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

A. 510(k) Number:

k110182

B. Purpose for Submission:

New device

C. Measurand:

Cannabinoids

D. Type of Test:

Qualitative lateral flow chromatographic immunoassay

E. Applicant:

Guangzhou Wondfo Biotech Co., Ltd.

F. Proprietary and Established Names:

Cannabinoids Urine Test

G. Regulatory Information:

1. Regulation section:

   21 CFR §862.3870, Cannabinoid Test System

2. Classification:

   Class II

3. Product code:

   LDJ – Enzyme Immunoassay Cannabinoids

4. Panel:

   Toxicology (91)

H. Intended Use:

1. Intended use(s):

   See indications for use below.

2. Indications(s) for use:

   Wondfo Cannabinoids Urine Test is an immunochromatographic assay for the qualitative determination of Cannabinoids in human urine. The test is available in a cassette format and a strip format. The test has a cutoff of 50 ng/mL of Cannabinoids. 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 Wondfo Biotech Co., Ltd. Cannabinoids Test utilizes two formats: cassette and strip. The strip format involves immersing the absorbent end of the device into a urine sample. While the cassette is composed of a functional strip and a plastic cover and four drops of urine are added to the sample well. The devices are single-used and are used to obtain visual qualitative results for cannabinoids.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   ACON One Step Drug Screen Test

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

   k020771

3. Comparison with predicate:

Reagent Similarities and Differences		
Feature	**Candidate Device: Wondfo Cannabinoids Urine Test (k110182)**	**Predicate Device: ACON One Step Drug Screen Card (k020771)**
Intended/Indications for Use	Competitive binding, lateral flow immunochromatographic assay for the qualitative determination of cannabinoids in human urine.	Same
Methodology	Utilizes mouse monoclonal antibodies to selectively detect elevated levels of cannabinoids in human urine in a qualitative, visual manner.	Same
Specimen Type	Human Urine	Same
Analyte	Cannabinoids	Same
Testing Format	Strip: Dip test strip into urine samples Cassette: Add drops of urine onto test strips enclosed in a plastic container.	Same
Cut Off Value	50 ng/ml of Cannabinoids	Same
Device Design	Negative Result: One Colored Line in the Test Region, One colored line in the control region Positive Result: No Colored Line in the Test Region, One colored line in the control region	Same

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:

The Wondfo Biotech Co., Ltd. Cannabinoids is a competitive binding, lateral flow immunochromatographic assay for the qualitative determination of Cannabinoids and their metabolites in human urine. The test utilizes two formats: cassette and strip. For both formats, the urine is absorbed via capillary action and mixes with the anti-mouse IgG polyclonal antibody-dye conjugate. It then flows across a pre-coated membrane and if the sample is below the target cut off, the antibody-dye conjugate binds to the drug protein

conjugate immobilized in the test region. This produces a color test line indicating a negative result. When the Cannabinoids level is at or above the target cutoff, the free drug in the sample binds to the antibody-dye conjugate preventing the antibody-dye conjugate from binding to the drug-protein conjugate immobilized in the test region. This prevents the development of a colored line in the test region, indicating a positive result. A control line is also present that appears in the control region to indicate that the test has performed properly.

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 100, 87.5, 75, 62.5, 37.5, 25, 12.5 and 0 ng/ml of cannabinoid, corresponding to ± 25, ±50, ±75, and ±100% of the 50 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 Cannabinoids 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

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	5/45	0/50	0/50	0/50	0/50

b. Cassette Format

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	5/45	0/50	0/50	0/50	0/50

b. Linearity/assay reportable range:

Not Applicable, the assay is intended for qualitative use only

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 Cannabinoid Urine Drug Tests. Accelerated stability was performed at 50°C for 85 days, and the data support a greater than 18 month shelf life. Real time studies were performed by storing three lots of strips and cassettes at 4°C and 30°C. Performance tests were completed at defined intervals and the results support a 23 month shelf life at 4°C and 30°C.

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 Cannabinoid Urine Test. 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 several Cannabinoid-related compounds into drug-free urine at three different concentrations and measuring the samples in duplicate using three batches of strips and three batches of cassettes.

