FEN2

Fentanyl II Enzyme Immunoassay

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
09444360190	Fentanyl II Enzyme Immunoassay (240 tests)	System-ID 2114 001	**cobas c** 303, **cobas c** 503

Materials required (but not provided):

09330097190	Fentanyl Qualitative Calibrator Set FEN Cutoff Calibrator 5 ng/mL (1 x 5 mL)	Code 20710	
09330119190	DAT Opiates Multi Control I Set Negative Control 3.75 ng/mL (2 x 15 mL) Positive Control 6.25 ng/mL (2 x 15 mL)	Code 20159 Code 20160	
04908856160	Open/Close tool (5 pieces)		

English

For use in the USA only

Roche does not hold the product registration for Partner Channels. The legal manufacturer indicated on the kit is solely responsible for all of the design, legal, and regulatory aspects of the product.

System information

FEN5Q: ACN 21140: for qualitative assay, 5 ng/mL

Intended use

The Fentanyl II Enzyme Immunoassay is an in vitro diagnostic test intended for the qualitative determination of norfentanyl in human urine. The cutoff for the assay is 5 ng/mL when calibrated against norfentanyl. The assay is designed for prescription use on Roche **cobas c** analyzers.

The assay provides only a preliminary analytical result. A more specific alternative analytical chemistry method must be used in order to obtain a confirmed analytical result. Gas or Liquid Chromatography/Mass Spectrometry (GC-MS or LC-MS) are the preferred confirmatory methods.[1,2] Clinical consideration and professional judgment should be exercised with any drug of abuse test result, particularly when the preliminary test result is positive.

Summary

Fentanyl is an important opioid analgesic used widely in surgical operations and is a controlled substance.[3] Fentanyl is most commonly encountered in the form of patches applied to the skin, as “lollipops” which can be dissolved in the mouth through the mucous membrane, or can be administered intravenously. It is 50-100 times stronger than morphine[4,5] and cases of fentanyl abuse via intravenous injection, inhalation, oral, or nasal applications have been previously reported.[6] Fentanyl is used in the treatment of acute and chronic pain, usually in patients who no longer respond to high doses of less potent opioids such as morphine or oxycodone. Due to its potency and wide availability as a prescribed drug, fentanyl has been abused and misused by health professionals, pain management patients, and recreational abusers.[7]

Due to its short elimination half-life and approximately 90 % metabolism, fentanyl is difficult to detect in urine.[8] Fentanyl undergoes extensive hepatic biotransformation to metabolites coming from hydrolysis, N-dealkylation, or hydroxylation reactions.[9] In an intravenous dose of fentanyl, up to 85 % is excreted in urine over a 3- to 4-day period with 0.4-6 % eliminated as unchanged fentanyl and 26-55 % eliminated as the norfentanyl metabolite.[10]

Fentanyl analogs also have high potency analgesic activities. Numerous reports have been published with modified fentanyl-related compounds abused as designer drugs.[11,12,13] Other recently available fentanyl analogs associated with abuse and severe intoxication include butyryl fentanyl and 4-fluorobutyryl fentanyl.[14,15,16,17,18]

Test principle

The fentanyl assay is a homogeneous enzyme immunoassay ready-to-use liquid reagent. The assay is based on competition between drug in the sample and drug labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for a fixed amount of antibody in the reagent.[19] Enzyme activity decreases upon binding to the antibody, and the drug concentration in the sample is measured in terms of enzyme activity. In the absence of drug in the sample, fentanyl-labeled G6PDH conjugate is bound to antibody, and the enzyme activity is inhibited. On the other hand, when drug is present in the sample, antibody binds to the free drug; and the unbound fentanyl-labeled G6PDH then exhibits its maximal enzyme activity. Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that can be measured spectrophotometrically at a 340 nm primary wavelength.

