Status DS FYL

One-Step Fentanyl Test

For Forensic Use Only

Simple One-Step Immunoassay for the Qualitative Detection of Fentanyl and Analogues in Urine

LifeSign

Intended Use

Status DS FYL is a simple, one-step immunochromatographic assay for the rapid, qualitative detection of fentanyl at a cutoff concentration of 20 ng/mL in human urine.

The* Status DS FYL *test provides only a preliminary analytical result. A more specific alternative chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography, mass spectrometry (GC/MS) is the preferred confirmatory method. Other chemical confirmatory methods are available. Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

Summary and Explanation

Fentanyl is a potent synthetic opioid analgesic with a rapid onset and short duration that is similar to morphine but is 50 to 100 times more potent than morphine and some fentanyl analogues may be as much as 10,000 times more potent than morphine. Fentanyl is a typically used to treat patient with severe pain or to manage pain after surgery. In the United States, fentanyl is classified as Schedule II controlled substance. Fentanyl is excreted with the urine within 3 days and mainly metabolized into norfentanyl.

Principle

The **Status DS FYL** test uses solid-phase immunoassay technology for the qualitative detection of fentanyl in human urine. The test is based on the principle of the highly specific immunochemical reactions between antigens and antibodies which are used for the analysis of specific substances in biological fluids. The test relies on the competition for binding to the antibodies between drug conjugates and drugs which may be present in the urine sample. In the test procedure, a sample of urine is placed in the Sample well of the device and is allowed to migrate upward. If drug is present in the urine sample, it competes with the drug conjugate, which is immobilized on the membrane, for the limited antibodies present in the form of dye-antibody conjugate. When a sufficient amount of drug or drug metabolite above the cutoff level is present, the drug will saturate the antibodies, thus inhibiting the binding of dye-antibody conjugate to the drug conjugate on the membrane. This prevents the formation of a line on the membrane. Therefore, a drug-positive urine sample will not generate a line at the Test position in the Result Window, indicating a positive result from positive drug competition, while a negative urine sample will generate a line at the Test position in the Result Window, indicating a negative result from an absence of competition with free drug.

In addition to the Test line that may appear in the Result Window, a Control line is present at the Control position (C) to confirm the viability of the test. This Control line (validation line) should always appear if the test is conducted properly. The Control line is immobilized with monoclonal anti-mouse antibody; therefore, it will capture the monoclonal antibody-dye conjugates that pass the region, producing a colored line at the Control position (validation line). This works as a procedural control, confirming that proper sample volume was used and the reagent system worked. If insufficient sample volume is used, there may not be a Control line, indicating the test is invalid.

Materials Provided

The **Status DS FYL** test kit contains all the reagents necessary to perform the tests.

- **Status DS FYL** device. The test device contains a membrane strip coated with fentanyl conjugated with a protein and a pad containing monoclonal anti-fentanyl antibody-dye conjugate in a protein matrix.
- Disposable specimen dispensers.
- Instructions for use.

