BioSieve™ Fentanyl FIA Pro Test Kit
(Fluorescence Immunochromatographic Assay)

For *in vitro* diagnostic use.

This Package Insert must be read carefully prior to use. Package Insert must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the Package Insert.

INTENDED USE

BioSieve™ Fentanyl FIA Pro Test Kit is a fluorescence immunoassay (FIA) for the qualitative determination of fentanyl in human urine at a cutoff concentration of 1.0 ng/mL. The assay is intended for use with BioSieve™ Toxismart Reader.
It is for in vitro diagnostic use only.
The tests provide only preliminary results. A more specific alternative chemical method must be used in order to obtain a confirmed analytical result. Gas Chromatography/Mass Spectrometry (GC/MS) or Liquid Chromatography/Tandem Mass Spectrometry (LC/MS-MS) is the preferred confirmatory method.
Clinical consideration and professional judgment should be exercised with any drug test result, particularly when the preliminary test result is positive.

SUMMARY AND EXPLANATION

Fentanyl, also spelled fentanil, is a highly potent synthetic piperidine opioid drug primarily used as an analgesic. It is approximately 100 times more potent than morphine and 50 times more potent than heroin as an analgesic. It is prescribed for patients with chronic pain and is used to manage pain after surgery or for treatment of breakthrough pain in cancer patients. [1] Tolerance occurs when you need a higher and/or more frequent amount of a drug to get the desired effects. Because fentanyl is a powerful prescription opioid it can be misused, abused and cause overdose deaths.

PRINCIPLE OF THE TEST

BioSieve™ Fentanyl FIA Pro Test Kit uses the principle of competitive and fluorescence immunochromatography assay. The nitrocellulose membrane test area (T) of the test strip is correspondingly coated with fentanyl-bovine serum albumin conjugate, and the quality control area (C) is coated with goat anti-rabbit IgG polyclonal antibody. Both Fentanyl monoclonal antibody and rabbit IgG polyclonal antibody labeled with fluorescent microspheres are embedded on the conjugate pad. The labeled antibody will flow forward with the sample, when the urine sample is applied to the sample well of the test device. When the concentration of fentanyl in the sample is higher than or equal to the cut-off of the product, it will compete with the corresponding conjugate coated on the test area (T) to bind to the fluorescently labeled monoclonal antibody, the fluorescence signal rendering of the test line is inhibited and the result is positive; while when the sample does not contain fentanyl or its concentration is lower than the cut-off of the product, the corresponding conjugate on the test line reacts with sufficient fluorescently-labeled monoclonal antibodies, the test line will have fluorescence signal and the result is negative. The quality control area (C) will develop fluorescence signal, which is the criteria for judging whether the test process is normal or not. Signal intensity of fluorescence is detected by BioSieve™ Toxismart Reader.

REAGENTS AND MATERIALS SUPPLIED

1. Main components:

Components	Specification
Test Device (code chip on the back)	25
Dropper	25
Package Insert	1

2. Ingredients of the Test Device

The Test Device is packaged in aluminum foil pouch for single serving, containing desiccant, and consists of a test strip, a plastic cassette and a code chip. The main components on the test strip are:

Fentanyl-bovine serum albumin conjugate	Fixed on T zone of nitrocellulose membrane

Goat anti Rabbit IgG polyclonal antibodies	Fixed on C zone of nitrocellulose membrane
Fluorescent labeled fentanyl monoclonal antibodies and Rabbit IgG polyclonal antibodies	Embedded on the conjugate pad

***Note**: The* plastic cassette *of the test device cannot be disassembled, which may affect the operation of code chip. The code chip stores information such as the batch number of the kit, and cut-off signal value.*