Reagents - working solutions

R1 Contains a mouse monoclonal anti-fentanyl antibody, glucose-6-phosphate (G6P), nicotinamide adenine dinucleotide (NAD), stabilizers, and sodium azide (0.09 %) as a preservative.

R2 Contains glucose-6-phosphate dehydrogenase (G6PDH) labeled with fentanyl in buffer with sodium azide (0.09 %) as a preservative.

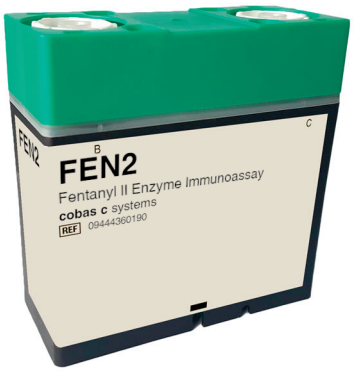
Precautions and warnings

- This test is for *in vitro* diagnostic use only. Harmful if swallowed.
- Reagents used in the assay contain sodium azide as a preservative, which may react with lead or copper plumbing to form potentially explosive metal azide. When disposing of such reagents or wastes always flush with a large volume of water to prevent azide build-up. See National Institute for Occupational Safety and Health Bulletin: Explosive Azide Hazards.[20]
- Do not use the reagents beyond their expiration dates.

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

Reagent handling

Ready for use



Filling the **cobas c** pack:

1. Turn the **cobas c** pack toward you.
2. Position B of the **cobas c** pack is on the left side and position C on the right side.
3. Unscrew the screw cap of the bottle in position B on the left side of the **cobas c** pack using the Open/Close tool.
4. Use one of the enclosed funnels to pour the content of the R1 bottle (24.0 mL) into the opened bottle of the **cobas c** pack (position B). Discard the funnel.
5. Close the bottle tightly using the Open/Close tool.
6. Unscrew the screw cap of the bottle in position C on the right side of the **cobas c** pack using the Open/Close tool.

FEN2

Fentanyl II Enzyme Immunoassay

7. Use one of the enclosed funnels to pour the content of the R2 bottle (10.1 mL) into the opened bottle of the **cobas c** pack (position C). Discard the funnel.
8. Close the bottle tightly using the Open/Close tool.

The FEN2 **cobas c** pack is now ready for use.

NOTE: Solutions must be at the reagent compartment storage temperature of the analyzer before performing assays.

Note
Always use a new **cobas c** pack when preparing fresh reagent. Never reuse accessories designed for single use, as this may result in reagent contamination and could affect test results. If the **cobas c** pack bottles are not filled correctly, this may result in faulty reagent pipetting and could cause erroneous results.

Storage and stability

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label
On-board in use and refrigerated on the analyzer:	145 days

Do not freeze.

Specimen collection and preparation
Use fresh urine specimens for the test. If a sample cannot be analyzed immediately, it may be refrigerated at 2-8 °C for up to 4 weeks[21] or at room temperature for up to 4 weeks.[22] For longer storage, keep samples frozen at -20 °C and then thaw before use. Studies have shown norfentanyl analytes in urine are stable at -20 °C for up to 6 months.[23]

Samples should be equilibrated to room temperature (18-25 °C) for testing. Samples with high turbidity should be centrifuged before analysis. Adulteration may cause erroneous results. If sample adulteration is suspected, obtain a new sample and forward both samples to the laboratory for testing.

See the limitations and interferences section for details about possible sample interferences.

Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

Handle all urine specimens as if they were potentially infectious.

Materials provided
See “Reagents – working solutions” section for reagents.

cobas c pack, funnels

Materials required (but not provided)
See “Order information” section

General laboratory equipment

Assay
For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator’s manual for analyzer-specific assay instructions.