Precautions

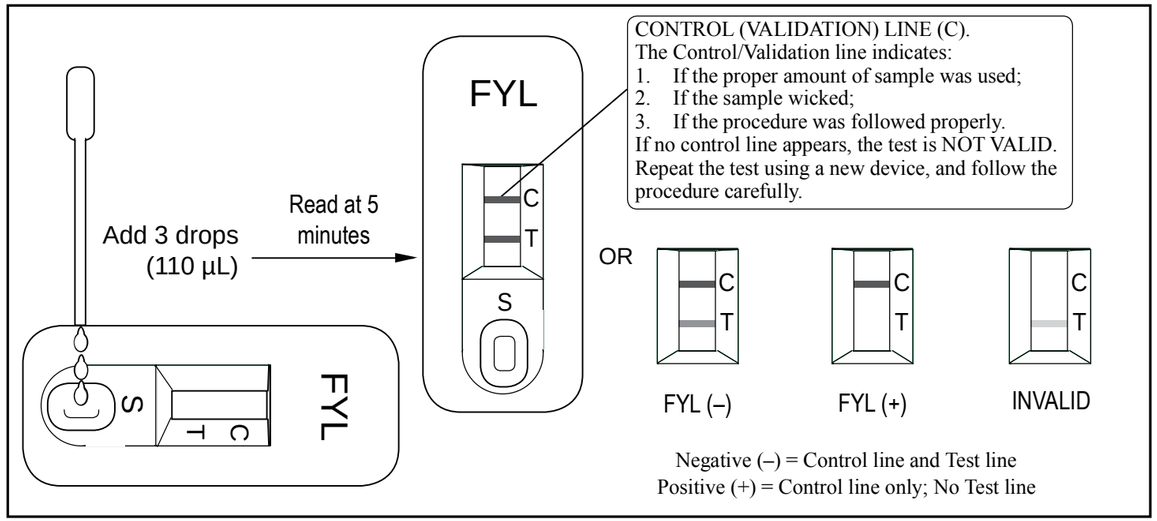
- For forensic use only.
- Avoid cross contamination of urine samples by using a new urine specimen container and dropper for each urine sample.
- The test kit does not contain any HIV or hepatitis infective components.
- Urine specimens are potentially infectious. Proper handling and disposal methods should be followed according to good laboratory practices.
- The **Status** device should remain in its original sealed pouch until ready for use. Do not use the test if the pouch is damaged or the seal is broken.
- Do not use the test kit after the expiration date.

Storage and Stability

The **Status DS FYL** test kit should be stored at 2–30°C (35–86°F) in the original sealed pouch. The expiration dating was established under these storage conditions.

Specimen Collection and Preparation

Approximately 110 µL of urine sample is required for each test. Fresh urine specimens do not require any special handling or pretreatment. Specimens should be collected in a clean glass or plastic container. If testing will not be performed immediately, specimens should be refrigerated (2–8°C) or frozen. Frozen specimens must be completely thawed and thoroughly mixed before using.



Specimens containing a large amount of particulate matter may give inconsistent test results. Such specimens should be clarified by centrifuging or allowing to settle before testing.

Test Procedure

The test procedure consists of adding the urine sample to the Sample well of the device and watching for the appearance of colored lines in the result window.

Test Protocol

1. For each test, open one **Status DS FYL** pouch and label the **Status** device with the patient ID.
2. Holding the dropper vertically, dispense 3 drops of the urine sample into the Sample well (**S**).
3. Read the result at 5 minutes.

Interpretation of Results

Negative: The appearance of a reddish-purple Control line (**C**) and a line next to T indicates a negative test result; i.e., no drug above the cutoff level has been detected. The color intensities of the Control line and the Test line may not be equal. *Any faint line at the T position in the Result window, visible at 5 minutes, should be interpreted as negative. A negative test result does not indicate the absence of drug in the sample; it only indicates the sample does not contain drug above the cutoff level in qualitative terms.*

Positive: The appearance of only a reddish-purple Control line and no distinct line next to T indicates the test result is positive for FYL (i.e., the specimen contains FYL at a concentration above the cutoff level). *A positive test result does not provide any indication of the level of intoxication or urinary concentration of the drug in the sample; it only indicates the sample contains drug above the cutoff level in qualitative terms.*

Invalid: A distinct Control line (**C**) should always appear. The test is invalid if no Control line forms at the **C** position. Such tests should be repeated with a new **Status DS FYL** test device.

Limitations

- The test is designed for use with unadulterated urine only.
- There is a possibility that factors such as technical or procedural errors, as well as certain substances in the urine sample may interfere with the test and cause erroneous results.
- Adulterants, such as bleach and/or alum, in urine specimens may produce erroneous results. If adulteration is suspected, the test should be repeated with a new sample.
- This test detects only the presence of fentanyl in human urine. A positive test result does not provide any indication of the level of intoxication or urinary concentration.
- The test result read after 10 minutes may not be consistent with the original reading obtained within the 10 minute reading period. The test must be read within 10 minutes of sample application.