THC (Cannabinoids)	**Result**	**% Cross Reactivity**
(11-nor-D9-THC-9-COOH, cut-off=50 ng/mL)	Positive at 50 ng/mL	100%
11-nor-D8-THC-9-COOH	Positive at 30 ng/mL	167%
11-hydroxy-D9-	Positive at 2,500 ng/mL	2%

Tetrahydrocannabinol		
D8-Tetrahydrocannabinol	Positive at 7,500 ng/mL	<1%
D9-Tetrahydrocannabinol	Positive at 10,000 ng/mL	<1%
Cannabinol	Positive at 100,000 ng/mL	<1%
Cannabidiol	Positive at 100,000 ng/mL	<1%

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 ± 50% of the cut-off concentration of Cannabinoid:

Acetophenedtidin	Deoxycorticosterone	Labetalol	D-Propoxyphene
Nalidixic Acid	Dextromethorphan	Loperamide	D-Pseudoephedrine
Acetylsalicylic acid	Diclofenac	Meprobamate	Quinidine
Aminopyrine	Diflunisal	Methoxyphenamine	Quinine
Amoxicillin	Digoxin	Nalidixic acid	Rantidine
Ampicillin	Diphenhydramine	Naloxone	Salicylic acid
L-Pheynylephrine	L-Ephedrine	Naltrexone	Serotonin
Apomorphine	Ecgonine methylester	Naproxen	Sulfamethazine
Aspartame	Ethyl-p-aminobenzoate	Niacinamide	Sulindac
Atropine	Estradiol	Nifedipine	Tetracycline
Benzilic acid	Estrone-3-sulfate	Norethindrone	Tetrahydrocortisone
Benzoic acid	Erythromycin	D-Norphpoxyphene	3-Acetate
Benzphetamine	Fenoprofen	Noscapine	D-glucuronide
Bilirubin	Furosemide	D,L-Octopamine	Tetrahydrozoline
Deoxycorticosterone	Gentisic acid	Oxalic acid	Thiamine
Caffeine	Hemoglobin	Oclolinic acid	Thioridazine
Cholarlhydrate	Hydralazine	Oxymetazoline	D,L-Tyrosine
D,L-Chlolrpheniramine	Hydrochlorothiazide	Papaverine	Tolbutamide
Chlorpromazine	Hydrocortisone	Penicillin-G	Triamterene
Chlorquine	O-Hydroxyhippuric acid	Perphenazine	Trifluoperazine
Cholesterol	3-Hydroxytyramine	Phenelzine	Trimethoprim
Clonidine	D,L-Isoproterenol	Phenylethylamine	Tyramine
Cortisone	Isoxsuprine	Phenylpropanolamine	D,L-Tryptophan
L-Cotinine	Ketamine	Prednisone	Urine acid
Creatinine	Ketoprofen	D,L-Propanolol	Verapamil
			Zomepirac

The pH of a drug-free urine pool was adjusted to pH 4, pH 5, pH6, pH 7, pH 8 or pH 9; the urine was spiked with ± 50% of the cut-off concentration of Cannabinoid and tested using three batches of strips and three batches of cassettes of the Wondfo Cannabinoid Urine Test. The spiked, pH-adjusted urine was tested in duplicate. 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 ± 50% of the cut-off concentration of Cannabinoid and tested using three batches of strips and three batches of cassettes of the Wondfo Cannabinoid Urine Test. The spiked urines of different specific gravities were tested in duplicate. The results indicate that there is stable performance of the Wondfo Cannabinoid Urine Test 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 Cannabinoids Urine Test to test 150 urine samples. These samples came from the Shezhen Drug Addiction Recovery Center for cannabinoids (25 Drug urine samples) and from drug-free urine samples (125 samples) spiked with cannabinoid 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

[Drug] (ng/ml)	**Cut-off range**	**n**	**Batch 1**		**Batch 2**		**Batch 3**		**Total**	
			-	**+**	**-**	**+**	**-**	**+**	**-**	**+**
25	-50% Cutoff	30	30	0	30	0	30	0	90	0
38	-25% Cutoff	30	30	0	30	0	30	0	90	0
50	Cut-off	30	5	25	4	26	3	27	12	78
63	+25% Cut-off	30	0	30	0	30	0	30	0	90
75	+50% Cut-off	30	0	30	0	30	0	30	0	90

b. Cassette:

[Drug] (ng/ml)	**Cut-off range**	**n**	**Batch 1**		**Batch 2**		**Batch 3**		**Total**	
			-	**+**	**-**	**+**	**-**	**+**	**-**	**+**
25	-50% Cutoff	30	30	0	30	0	30	0	90	0
38	-25% Cutoff	30	30	0	30	0	30	0	90	0
50	Cut-off	30	5	25	4	26	3	27	12	78
63	+25% Cut-off	30	0	30	0	30	0	30	0	90

75	+50% Cut-off	30	0	30	0	30	0	30	0	90

The cut-off value was determined to be 50 ng/ml.