Application for urine

Test definition - 5 ng/mL cutoff assay

	Qualitative		
Reporting time	10 min		
Wavelength (sub/main)	415/340 nm		
Reagent pipetting			
R1	65 µL		
R3	25 µL		
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (H_2O)*
Normal	10.9 µL	–	–
Decreased	10.9 µL	–	–
Increased	10.9 µL	–	–

Calibration

Calibrators	*Qualitative application* *5 ng/mL cutoff assay* S1: Fentanyl Calibrator 5 The drug concentration of the calibrator has been verified by GC-MS or LC-MS.
Calibration K Factor	For the qualitative application, a K factor of 1000 is predefined in the application settings.
Calibration mode	*Qualitative application* Linear
Calibration frequency	Full calibration • every 30 days • after reagent lot change • as required following quality control procedures

For the cutoff calibrator a value of “0” is encoded in the e-barcode in order to ensure flagging of positive samples with >Test and negative absorbance values for negative samples.

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: This method has been standardized against a primary reference method (GC-MS or LC-MS).

Quality control
Good laboratory practices recommend the use of at least two levels of control specimens (one positive and one negative control near the cutoff) to ensure proper assay performance. Controls should be run with each new calibration and after specific maintenance or troubleshooting procedures as detailed in the instrument manual. Each laboratory should establish its own control frequency. If any trends or sudden change in control value are observed, review all operating parameters, or contact Lin-Zhi International, Inc. (LZI) technical support for further assistance. Laboratories should comply with all federal, state, and local laws, as well as all guidelines and regulations.

For quality control, use control materials as listed in the "Order information" section.

In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory’s individual requirements. It is recommended to perform quality control always after lot calibration and subsequently at least every 30 days.

Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Results
NOTE: A preliminary positive test result does not necessarily mean that a person took illegal drugs and a negative test result does not necessarily mean that a person did not take illegal drugs. There are a number of factors that influence the reliability of drug tests.

Qualitative: The cutoff calibrator, which contains 5 ng/mL norfentanyl, is used as a reference for distinguishing preliminary positive from negative samples. Samples producing a positive or "0" absorbance value are considered preliminary positive. Preliminary positive samples are flagged with the >Test. Samples producing a negative absorbance value are considered negative. Negative samples are preceded by a minus sign. Results of this assay distinguish preliminary positive (≥ 5 ng/mL) from negative samples only. The amount of drug detected in a preliminary positive sample cannot be estimated.

As with any sensitive test for drugs of abuse on automated clinical chemistry analyzers, the possibility exists for analyte carry-over from a sample with an extremely high concentration to a normal (negative) sample which immediately follows it.

FEN2

Fentanyl II Enzyme Immunoassay

Use caution when reporting results as there are various factors that influence a urine test result, such as fluid intake and other biological factors.

Limitations

1. Boric Acid at 1 % w/v may cause false negative results.
2. Dextromethorphan may cause false positive results at concentrations greater than 40000 ng/mL.
3. A preliminary positive result from the assay indicates only the presence of norfentanyl.
4. The test is not intended for quantifying this single analyte in patient samples.
5. A preliminary positive result does not necessarily indicate drug abuse.
6. A negative result does not necessarily mean a person did not take illegal drugs.
7. There is a possibility that other substances and/or factors not listed above may interfere with the test and cause incorrect results (e.g., technical or procedural error, fluid intake, endogenous or exogenous interferents).
8. Preliminary positive results should be confirmed by other affirmative, analytical chemistry methods (e.g., chromatography), preferably GC-MS or LC-MS.
9. The test is designed for use with human urine only.
10. The test is not for therapeutic drug monitoring.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet. For further instructions, refer to the operator's manual.

Specific performance data

Representative performance data on a Beckman AU480 analyzer are given below. Data was validated on a Roche **cobas c** 501, **cobas c** 502, **cobas c** 303, and **cobas c** 503 analyzer and did not show any differences between the analyzers. Results obtained in individual laboratories may differ.