MATERIALS REQUIRED BUT NOT PROVIDED

- BioSieve™ Toxismart Reader
- Timer
- Specimen collection container

WARNINGS AND PRECAUTIONS

1. For professional *in vitro* diagnostic use only.
2. Please follow the Package Insert when testing.
3. All specimens that have a positive test result using this Test Device must be further confirmed.
4. The Test Device should remain sealed in its original pouch until just before use. Do not use the Test Device, if pouch is damaged or has already been opened.
5. Do not reuse the Test Device and do not use your test kits beyond the expiration date. Biological materials used beyond their expiry date can become unstable and fail.
6. This product is suitable for qualitative test of urine samples only, and abnormal results may be obtained if other samples or solutions are tested.
7. There is drying agent in the aluminum foil pouch, which is inedible.
8. Errors may be caused by diluted and unclean samples or improper operation. Every sample should be collected using a new container to avoid contamination.
9. Errors may be caused if the results are read too early or too late.
10. Urine specimen, laboratory waste and disposable article should be considered potentially hazardous and handled in the same manner as an infectious agent.
11. Comply with safety operation regulations and correctly wear work clothes and gloves during operation.
12. As with all diagnostic reagents, the final diagnosis should be made after the comprehensive test indicators and clinical symptoms.

SPECIMEN COLLECTION AND PREPARATION

1. Urine Assay

The urine specimens must be collected in a clean and dry container or the test cup provided. Urine collected at any time of the day may be used. Urine specimens exhibiting visible precipitates should be centrifuged, filtered, or allowed to settle to obtain a clear supernatant for testing.

2. Specimen Storage

Test with fresh urine at room temperature. Urine specimens may be stored at 35.6 ~ 46.4°F (2 ~ 8°C) for up to 48 hours prior to assay. For prolonged storage, specimens may be frozen and stored below -4°F (-20°C). Frozen specimens can be frozen and thawed up to 3 times when below -4°F (-20°C). It should be thawed and mixed before testing.
Urine samples, experimental waste, disposable items, etc. shall be treated as potential infectious agents.

KIT STORAGE AND STABILITY

1. Store the test kit in a cool, dry place between 35.6 ~ 86°F (2 ~ 30°C). Keep away from light. Exposure to temperature and/or humidity outside the specified conditions may cause inaccurate results.

2. Do not freeze. Use the test kit at temperatures between 64.4 ~ 77°F (18 ~ 25°C).

3. Do not open the pouch until ready to perform the assay. Once the pouch is opened, the Test Device should be used in 1 hour.

4. Do not use the test kit beyond the expiration date (printed on the foil pouch and box). The validity is 24 months. All expiration dates are printed in Year-Month-Day format. Example: 2024-06-18 indicates June 18, 2024.

APPLICABLE EQUIPMENT

This Test Kit is only applicable to the BioSieve™ Toxismart Reader, produced by VivaChek Biotech (Hangzhou) Co., Ltd.

QUALITY CONTROL

User should follow federal, state and local guidelines for testing quality control materials.

External Control (positive and negative) should be run with each new lot of tests received, each new shipment, each new operator and monthly to determine that tests are working properly. This will ensure that the end user has clear understanding of when to

perform quality control testing.

Control materials are not provided with BioSieve™ Fentanyl FIA Pro Test Kit. However, it is recommended that positive and negative controls be tested as good laboratory practice to confirm the test procedure and to verify proper test performance. Our recommended quality control material available to users is the VivaChek BioSieve™ Fentanyl Control (Ordering Product Code : VC-FYCTL) Please contact your local distributor or VivaChek Diagnostics Inc. USA Technical Support for more information.

TEST PROCEDURE

Preparation of the Test

1. Press the right button "  " for 5 seconds to turn on the Reader, initial test interface screen appears.
2. If the test kit has been stored in a refrigerator, place it on a clean and flat surface at 64.4 ~ 77°F (18 ~ 25°C) for at least 30 minutes before testing.
3. Check the contents of BioSieve™ Fentanyl FIA Pro Test Kit: 'Test Device (packaged in pouch with desiccant)', 'Dropper', and 'Package Insert'.
4. Check the label information on the Test Device to ensure it is consistent with the box label.