User Quality Control

Internal Control: Each **Status** test device has built-in controls. The Control line is an internal positive procedural control. A distinct reddish-purple Control line should always appear at the C position, if the test procedure is performed properly, an adequate sample volume is used, the sample and reagent are wicking on the membrane, and the test reagents at the control line and the conjugate-color indicator are reactive. In addition, if the test has been performed correctly and the device is working properly, the background in the result window will become clear and provide a distinct result. This may be considered an internal negative procedural control.

The positive and negative procedural controls contained in each **Status** test device satisfy the requirements of testing a positive control and a negative control on a daily basis. If the Control line does not appear at the Control position, the test is invalid and a new test should be performed. If the problem persists, contact LifeSign for technical assistance.

External Control: External controls may also be used to assure that the reagents are working properly and that the assay procedure is followed correctly. It is recommended that a control be tested at regular intervals as good laboratory testing process. For information on how to obtain controls, contact LifeSign's Technical Services.

Expected Values

Status DS FYL is a qualitative assay. The amount of fentanyl present in the urine cannot be estimated by the assay. The assay results distinguish positive from negative samples. Positive results indicate the samples contain fentanyl and/or fentanyl metabolite above the cutoff concentration.

Performance Characteristics

Precision and Accuracy

The precision of the **Status DS FYL** assay was determined by carrying out the test with urine samples containing fentanyl at the concentrations of 0 ng/ml, 10 ng/ml, 15 ng/ml, 20 ng/ml, 25 ng/ml, 30 ng/ml, and 40 ng/ml. The results demonstrates > 99% accuracy at 50% above and 50% below the cutoff concentration. The data are summarized below.

Table 1. Precision

Fentanyl concentration (ng/ml)	Percent of Cutoff	# of Tested	# of Positive	# of Negative	% Agreement
0	0%	60	0	60	100
10	-50%	60	0	60	100
15	-25%	60	6	54	90
20	Cutoff	60	30	30	N/A
25	+25%	60	57	3	95
30	+50%	60	60	0	100
40	+100%	60	60	0	100

Effect of Urinary pH

The effects of the urine pH on the performance of the **Status DS FYL** test devices were evaluated using 10 ng/ml (50% cutoff) and 30 ng/ml (150% cutoff) of fentanyl urine samples with pH values of 4.5, 6.5, and 9.0. Each sample was tested in ten (10) replicates. The results demonstrate that the urine in the pH range of 4.5 – 9.0 doesn't affect the performance of the **Status DS FYL**.

Effect of Urinary Specific Gravity

Urine samples with a specific gravity of 1.002 and 1.04 were tested at 50% cutoff level and 150% cutoff level of fentanyl to examine the effect of specific gravity of the urine sample on the results. At each specific gravity and each concentration, the test was repeated ten times. There was no discrepancy in the results with different specific gravity.

Specificity

Compounds that are detected by the **Status DS FYL** test are listed below. The specificity of the **Status DS FYL** test was determined by adding the drugs and drug metabolites listed to drug-negative urine specimens and testing with the **Status DS FYL** test kit. The results are expressed in terms of the concentration required to produce a positive result (Table 2).

Table 2. Specificity

Compound	Concentration (ng/mL)
Alfentanyl	> 100,000
Butyryl fentanyl	50
Fentanyl	20
Furanyl fentanyl	100
Para-Fluorobutyl fentanyl (PFBF)	50
Heroin	> 100,000
Hydrocodone	> 100,000
Hydromorphone	> 100,000
(±) cis 3-methyl fentanyl	500
Morphine	> 100,000
Norfentanyl	20
Oxycodone	> 100,000
Oxymorphone	> 100,000
Sufentanyl	> 50,000

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