Precision

Qualitative analysis: The following concentrations were evaluated. Typical qualitative results (measured by ΔOD, mAU) are as follows:

5 ng/mL Cutoff		Within run		Total precision	
Sample [ng/mL]	**% of Cutoff**	**# Samples**	**EIA result**	**# Samples**	**EIA result**
0 ng/mL	0	22	22 Neg	88	88 Neg
1.25 ng/mL	25	22	22 Neg	88	88 Neg
2.5 ng/mL	50	22	22 Neg	88	88 Neg
3.75 ng/mL	75	22	22 Neg	88	88 Neg
5 ng/mL	100	22	13 Neg/ 9 Pos	88	59 Neg/ 29 Pos
6.25 ng/mL	125	22	22 Pos	88	88 Pos
7.5 ng/mL	150	22	22 Pos	88	88 Pos
8.75 ng/mL	175	22	22 Pos	88	88 Pos
10 ng/mL	200	22	22 Pos	88	88 Pos

Accuracy

A total of 100 unaltered clinical urine specimens were tested with the Fentanyl II Enzyme Immunoassay and confirmed with LC-MS. Specimens having a norfentanyl concentration greater than or equal to 5 ng/mL by LC-MS are defined as positive, and specimens with concentrations lower than 5 ng/mL by LC-MS are defined as negative in the table below. The correlation results are summarized as follows (near cutoff samples are defined as ± 50 % of the cutoff value):

Norfentanyl Results 5 ng/mL Cutoff	Negative (Below the cutoff by LC-MS analysis)	Positive (At or above the cutoff by LC-MS analysis)	% Agree-ment
Positive (at or above the cutoff by EIA)	9*	50	100
Negative (below the cutoff by EIA)	41	0	82

The following table summarizes the results for the discordant samples:

Sample #	NFEN LC-MS (ng/mL)	LC-MS Pos/Neg Result	AU480 EIA Qualitative Result (mAU)	LZI FEN II EIA Pos/Neg Result
37*	1.5	-	85.9	+
41*	2.7	-	111.3	+
43*	3.0	-	207.9	+
44*	3.0	-	107.7	+
45*	3.3	-	124.7	+
46*	3.5	-	169.6	+
47*	3.8	-	204.6	+
48*	3.9	-	113.6	+
49*	4.2	-	263.1	+

Qualitative Cutoff Rate = 83.0 mAU

* Discrepant below the cutoff concentration (0 ng/mL – 4.9 ng/mL)

These samples contained levels of fentanyl that contributed to the false positive result.

Analytical specificity

Various potentially interfering substances were tested for cross-reactivity with the assay. Test compounds were spiked into the drug-free urine calibrator matrix individually to various concentrations and evaluated against the cutoff calibrator. The following table summarizes the approximate quantity of each compound that is equivalent in assay reactivity to the 5 ng/mL cutoff or the maximal concentration of the compound tested that gave a response with cross-reactivity below the response of the cutoff calibrator. Compounds tested at high concentration with results below the cutoff value were listed as Not Detected (ND).

Cross-reactant	Concentration tested [ng/mL]	% cross-reactivity	Result
Fentanyl	3.8	131.58	Positive
Norfentanyl	5	100.00	Positive

Structurally related compounds:

Cross-reactant	Concentration tested [ng/mL]	% cross-reactivity
4-Fluoro-Isobutyryl Fentanyl	20.0	25.00
9-Hydroxy Risperidone	100000	ND
Acetyl Fentanyl	7.0	71.43
Acetyl Norfentanyl	100.0	5.00
Acryl Fentanyl	4.0	125.00
Alfentanil	100000	ND