***Note**: Please refer to the User Manual of BioSieve™ Toxismart Reader for more operating instructions. The Reader control procedure shall be conducted once a week to ensure that the Reader can be used normally.*

Standard Test:

a. Take the Test Device out of the foil pouch and place it on a clean, dust-free and flat surface.
b. Apply 3 drops (~75 µL) urine to the sample well of the Test Device using the Dropper.
c. Insert the Test Device into the slot of BioSieve™ Toxismart Reader immediately. Ensure proper orientation of the Test Device before pushing it into the slot.
d. The Reader will automatically display the result after 5 minutes countdown.

INTERPRETATION OF TEST RESULTS

BioSieve™ Fentanyl FIA Pro Test Kit with BioSieve™ Toxismart Reader can only perform qualitative analysis.

1. **Positive:** This indicates that the drug concentration in the urine specimen exceeds the designated cut-off and is a preliminary positive result for fentanyl.
2. **Negative:** This indicates that the drug concentration in the urine specimen is zero or below the designated cut-off level for fentanyl.
3. **Invalid:** The test is invalid. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test using a new test panel. If the problem persists, discontinue using the lot immediately and contact your local distributor.

The preliminary positive test result does not always mean that a person took illegal drugs.

The negative test result does not always mean that a person did not take illegal drugs.

There could be a number of factors that affect the reliability of drug tests. Certain drugs of abuse tests are more accurate than others.

LIMITATIONS

1. The performance of this product has been established for human urine only, and abnormal results may be obtained if other samples or solutions are tested.
2. A false positive result might be obtained from relevant drugs and foods.
3. There is a possibility that substances and/or factors may interfere with the test and cause false results. Technical or procedural errors may also contribute to erroneous results.
4. A positive result indicates presence of the drug or its metabolites but does not indicate level of 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. 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.
7. The test does not distinguish between drugs of abuse and certain medications.
8. BioSieve™ Fentanyl FIA Pro Test Kit provides only a preliminary analytical result. A more specific chemical method must be used to obtain a confirmed result. GC/MS or LC/MS-MS is the preferred confirmatory method.

PERFORMANCE CHARACTERISTICS

1. ACCURACY

Eighty clinical urine specimens were analyzed by LC-MS/MS and the BioSieve™ Fentanyl FIA Pro Test Kit. Each sample was

performed by three operators in three clinical sites with three lots of test kits. Samples were divided by concentration into five categories: drug-free, low negative (less than -50%), near cutoff negative (Between -50% and the cutoff), near cutoff positive (between cutoff and +50%) and high positive (greater than +50%).

Results were as follows:

Site	Result	Drug-free	Low Negative	Near Cutoff Negative	Near Cutoff Positive	High Positive
Site 1	+	0	0	4	19	20
	-	10	18	8	1	0
Site 2	+	0	0	3	18	20
	-	10	18	9	2	0
Site 3	+	0	0	2	19	20
	-	10	18	10	1	0
%Agreement among Positive is 96.7% %Agreement among Negative is 92.5%						

2. PRECISION

To investigate the analytical sensitivity and precision, drug samples at concentrations of -100% cutoff, -75% cutoff, -50% cutoff, -25% cutoff, cutoff, +25% cutoff, +50% cutoff, +75% cutoff and +100% cutoff were tested using three lots of device by three different operators. Each of the 3 operators tested 2 aliquots at each concentration for each lot per day (2 runs/ day), for a total of 60 determinations per concentration per lot of device.

