Rapid Response™

Fentanyl Test Cassette
(Urine)
REF FYL-1CAP156-25 Product Insert

A rapid test for the qualitative detection of FYL (Fentanyl) in human urine.
CLIA Waived
Medical and other professional *in vitro* diagnostic use labeling.

Intended Use

Rapid Response™ Fentanyl Test Cassette is an immunoassay intended for the qualitative detection of fentanyl in human urine at a cutoff concentration of 1 ng/mL.
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 or LC/MS is the preferred confirmatory method. For *in vitro* diagnostic use only.

Summary

Fentanyl, belongs to powerful narcotics analgesics, and is a μ special opiates receptor stimulant. Fentanyl is one of the varieties that been listed in management of United Nations "Single Convention of narcotic drug in 1961". Among the opiates agents that under international control, fentanyl is one of the most commonly used to cure moderate to severe pain.[1] After continuous injection of fentanyl, the sufferer will have the performance of protracted opioid abstinence syndrome, such as ataxia and irritability etc,[2,3] which presents the addiction after taking fentanyl in a long time. Compared with drug addicts of amphetamine, drug addicts who take fentanyl mainly have got the possibility of higher infection rate of HIV, more dangerous injection behavior and more lifelong medication overdose.[4]
The Rapid Response™ Fentanyl Test Cassette is a rapid urine screening test that can be performed without the use of an instrument. The test utilizes a monoclonal antibody to selectively detect elevated levels of Fentanyl in urine. The Rapid Response™ Fentanyl Test Cassette yields a positive result when Fentanyl in urine exceeds 1 ng/mL.

Principle

The Rapid Response™ Fentanyl Test Cassette is an immunoassay based on the principle of competitive binding. Drugs which may be present in the urine specimen compete against the drug conjugate for binding sites on the antibody.
During testing, a urine specimen migrates upward by capillary action. Fentanyl, if present in the urine specimen below 1 ng/mL, will not saturate the binding sites of antibody-coated particles in the test device. The antibody-coated particles will then be captured by immobilized FYL conjugate and a visible colored line will show up in the test line region. The colored line will not form in the test line region if the FYL level exceeds 1 ng/mL because it will saturate all the binding sites of anti-FYL antibodies.
A drug-positive urine specimen will not generate a colored line in the test line region, while a drug-negative urine specimen or a specimen containing a drug concentration less than the cut-off will generate a line in the test line region. To serve as a procedural control, a colored line will always appear at the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.

Reagents

The test cassette contains mouse monoclonal anti-FYL antibody-coupled particles and FYL-protein conjugate. A goat antibody is employed in the control line system.

Precautions

For medical and other professional *in vitro* diagnostic use only. Do not use after the expiration date.
- The test should remain in the sealed pouch until use.
- All specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
- The used test should be discarded according to local regulations.

Materials

Materials provided
- Test cassettes and droppers (sealed in foil pouch with a desiccant)
- Product Insert

Materials required but not provided
- Specimen Collection Containers
- Timer

Storage and Stability

Store as packaged in the sealed pouch at 35.6-86°F (2-30°C). The test is stable through the expiration date printed on the sealed pouch. The test cassettes must remain in the sealed pouch until use. **DO NOT FREEZE.** Do not use beyond the expiration date.

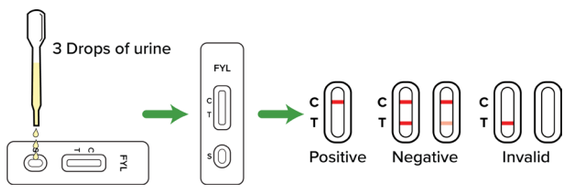
Specimen Collection and Preparation

Urine Assay
The urine specimen must be collected in a clean and dry container. Urine collected at any time of the day may be used.

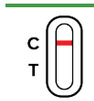
Specimen Storage
Urine specimens may be stored at 35.6-46.4°F (2-8°C) for up to 48 hours prior to testing. For long-term storage, specimens may be frozen and stored below -4°F (-20°C). Frozen specimens should be thawed and mixed before testing.

Test Procedure

Allow the test, urine specimen and/or controls to reach room temperature 59-86°F (15-30°C) prior to testing.
1. Remove the test cassette from the sealed pouch and use it within one hour.
2. Place the test cassette on a clean and level surface. Hold the dropper vertically and transfer 3 full drops of urine (approx. 120 μL) to the specimen well (S) of the test cassette, and then start the timer. Avoid trapping air bubbles in the specimen well (S). See the illustration below.
3. Wait for the colored line(s) to appear. **Read results at 5 minutes.** Do not interpret the result after 10 minutes.
4. If preliminary positive results are observed, please send the urine sample to the laboratory for confirmation.