FEN2

Fentanyl II Enzyme Immunoassay

Cross-reactant	Concentration tested [ng/mL]	% cross-reactivity
Butyryl Fentanyl	6.0	83.33
Butyryl Norfentanyl	40.0	12.50
Carfentanil Oxalate	100000	ND
Cis-*d,l* 3-Methyl Fentanyl	8.0	62.50
Cyclopropyl Fentanyl	3.2	156.25
Cyclopropyl Norfentanyl	25.0	20.00
Despropionyl Fentanyl (4-ANPP)	100000	ND
Furanyl Fentanyl	5.5	90.91
Furanyl Norfentanyl	180.0	2.78
(±) β-Hydroxy Thiofentanyl	5.0	100.00
Isobutyryl Fentanyl	15.0	33.33
Isobutyryl Norfentanyl	500.0	1.00
Labetalol Hydrochloride	100000	ND
Methoxyacetyl Fentanyl	3.5	142.86
MT-45	100000	ND
N-benzyl Furanyl Norfentanyl	11.0	45.45
N-benzyl Para-fluoro Norfentanyl	4.0	125.00
Norcarfentanil Oxalate	100000	ND
Ocfentanil	3.8	131.58
Para-fluoro Butyryl Fentanyl (p-FBF)	4.5	111.11
Para-fluoro Fentanyl	3.2	156.25
Remifentanil	100000	ND
Risperidone	100000	ND
Sufentanil	100000	ND
Thienyl Fentanyl	4.0	125.00
Thiofentanyl	3.2	156.25
Trans-*d,l* 3-Methyl Fentanyl	6.0	83.33
Trazodone	100000	ND
U-47700	100000	ND
Valeryl Fentanyl	70.0	7.14
ω-1-Hydroxy Fentanyl	300.0	1.67

Structurally unrelated compounds:

Cross-reactant	Spiked [ng/mL]	Spiked Norfentanyl Concentration		
		0 ng/mL	3.75 ng/mL Control	6.25 ng/mL Control
Acetaminophen	100000	ND	Neg	Pos
6-Acetylmorphine	100000	ND	Neg	Pos
Acetylsalicylic Acid	100000	ND	Neg	Pos
Amitriptyline	100000	ND	Neg	Pos
Amlodipine Besylate	100000	ND	Neg	Pos
Amoxicillin	100000	ND	Neg	Pos
d-Amphetamine	100000	ND	Neg	Pos
Atorvastatin	100000	ND	Neg	Pos
Benzoylecgonine	100000	ND	Neg	Pos
Buprenorphine	100000	ND	Neg	Pos
Bupropion	100000	ND	Neg	Pos
Caffeine	100000	ND	Neg	Pos
Carbamazepine	100000	ND	Neg	Pos
Cetirizine	100000	ND	Neg	Pos
Chlorpheniramine	100000	ND	Neg	Pos
Chlorpromazine	100000	ND	Neg	Pos
Clomipramine	100000	ND	Neg	Pos
Codeine	100000	ND	Neg	Pos
Desipramine	100000	ND	Neg	Pos
Dextromethorphan	40000	0.01 %	Pos	Pos
Diphenhydramine	100000	ND	Neg	Pos
Duloxetine	100000	ND	Pos	Pos
Fluoxetine	100000	ND	Neg	Pos
Fluphenazine	100000	ND	Neg	Pos
Gabapentin	100000	ND	Neg	Pos
Hydrocodone	100000	ND	Neg	Pos
Hydromorphone	100000	ND	Neg	Pos
Ibuprofen	100000	ND	Neg	Pos
Imipramine	100000	ND	Neg	Pos
Lisinopril	100000	ND	Neg	Pos
Losartan	100000	ND	Neg	Pos
Loratadine	100000	ND	Neg	Pos
MDA (3,4-methylenedioxyamphetamine)	100000	ND	Neg	Pos
MDEA	100000	ND	Neg	Pos
MDMA (3,4-methylenedioxymethamphetamine)	100000	ND	Neg	Pos
Meperidine	100000	ND	Neg	Pos
Metformin	100000	ND	Neg	Pos
Metoprolol	100000	ND	Neg	Pos
Methadone	100000	ND	Neg	Pos
d-Methamphetamine	100000	ND	Neg	Pos
Morphine	100000	ND	Neg	Pos
Nalmefene	100000	ND	Neg	Pos
Nicotine	100000	ND	Neg	Pos
Nortriptyline	100000	ND	Neg	Pos
Omeprazole	100000	ND	Neg	Pos
Oxazepam	100000	ND	Neg	Pos
Oxycodone	100000	ND	Neg	Pos
Oxymorphone	100000	ND	Neg	Pos
Phenobarbital	100000	ND	Neg	Pos
(1S,2S)-(+)-Pseudoephedrine	100000	ND	Neg	Pos