The results are given below:

Drug	Concentration (ng/mL)	n	Result Negative/ Positive		
			Lot 1	Lot 1	Lot 3
Fentanyl	0	60	60/0	60/0	60/0
	0.25	60	60/0	60/0	60/0
	0.5	60	60/0	60/0	60/0
	0.75	60	58/2	60/0	59/1
	1	60	28/32	28/32	29/31
	1.25	60	0/60	0/60	0/60
	1.5	60	0/60	0/60	0/60
	1.75	60	0/60	0/60	0/60
	2	60	0/60	0/60	0/60

3. ANALYTICAL SPECIFICITY

To investigate the analytical specificity, drug metabolites and other components that are likely to interfere in urine samples were tested using three lots of device. The lowest concentration that caused a positive result for each compound is listed below:

Drug	Concentration (ng/mL)	% Cross-Reactivity
Acetyl fentanyl	1.2	83.33
Acrylfentanyl	1.2	83.33
ω-1-Hydroxyfentanyl	20000	0.01
Isobutyryl fentanyl	1.5	66.67
Ocfentanil	1.5	66.67
Butyryl fentanyl	1.6	62.50
Furanyl fentanyl	1.75	57.14
Valeryl fentanyl	2.5	40.00
(±) β-hydroxythiofentanyl	2.8	35.71
4-Fluoro-isobutyrylfentanyl	3	33.33

Drug	Concentration (ng/mL)	% Cross-Reactivity
Para-fluorobutyryl fentanyl	3	33.33
Para-fluoro fentanyl	3	33.33
(±)-3-cis-methyl fentanyl	5	20.00
Carfentanil	500	0.20
Sufentanil	625	0.16
Alfentanil	100000	0.00
Despropionyl fentanyl (4-ANPP)	50000	0.00

The following other Metabolites and opioids compounds were tested at a concentration of 100 µg/mL. Negative results were obtained for all these compounds. There is no cross-reactivity for these compounds using the BioSieve™ Fentanyl FIA Pro Test Kit.

Remifentanil	Norfentanyl	Acetyl norfentanyl
Norcarfentanil	6-Acetyl morphine	Amphetamine
Buprenorphine	Buprenorphineglucuronide	Codeine
Dextromethorphan	Dihydrocodeine	EDDP
EMDP	Fluoxetine	Heroin
Hydrocodone	Hydromorphone	Ketamine
Levorphanol	Meperidine	Methadone
Morphine	Morphine-3-glucuronide	Naloxone
Naltrexone	Norbuprenorphine	Norcodeine
Norketamine	Normeperidine	Normorphine
Noroxycodone	Oxycodone	Oxymorphone
Pentazocine (Talwin)	Pipamperone	Risperidone
Tapentadol	Thioridazine	Tilidine
Tramadol	Tramadol-O- Desmethyl	Tramadol-N- Desmethyl
Trazodone		

4. EFFECT OF URINARY DENSITY

Twelve urine samples with density ranges from 1.000 to 1.035 were spiked with -50% cutoff and +50% cutoff of fentanyl. Each sample was tested by three different operators per lot of device, with a total of three lots. The results demonstrate that varying ranges of urinary specific gravity do not affect the test results.

5. EFFECT OF THE URINARY PH

The pH of an aliquoted negative urine pool was adjusted to a pH range of 4 to 9 in 1 pH unit increments and spiked with -50% cutoff and +50% cutoff of fentanyl. The spiked, pH-adjusted urine samples were tested by three different operators per lot of device, with a total of three lots. The results demonstrate that varying ranges of pH does not interfere with the performance of the test.

6. CROSS-REACTIVITY

A study was conducted to determine the cross-reactivity of the test with compounds in either drug-free urine or drug positive urine containing Fentanyl. The following compounds show no cross-reactivity when tested with the Fentanyl Test at a concentration of 100µg/ml or specified concentrations. There are no differences observed between different lots.

