20	401.6	18/2	90.0
+50 % Cutoff	20	466.5	20/0	100
+75 % Cutoff	20	560.8	20/0	100

Oxazepam:

% of Cutoff	Number of Samples	Oxazepam GS/MS Concentration (ng/mL)	Lay person results: Pos/Neg	Percentage of Correct Results
-100% of the Cut-off	20	0	0/20	100
-75 % Cutoff	20	70.2	0/20	100
-50 % Cutoff	20	168.5	0/20	100
-25 % Cutoff	20	253.4	3/17	85.0
+25% Cutoff	20	356.8	19/1	95.0
+50 % Cutoff	20	482.5	20/0	100
+75 % Cutoff	20	501.6	20/0	100

The following are the results of the lay-user study pooled together from all three sites for the cassette format:

d- Amphetamine:

% of Cutoff	Number of Samples	d-Amphetamine GS/MS Concentration (ng/mL)	Lay person results: Pos/Neg	Percentage of Correct Results
-100% of the Cut-off	20	0	0/20	100
-75 % Cutoff	20	240.1	0/20	100
-50 % Cutoff	20	523.4	0/20	100
-25 % Cutoff	20	721.2	2/18	90.0
+25% Cutoff	20	1320.5	19/1	950.0
+50 % Cutoff	20	1465.5	20/0	100
+75 % Cutoff	20	1830.2	20/0	100

Secobarbital:

% of Cutoff	Number of Samples	Secobarbital GS/MS Concentration (ng/mL)	Lay person results: Pos/Neg	Percentage of Correct Results
-100% of the Cut-off	20	0	0/20	100
-75 % Cutoff	20	86.2	0/20	100
-50 % Cutoff	20	173.5	0/20	100
-25 % Cutoff	20	203.4	2/18	90.0
+25% Cutoff	20	401.6	18/2	90.0
+50 % Cutoff	20	466.5	20/0	100
+75 % Cutoff	20	560.8	20/0	100

Oxazepam:

% of Cutoff	Number of Samples	Oxazepam GS/MS Concentration (ng/mL)	Lay person results: Pos/Neg	Percentage of Correct Results
-100% of the Cut-off	20	0	0/20	100
-75 % Cutoff	20	70.2	0/20	100
-50 % Cutoff	20	168.5	0/20	100
-25 % Cutoff	20	253.4	2/18	90.0
+25% Cutoff	20	356.8	19/1	95.0
+50 % Cutoff	20	482.5	20/0	100
+75 % Cutoff	20	501.6	20/0	100

The overall percent agreement between the Lay person and the GC/MS method was:

% Agreement	AMP (Strip)	AMP (Cassette)	BAR (Strip)	BAR (Cassette)	BZO (Strip)	BZO (Cassette)
Total	97.1%	97.8%	96.4%	97.1%	97.1%	97.8%

C. The subject's were to fill out a questionnaire to evaluate labeling access.

i. Strip format

d-Amphetamine:
The participant's ages ranged from 22-65 and there were slightly more males than females. They come from a variety of career and education backgrounds and none had used a home drug kit before. They all understood the storage and expiration of the device and that the test could not be reused. They all understood that the strip couldn't be immersed in urine above the marker line and to read the result within 5 minutes and how to interpret the results. None of the participants responded that the strip test was difficult, 104/140 said the test was very easy and 36/140 said it was easy. 80/140 said the instructions were very clear and 60/140 said they were clear. 108/140 said the interpretation of the results was very clear and 32/140 said they were clear. When asked what they would do if the device failed to show a result, 105/140 said they would repeat the test with a new test device, 5/140 said they would turn to another method, and 30/140 said they would call the manufacturer if the device failed to show a result.