FEN2

Fentanyl II Enzyme Immunoassay

Cross-reactant	Spiked [ng/mL]	Spiked Norfentanyl Concentration		
		0 ng/mL	3.75 ng/mL Control	6.25 ng/mL Control
Quetiapine	100000	ND	Neg	Pos
Ranitidine	100000	ND	Neg	Pos
Salbutamol (Albuterol)	100000	ND	Neg	Pos
Sertraline	100000	ND	Neg	Pos
THC-COOH (11-Nor-Delta-9-THC-9-carboxylic acid)	100000	ND	Neg	Pos
l-Thyroxine	100000	ND	Neg	Pos
Tramadol	100000	ND	Neg	Pos
Zolpidem	100000	ND	Neg	Pos
Phencyclidine	100000	ND	Neg	Pos

It is possible that other substances and/or factors not listed above may interfere with the test and cause false results.

Interference: Endogenous and Preservatives Substances

The following potentially interfering compounds were spiked into a pool of processed drug free urine to the desired concentrations and then norfentanyl was spiked to a final concentration of 0 ng/mL or the negative control concentration of 3.75 ng/mL, or the positive control concentration of 6.25 ng/mL. The spiked solution was evaluated against the cutoff calibrator. Interference was observed for boric acid. No other major interference with these compounds at physiologically relevant concentrations was observed as all spiked samples gave correct corresponding preliminary positive/ negative results against the cutoff value of 5 ng/mL. The table listed below shows the maximal concentration of the compound tested without interference.

Endogenous & Preservative Substance	Spiked (mg/dL)	Spiked Norfentanyl Concentration (ng/mL)		
		0 ng/mL	3.75 ng/mL Control	6.25 ng/mL Control
Acetone	1000	Neg	Neg	Pos
Ascorbic Acid	500	Neg	Neg	Pos
Bilirubin	2	Neg	Neg	Pos
Biotin	0.5	Neg	Neg	Pos
Boric Acid	1000	Neg	Neg	Neg
Calcium Chloride ($CaCl_2$)	300	Neg	Neg	Pos
Citric Acid (pH 3)	200	Neg	Neg	Pos
Creatinine	500	Neg	Neg	Pos
Ethanol	1000	Neg	Neg	Pos
Galactose	10	Neg	Neg	Pos
γ-Globulin	500	Neg	Neg	Pos
Glucose	3000	Neg	Neg	Pos
Hemoglobin	300	Neg	Neg	Pos
Human Serum Albumin	500	Neg	Neg	Pos
Human Urine (pooled)	N/A	Neg	Neg	Pos
β-hydroxybutyric Acid	100	Neg	Neg	Pos
Oxalic Acid	100	Neg	Neg	Pos
Potassium Chloride	1000	Neg	Neg	Pos
Riboflavin	7.5	Neg	Neg	Pos
Sodium Azide	1000	Neg	Neg	Pos
Sodium Chloride	1000	Neg	Neg	Pos
Sodium Fluoride	1000	Neg	Neg	Pos
Sodium Phosphate	300	Neg	Neg	Pos
Urea	6000	Neg	Neg	Pos
Uric Acid	10	Neg	Neg	Pos
LZI Urine-Based Calibrator Buffer	N/A	Neg	Neg	Pos