Results Interpretation



POSITIVE: One colored line appears in the control line region (C). No line appears in the test line region (T). A positive result indicates that the Fentanyl concentration exceeds the detectable level (1 ng/mL).

NEGATIVE:* Two colored lines appear. One colored line should be in the control line region (C) and another colored line should be in the test line region (T). A negative result indicates that the Fentanyl concentration is below the detectable level (1 ng/mL).
***NOTE:** The shade of color in the test line region (T) may vary, but it should be considered negative whenever there is even a faint colored line.

INVALID: Control line fails to appear. 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. If the problem persists, discontinue using the lot immediately and contact your local distributor.

Quality Control

A procedural control is included in the test. A colored line appearing in the control line region (C) is considered an internal procedural control. It confirms sufficient specimen volume, adequate membrane wicking and correct procedural technique. Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as good laboratory testing practice to confirm the test procedure and to verify proper test performance. Our recommended quality control material available to users is Fentanyl Cerilliant F-013 at 1.0 mg/mL, which is same QC material used for our performance studies. User should follow federal, state and local guidelines for testing quality control materials.
Laboratories should comply with all federal state, and local laws, as well as all guidelines and regulations.

Limitations

1. The Rapid Response™ Fentanyl Test Cassette provides only a qualitative, preliminary result. A secondary analytical method must be used to obtain a confirmed result. GC/MS or LC/MS is the preferred confirmatory method.
2. It is possible that technical or procedural errors, as well as other interfering substances in the urine specimen may cause erroneous results.
3. A confirmed positive result indicates presence of the drug but does not indicate level of intoxication, administration route or concentration in urine.
4. 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.
5. Test does not distinguish between drugs of abuse and certain medications.

Performance Characteristics

Accuracy
About 80 clinical urine specimens fentanyl with known LC/MS values and tested by Rapid Response™ Fentanyl Test Cassette. Each test was performed by three operators. Results were as follows:

Site	Rapid Response™ Fentanyl Test Cassette	Concentration by LC/MS (ng/mL)			
		≤ -50% Cut-off	-50% Cut-off to the cut-off	Cut-off to +50% cut-off	≥ +50% Cut-off
Site 1	Positive	0	3	22	16
	Negative	25	12	2	0
	Total Results	100%	80.0%	91.7%	100%
Site 2	Positive	0	3	22	16
	Negative	25	12	2	0
	Total Results	100%	80.0%	91.7%	100%
Site 3	Positive	0	2	21	16
	Negative	25	13	3	0
	Total Results	100%	86.7%	87.5%	100%

Analytical Specificity
The following table list compounds that are positively detected

in urine by Rapid Response™ Fentanyl Test Cassette.

Fentanyl (Cut-off=1 ng/mL)	Concentration (ng/mL)	Cross- Reactivity (%)
Acetyl fentanyl	1	100%
Acrylfentanyl	1	100%
ω-1-Hydroxyfentanyl	20,000	0.005%
Isobutyryl fentanyl	1	100%
Ocfentanil	2.5	40%
Butyryl fentanyl	2.5	40%
Furanyl fentanyl	5	20%
Valeryl fentanyl	10	10%
(±) β-hydroxythiofentanyl	2	50%
4-Fluoro-isobutyrylfentanyl	50	2%
Para-fluorobutyryl fentanyl	4	25%
Para-fluoro fentanyl	3	33.3%
(±)-3-cis-methyl fentanyl	50	2%
Carfentanil	2	50%
Sufentanil	7.5	13.3%
Alfentanil	5,000	0.02%
Despropionyl fentanyl (4-ANPP)	2,500	0.04%
Trazodone	1,000	0.1%
Remifentanil	>100μg/mL	< 0.001%
Norfentanyl	>100μg/mL	< 0.001%
Acetyl norfentanyl	>100μg/mL	< 0.001%
Norcarfentanil	>100μg/mL	< 0.001%

The following 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 Rapid Response™ Fentanyl Test Cassette.