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

   1. Performance of the Wondfo Cannabinoids Urine Test (strip and cassette formats) was established by comparing 80 samples against GC/MS. These samples came from a drug addiction recovery center for cannabinoids and from drug-free urine 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

Wondfo Device Result		**< -50% of the cut-off by GC/MS concentration**	**-50% of the cut-off to the cut-off by GC/MS concentration**	**cut-off to +50% of the cut-off by GC/MS concentration**	**> +50% of the Cut-off by GC/MS concentration**
Viewer A	+	0	1	18	22
	-	22	17	0	0
Viewer B	+	0	1	18	22
	-	22	17	0	0
Viewer C	+	0	2	18	22
	-	22	16	0	0
Lay Person	+	0	1	18	22
	-	22	17	0	0

      b. Cassette

Wondfo Device Result		**< -50% of the cut-off by GC/MS concentration**	**-50% of the cut-off to the cut-off by GC/MS concentration**	**cut-off to +50% of the cut-off by GC/MS concentration**	**> +50% of the Cut-off by GC/MS concentration**
Viewer A	+	0	1	18	22
	-	22	17	0	0
Viewer B	+	0	1	18	22
	-	22	17	0	0

Viewer C	+	0	2	18	22
	-	22	16	0	0
Lay Person	+	0	1	18	22
	-	22	17	0	0

The discordant results are listed in the table below.

Viewer	**Sample Number**	**GC/MS result**	**Viewer Result**
Viewer A	MC34	39	positive
Viewer B	GDA 20	49	positive
Viewer C	MC34	39	positive
Viewer C	GDA19	47	positive
Lay Person	MC34	39	positive
Lay Person	GDA19	47	positive

Both the test and strip formats resulted in a 100% Positive agreement and a 97.5% negative agreement between the Wondfo Devices and the GC/MS method. Additionally, the agreement lay user had 100% positive agreement and 94% negative agreement between the Wondfo Devices and the GC/MS method.

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 with 12.5, 25, 37.5, 62.5, 75, and 87.5 ng/ml of target drug (11-nor-D9-THC-9-COOH) corresponding to ± 25%, ± 50%,± 75% of the THC cutoff (50 ng/mL), 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 242 blinded consumers divided between three sites. The lay users were chosen from likely intended users at the Shenzhen Drug Addiction Recovery Center, Baiyun Drug Addition Recovery Volunteer Center, and the No. 177 Hospital of the People's Liberation Army. Each participant received the package inset in English, 1 sample, and a either 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:

% of Cutoff	Number of Samples	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	16	13.6	0/16	100
-50 % Cutoff	17	24.3	0/17	100
-25 % Cutoff	17	38.4	3/14	82.4
+25% Cutoff	16	64.5	14/2	87.5
+50 % Cutoff	17	72.2	17/0	100
+75 % Cutoff	16	91.2	16/0	100

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

% of Cutoff	Number of Samples	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	17	13.6	0/17	100
-50 % Cutoff	17	24.3	0/17	100
-25 % Cutoff	18	38.4	2/16	88.9
+25% Cutoff	17	64.5	15/2	88.2
+50 % Cutoff	18	72.2	18/0	100
+75 % Cutoff	16	91.2	16/0	100

The overall percent agreement was 114/119 (95.8%) for the strip format and 119/123 (96.7%) for the cassette format.

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

i. Strip format

The participant's ages ranged from 21-61 and there were slightly more males than females. They come from a variety of career and education backgrounds and 2.7% 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 person responded that the strip test was difficult, 84/119 said the test was very easy and 35/119 said it was easy. 76/119 said the instructions were very clear and 43/119said they were clear. 86/119 said the interpretation of the results was very clear and 33/119 said they were clear. When asked what they would do if the device failed to show a result, 104/119 said they would repeat the test with a new test device, 5/119 said they would turn to another method, and 10/119 said they would call the manufacturer if the device failed to show a result.

ii. Cassette format

The participant's ages ranged from 22-60 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 4 drops of sample were required to add to the sample well, and to read the result within 5 minutes and how to interpret the results. 78/123 said the test was very easy and 45/123 said it was easy. 76/123 said the instructions were very clear and 43/123 said they were clear. 80/123 said the interpretation of the results was very clear and 43/123 said they were clear. When asked what they would do if the device failed to show a result, 100/123 said they would repeat the test with a new test device, 3/123 said they would turn to another method, and 20/123 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.