The following endogenous compound which showed interference at ± 25 % of cutoff concentrations was then spiked into negative urine and at ± 50 % of cutoff concentrations (2.5 ng/mL and 7.5 ng/mL) for the assay. Interference was still observed with Boric Acid at 1 % w/v. Results are summarized in the following table:

Endogenous & Preservative Substance	Spiked (mg/dL)	Spiked Norfentanyl Concentration (ng/mL)		
		0 ng/mL	2.5 ng/mL	7.5 ng/mL
Boric Acid	1000	Neg	Neg	Neg

Interference: pH

Negative urine and urine spiked with analyte to the 2 levels of controls (3.75 ng/mL and 6.25 ng/mL) were adjusted to the following pH levels and tested by the assay. The pH adjusted solutions were evaluated against the cutoff calibrator. No major interference with these pH levels was observed as all pH adjusted levels gave correct corresponding preliminary positive/negative results against the cutoff value of 5 ng/mL. Results are summarized in the following table:

pH	Spiked Norfentanyl Concentration (ng/mL)		
	0 ng/mL	3.75 ng/mL Control	6.25 ng/mL Control
pH 3	Neg	Neg	Pos
pH 4	Neg	Neg	Pos
pH 5	Neg	Neg	Pos
pH 6	Neg	Neg	Pos
pH 7	Neg	Neg	Pos
pH 8	Neg	Neg	Pos
pH 9	Neg	Neg	Pos
pH 10	Neg	Neg	Pos
pH 11	Neg	Neg	Pos

Specific gravity: Samples ranging in specific gravity from 1.000 to 1.027 were split into 3 portions each and either left un-spiked or further spiked to a final norfentanyl concentration of either 3.75 ng/mL or 6.25 ng/mL (the negative and positive control concentrations, respectively). These samples were then evaluated in qualitative mode. No interference was observed. Results are summarized in the following table:

Specific Gravity Value	Spiked Norfentanyl Concentration (ng/mL)		
	0 ng/mL	3.75 ng/mL Control	6.25 ng/mL Control
1.000	Neg	Neg	Pos
1.003	Neg	Neg	Pos
1.005	Neg	Neg	Pos

FEN2

Fentanyl II Enzyme Immunoassay

Specific Gravity Value	Spiked Norfentanyl Concentration (ng/mL)		
	0 ng/mL	3.75 ng/mL Control	6.25 ng/mL Control
1.008	Neg	Neg	Pos
1.010	Neg	Neg	Pos
1.012	Neg	Neg	Pos
1.015	Neg	Neg	Pos
1.018	Neg	Neg	Pos
1.020	Neg	Neg	Pos
1.022	Neg	Neg	Pos
1.025	Neg	Neg	Pos
1.027	Neg	Neg	Pos

References

1 Urine Testing for Drug of Abuse, National Institute on Drug Abuse (NIDA) Research Monograph 73 (1986).

2 Mandatory Guidelines for Federal Workplace Drug Testing Programs. Fed Regist 2017 Jan 23;82:7920-7970.

3 Ikeda R, Pelton C. Diversion programs for impaired physicians. West J Med. 1990 May;152(5):617-621.

4 Poklis A., Fentanyl: a review for clinical and analytical toxicologists. J Toxicol Clin Toxicol 1995;33(5):439-447.

5 Jaffe JH, Martin WR. Opioid analgesics and antagonists. In: Pharmacological Basis of Therapeutics, 7th ed. Gilman AG, Goodman LS, Rall TW, Murad F, eds. New York: MacMillian, 517 (1985).

6 Ellenhorn MD, Schonwald S, Ordog G, Wasserberger J, Fentanyl, in: Ellenhorns Medical Toxicology, Williams & Wilkins, Baltimore, Philadelphia, London, Paris, Bangkok, Buenos Aires, Hong Kong, Munich, Sydney, Tokyo, Wroclaw, 416-420 (1997).