Secobarbital:
The participant's ages ranged from 22-66 and there were slightly more males than females. They come from a variety of career and education backgrounds and only 1 participant had used a home drug kit before. They all understood the storage and expiration of the device and that the test could not be reused. They all understood that the strip couldn't be immersed in urine above the marker line and to read the result within 5 minutes and how to interpret the results. None of the participants responded that the strip test was difficult, 81/140 said the test was very easy and 59/140 said it was easy. 75/140 said the instructions were very clear and 65/140 said they were clear. 108/140 said the interpretation of the results was very clear and 32/140 said they were clear. When asked what they would do if the device failed to show a result, 111/140 said they would repeat the test with a new test device, 7/140 said they would turn to another method, and 22/140 said they would call the manufacturer if the device failed to show a result.

Oxazepam:
The participant's ages ranged from 22-65 and there were slightly more males than females. They come from a variety of career and education backgrounds and only 1 participant had used a home drug kit before. They all understood the storage and expiration of the device and that the test could not be reused. They all understood that the strip couldn't be immersed in urine above the marker line and to read the result within 5 minutes and how to interpret the results. Only 1 of the participants responded that the strip test was difficult, 81/140 said the test was very easy and 58/140 said it was easy. 66/140 said the instructions were very clear and 74/140 said they were clear. 86/140 said the interpretation of the results was very clear and 54/140 said they were clear. When asked what they would do if the device failed to show a result, 107/140 said they would repeat the test with a new test device, 7/140 said they would turn to another method, and 26/140 said they would call the manufacturer if the device failed to show a result.

ii. Cassette format

d-Amphetamine:
The participant's ages ranged from 23-64 and there were slightly more males than females. They come from a variety of career and education backgrounds and only 1 subject had used a home drug kit before. They all understood the storage and expiration of the device and that the test could not be reused. They all understood that the strip couldn't be immersed in urine above the marker line and to read the result within 5 minutes and how to interpret the results. None of the participants responded that the strip test was difficult, 77/140 said the test was very easy and 63/140 said it was easy. 79/140 said the instructions were very clear and 61/140 said they were clear. 92/140 said the interpretation of the results was very clear and 48/140 said they were clear. When asked what they would do if the device failed to show a result, 113/140 said they would repeat the test with a new test device, 6/140 said they would turn to another method, and 21/140 said they would call the manufacturer if the device failed to show a result.

Secobarbital:
The participant's ages ranged from 23-64 and there were slightly more males than females. They come from a variety of career and education backgrounds and only 2 participants had used a home drug kit before. They all understood the storage and expiration of the device and that the test could not be reused. They all understood that the strip couldn't be immersed in urine above the marker line and to read the result within 5 minutes and how to interpret the results. Only 1 of the participants responded that the strip test was difficult, 76/140 said the test was very easy and 63/140 said it was easy. 71/140 said the instructions were very clear and 69/140 said they were clear. 66/140 said the interpretation of the results was very clear and 74/140 said they were clear. When asked what they would do if the device failed to show a result, 108/140

said they would repeat the test with a new test device, 6/140 said they would turn to another method, and 26/140 said they would call the manufacturer if the device failed to show a result.

Oxazepam:
The participant's ages ranged from 23-69 and there were slightly more males than females. They come from a variety of career and education backgrounds and only none of the participants had used a home drug kit before. They all understood the storage and expiration of the device and that the test could not be reused. They all understood that the strip couldn't be immersed in urine above the marker line and to read the result within 5 minutes and how to interpret the results. None of the participants responded that the strip test was difficult, 71/140 said the test was very easy and 69/140 said it was easy. 77/140 said the instructions were very clear and 63/140 said they were clear. 82/140 said the interpretation of the results was very clear and 58/140 said they were clear. When asked what they would do if the device failed to show a result, 103/140 said they would repeat the test with a new test device,11/140 said they would turn to another method, and 26/140 said they would call the manufacturer if the device failed to show a result.

Additionally, a Flesh-Kincaid reading analysis revealed that both package inserts had a reading grade level of 7.

b. Matrix comparison:

Not applicable.

3. Clinical studies:

a. Clinical Sensitivity:

Not Applicable

*b. Clinical specificity**:***

Not Applicable

c. Other clinical supportive data (when a. and b. are not applicable):

Not Applicable

4. Clinical cut-off:

Not Applicable

5. Expected values/Reference range

Specific ranges for each analyte/methodology are listed in the package insert.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.