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

Precision

This study is performed by three POC personnel who don't know the sample number system participate in the study, at 3 POC site. Three lots were run in consecutive business days at each concentration for each lot per day. The results as follows:

Concentration	n	Lot 1		Lot 2		Lot 3	
		+	-	+	-	+	-
0 ng/mL	60	0	60	0	60	0	60
0.25 ng/mL	60	0	60	0	60	0	60
0.5 ng/mL	60	0	60	0	60	0	60
0.75 ng/mL	60	2	58	1	59	0	60
1 ng/mL	60	35	25	28	32	26	34
1.25 ng/mL	60	60	0	60	0	60	0
1.5 ng/mL	60	60	0	60	0	60	0
1.75 ng/mL	60	60	0	60	0	60	0
2 ng/mL	60	60	0	60	0	60	0

Effect of Urinary Specific Gravity

Total 12 urine samples of specific gravities (SG) ranging from 1.000-1.035 were collected. Values of SG levels were determined by a refractometer. Target drugs were spiked to these urine samples at +50% cut-off and -50% cut-off concentrations. The results demonstrate that varying ranges of urinary specific gravity do not affect the test results.

Effect of 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 Fentanyl to 0.5 ng/mL and 1.5 ng/mL. The spiked, pH-adjusted urine was tested with the Rapid Response™ Fentanyl Test Cassette in duplicate. The results demonstrated that varying ranges of pH do not interfere with the performance of the test.

Interference

Potential interfering substances found in human urine of physiological or pathological conditions were added to drug-free urine and target drug fentanyl urine with concentrations at 50% below and 50% above cut-off levels. There urine samples were tested using three batches of each device. Compounds that showed no interference at a concentration of 100 μg/mL or specified concentrations are summarized in the following tables.

Non-Interference Compounds

Acetaminophen	Ketamine
Acetone (1000mg/dL)	Ketoprofen
Acetophenetidin	Labetalol
Acetylsalicylic acid	Lidocaine
Albumin (100mg/dL)	Loperamide
Albuterol	Maprotiline
Aminopyrine	Meperidine(50μg/mL)
Amitriptyline	Meprobamate
Amobarbital	Methapyrilene
Amoxicillin	Methaqualone
Ampicillin	Methoxyphenamine
Apomorphine	Metronidazole (300μg/mL)
Ascorbic acid	N-Acetylprocainamide
Aspartame	NaCl (4000mg/dL)
Atropine	Nalidixic acid
Benzilic acid	Naloxone
Benzoic acid	Naltrexone
Benzoylecgonine	Naproxen
Bilirubin	Niacinamide
Boric Acid (1%)	Nicotine
Bupropion	Nifedipine
Caffeine	Norethindrone
Carbamazepine	Nortriptyline
Chloral hydrate	Noscapine
Creatinine	Perphenazine
Cyclobenzaprine	Phencyclidine
Deoxycorticosterone	Phenelzine
Desipramine	Phenobarbital
Dextromethorphan	Prednisone
Diclofenac	Propoxyphene (50μg/mL)
Diflunisal	Propranolol
Digoxin	Pseudoephedrine
Diphenhydramine	Quinine
DL-Tryptophan	Ranitidine
DL-Tyrosine	Riboflavin (10mg/dL)
Doxepin	Salicylic acid
Ecgonine methyl ester	Secobarbital
Ephedrine	Serotonin (5-Hydroxytyramine)
Erythromycin	Sulfamethazine
Ethanol (1%)	Sulindac
Fenoprofen	Tetrahydrocortisone 3-(β-Dglucuronide)
Fluphenazine	Tetrahydrocortisone 3-acetate
Furosemide	Tetrahydrozoline
Galactose (10mg/dL)	Thiamine
Gamma Globulin (500mg/dL)	Thioridazine
Gentisic acid	Triamterene
Glucose (3000mg/dL)	Trifluoperazine
Hemoglobin	Trimethoprim

Bibliography

1. International Narcotics Control Board. Report of the International Narcotics Control Board for 2009[R].New York: UN, 2010
2. Lane JC, Tennison MB, Lawless ST, et al. Movement disorder after withdrawal of fentanyl infusion. J Pediatr, 1991, 119(4):649-651
3. Dominguez KD, Lomako DM，Katz RW，et al. Opioid withdraw in critically ill neonates.Ann Pharmacotherm, 2003, 37(4):473-477
4. European Monitoring Centre for Drugs and Drug Addiction.Annual Report 2009[R].Lisbon:EMCDDA, 2010

Glossary of Symbols

Consult instructions for use
Test per Kit
Do Not Reuse
Store between 35.6°F to 86°F (2-30°C)
Use by
REF Catalogue #
LOT Lot Number
IVD For *in vitro* diagnostic use only
Manufacturer
Do not use if package is damaged and consult instructions for use
UDI Unique device identifier

BTNX Inc.
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Hours of Operation: Monday – Friday 9:00AM-5:00PM (EST)