7 Atluri S, Sudarshan G, Manchikanti L. Assessment of the trends in medical use and misuse of opioid analgesics from 2004 to 2011. Pain Physician, Mar-Apr 2014;17(2): E119-E128.

8 McClain DA, Hug CC Jr. Intravenous fentanyl kinetics. Clin Pharmacol Ther 1980 Jul;28(1):106-114.

9 Hug CC, Murphy MR. Anesthesiology, 55 (1981) 369. Tateishi T, Wood AJJ, Guengerich FP and Wood M, Biochem Pharmacol, 50 (1995) 1921.

10 Baselt RC. Disposition of Toxic Drugs and Chemicals in Man. 10th ed. Biomedical Publications: Seal Beach, California 2014; 846-849.

11 Baum RM. New variety of street drugs poses growing problem. Chem Eng News 1985, Sep;9:7-16.

12 Suzuki S, Inoue, T. New abused drug "China White". Forensic Toxicol News 1987; 5:5-12.

13 Heagy JA. Identification of 4-propionoxy-4-phenyl-N-methylpiperidine. Microgram 1982;15:181-186.

14 Poklis J, Poklis A, Wolf C, Hathaway C, Arbefeville E, Chrostowski L, Devers K, Hair L, Mainland M, Merves M, and Pearson J. Journal of Analytical Toxicology 2016;40:703-708.

15 Backberg M, Beck O, Jonsson KH, Helander A.. Opioid intoxications involving butyryl fentanyl, 4-fluorobutyrfentanyl, and fentanyl from the Swedish STRIDA project. Clinical toxicology 2015;53:609-617.

16 EMCDDA. European Drug Report 2014: Trends and developments 2014. Available at: http://www.emcdda.europa.eu/attachements.cfm/att_228272_EN_TDAT14001ENN.pdf.

17 EMCDDA. (2015) New psychoactive substances in Europe. An update from the EU Early Warning System (March 2015). Available at: http://www.emcdda.europa.eu/attachements.cfm/att_235958_EN_TD0415135ENN.pdf.

18 Helander A, Backberg M, Hulten P, Al-Saffer Y, Beck O. Detection of new psychoactive substance use among emergency room patients: results from Swedish STRIDA project. Forensic Science International 2014;243:23-29.

19 Rubenstein KE, Schneider RS, Ullman EF. "Homogeneous" enzyme immunoassay. A new immunochemical technique. Biochem Biophys Res Commun 1972 May 26;47(4):846-851.

20 Sodium Azide. National Institute for Occupational Safety (NIOSH). Pocket Guide to Chemical Hazards. Third Printing, September 2007. Available online at: https://www.cdc.gov/niosh/npg/default.html

21 Hammargren WR and Henderson GL. Analyzing normetabolites of the fentanyls by gas chromatography/electron capture detection. J Anal Toxicol Jul-Aug 1988;12(4):183-191.

22 Thevis M, Geyer H, Bahr D, and Schänzer W. Identification of fentanyl, alfentanil, sufentanil, remifentanil and their major metabolites in human urine by liquid chromatography/tandem mass spectrometry for doping control purposes. Eur J Mass Spectrom 2005;11:419-427.

23 Gonzales E, Ng G, Pesce A, et al. Stability of pain-related medications, metabolites, and illicit substances in urine. Clin Chim Acta 2013;416:80-85.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
GTIN	Global Trade Item Number

FOR US CUSTOMERS ONLY: LIMITED WARRANTY

As a distributor, Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

Lin-Zhi International, Inc.
2945 Oakmead Village Court
Santa Clara, CA 95051, USA

EC REP
CEpartner4U
Esdoornlaan 13
3951 DB Maarn
The Netherlands
www.cepartner4u.eu

Distribution by:
Roche Diagnostics GmbH
Sandhofer Strasse 116
D-68305 Mannheim
www.roche.com

Distribution in USA by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support 1-800-428-2336