NON CROSS-REACTING COMPOUNDS

Acetaminophen	Doxepin (50 µg/mL)	Nortriptyline (25 µg/mL)
Acetone (1000 mg/dL)	Ecgonine methyl ester	Noscapine
Acetophenetidin	Ephedrine	O-Hydroxyhippuric acid

Acetylsalicylic acid	Erythromycin	Octopamine
Albumin (100 mg/dL)	Ethanol (1%)	Oxalic acid (100 mg/dL)
Albuterol	Fenoprofen	Oxazepam
Aminopyrine	Fluphenazine	Oxolinic acid
Amitriptyline (35 µg/mL)	Furosemide	Oxymetazoline
Amobarbital	Galactose (10 mg/dL)	Papaverine
Amoxicillin	Gamma Globulin (500 mg/dL)	Penicillin G
Ampicillin	Gentisic acid	Perphenazine
Apomorphine	Glucose (3000 mg/dL)	Phencyclidine
Ascorbic acid	Hemoglobin	Phenelzine
Aspartame	Hydralazine	Phenobarbital
Atropine	Hydrochlorothiazide	Prednisone
Benzilic acid	Hydrocortisone	Propoxyphene (50 µg/mL)
Benzoic acid	Hydroxytyramine	Propranolol
Benzoylecgonine	Ibuprofen	Pseudoephedrine
Bilirubin	Imipramine (30 µg/mL)	Quinine
Boric Acid (1%)	Isoproterenol	Ranitidine
Bupropion (50 µg/mL)	Isoxsuprine	Riboflavin (7.5 mg/dL)
Caffeine	Ketamine	Salicylic acid
Carbamazepine	Ketoprofen	Secobarbital
Chloral hydrate	Labetalol	Serotonin (5-Hydroxytyramine)
Chloramphenicol	Lidocaine (50 µg/mL)	Sulfamethazine
Chlorothiazide	Loperamide	Sulindac
Chlorpromazine	Maprotiline (50 µg/mL)	Tetrahydrocortisone 3-(β-Dglucuronide)
Cholesterol	Meperidine	Tetrahydrocortisone 3-acetate
Clomipramine (50 µg/mL)	Meprobamate	Tetrahydrozoline
Clonidine	Methapyrilene (10 µg/mL)	Thiamine
Cortisone	Methaqualone (50 µg/mL)	Thioridazine
Cotinine	Methoxyphenamine	Triamterene
Creatinine	Metronidazole (300 µg/mL)	Trifluoperazine
Cyclobenzaprine (10 µg/mL)	N-Acetylprocainamide	Trimethoprim
Deoxycorticosterone	NaCl (4000 mg/dL)	Tyramine
Desipramine (50 µg/mL)	Nalidixic acid	Urea (2000 mg/dL)
Dextromethorphan	Naloxone	Uric acid
Diclofenac	Naltrexone	Valproic acid (250 µg/mL)
Diflunisal	Naproxen	Venlafaxine
Digoxin	Niacinamide	Verapamil
Diphenhydramine	Nicotine (10 µg/mL)	Zomepirac
DL-Tryptophan	Nifedipine	β-Estradiol
DL-Tyrosine	Norethindrone	

REFERENCES

[1] Mystakidou, K. et al. 2005. Oral mucosal fentanyl citrate for the treatment of breakthrough pain in cancer patients: An overview of its pharmacological and clinical characteristics. J Opioid Manag. 1:36-40

INDEX OF SYMBOLS

	Consult instructions for use or consult electronic instructions for use
	Use-by date
	Contains sufficient for <n> tests
	In vitro diagnostic medical device
	Batch code
	Catalogue number
	Manufacturer
	Temperature limit
	Do not re-use

Manufactured by VivaChek Biotech (Hangzhou) Co., Ltd. Level 2, Block 2, 146 East Chaofeng Rd. Hangzhou, China

Made in China
VivaChek Diagnostics Inc. USA Technical Support
Toll Free No.: (888)860-3894
Our service time is Monday-Friday, 8:00AM-5:00PM, Eastern Standard Time.

Number: 1604xxxxxx
Effective date: 2024-06-24