| **Urine Fentanyl Screen** |
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| **Purpose** | This procedure provides instructions for FENTANYL SCREEN, URINE ON ABBOTT INSTRUMENTATION. The Urine Fentanyl Screen (also known as “FENTS” in SunQuest, or “FENTQ” on the Alinity analyzer) method is an *in vitro* diagnostic test for the qualitative analysis of fentanyl in human urine on the Abbott Alinity c automated chemistry analyzers. This test is an enzyme immunoassay with a cutoff of 1.0 ng/mL. This test provides only a preliminary screening test result; a more specific method such as GC-MS or LC-MS/MS is suggested for confirmatory testing.  |
| **Policy Statements** | This procedure applies to all personnel responsible for operating the Abbott Alinity c at Children’s Minnesota Laboratory. |
| **Principle** | The SEFRIA™ technology is based on artificial fragments of the E. coli enzyme β-galactosidase. A mutant enzyme, termed Enzyme Acceptor (EA), is created by deletion of a short sequence in the amino-terminal region of the sequence. EA is inactive, but can combine with peptides, termed Enzyme Donors (ED’s), containing the deleted sequence, to form active β-galactosidase. This process is termed complementation, and the active enzyme formed as a result can be measured by hydrolysis of a chromogenic substrate such as chlorophenolred β-D-galactopyranoside (CPRG). The ED peptides can be modified by attachment of a derivative of fentanyl, which does not interfere with the formation of active β-galactosidase. However, antibodies to fentanyl bind to the ED-fentanyl conjugate, and block complementation. The assay is based on the competition of fentanyl in a urine sample with the ED-fentanyl conjugate for the fixed amount of antibody binding sites. In the absence of the free drug in the sample, the antibody binds the ED-fentanyl conjugate, resulting in inhibition of enzyme formation. As the fentanyl concentration in the sample increases, ED-fentanyl becomes available for complementation, creating a dose response relationship between fentanyl concentration in the urine and enzyme formation. The β-galactosidase activity is determined spectrophotometrically at 570 nm by the conversion of CPRG (orange) to chlorophenol red (red) and galactose.Methodology: Enzyme Immunoassay |
| **Clinical Significance** | Fentanyl is a synthetic narcotic analgesic of high potency and short duration of action. Fentanyl is available as an injectable 50 μg/mL citrate salt solution typically used as an adjunct to surgical anesthesia. Fentanyl is also available as a transdermal patch containing 2.5-10 mg fentanyl providing doses of 25-100 μg/hr for 72 hours for management of chronic pain1. While fentanyl has all the properties of morphine, it is structurally different and therefore cannot be detected by morphine or oxycodonespecific opioid immunoassay screening tests2. Because of the potency of the drug, fentanyl urine concentrations are often in the low nanogram per milliliter range. |
| **Analyzer** | **Minneapolis: or Abbott Alinity ci (Sunquest method code: MACC)****St. Paul: Abbott Alinity c (Sunquest method code: SALIC)****Backup: Abbott Alinity c (Sunquest method code: MALIC)** |
| **Sunquest Test Codes** | **FENTS:** Fentanyl Screen, Urine |
| **Specimen** | Freshly voided urine specimens: collected in clean, polypropylene, unbreakable, leak-proof containers. If testing is not performed immediately on fresh urine, store refrigerated for up to 24 hours. Freeze if delayed more than 24 hours. Do not use polystyrene containers due to propensity for absorbing drugs.Specimens should be collected without preservatives or additives.**Minimum Volume:** 68 uL**Stability:** Store at 2-8 °C for up to 48 hours. Frozen, up to 7 days.**Rejection Criteria:**Unlabeled or mislabeled specimens, specimens with preservative or additives, specimens collected in polystyrene containers |
| **Reagents** | **Reagent Handling** Upon receipt, place reagent cartridges in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate. * Do not use reagents beyond the expiration date.
* Do not pool reagents within a kit or between kits.
* Do not use components from one lot with components from another lot.

Reagents may be stored on or off the system. If removed from the system, store reagents with new replacement caps in an upright position at 2 – 8°C. For reagents stored off the system, it is recommended that they be stored in their original trays or boxes to ensure they remain upright.

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| ***Product Description*** | ***Product Code*** | ***Stability*** |
| **Immunalysis SEFRIA™****Fentanyl Urine Reagent**CHC# TBDEnzyme Acceptor/Antibody Reagent (EA/R1), 25 mL - This contains EA protein and rabbit antibodies to fentanyl, in PIPES buffer with <0.1% w/v Sodium Azide as a preservative.Enzyme Donor/Substrate Reagent (ED/R2) 15 mL- This contains ED peptide labeled with fentanyl and CPRG substrate in malic acid buffer with <0.1% w/v Sodium Azide as a preservative. | 04Z44-20 | **Store at:** 2 – 8°C**Unopened:** Manufacturer’s printed expiration date**On-board:** 28 days**Opened, off the analyzer (with clean caps):** Manufacturer’s printed expiration date. (Reagents may be stored on or off the system. The system tracks time onboard.) |
| **Immunalysis****Fentanyl Urine Calibrator 1**CHC# TBDImmunalysis Fentanyl Urine Calibrator 1 containing 1 ng/mL of fentanyl, 1 x 5mL | 04Z44-01 | **Store at:** 2 – 8°C**Unopened:** Manufacturer’s printed expiration date**Opened:** 60 dayswhen opened and stored off the system.  |
| **Immunalysis****Fentanyl Negative Urine Calibrator**CHC# TBDImmunalysis Negative Urine Calibrator, 1 x 5mL | 04Z44-04 | **Store at:** 2 – 8°C**Unopened:** Manufacturer’s printed expiration date**Opened:** 60 dayswhen opened and stored off the system.  |
| **Immunalysis Fentanyl Urine Control Set**CHC# TBDImmunalysis Fentanyl Urine Control Set containing LOW (0.5 ng/mL) and HIGH (1.5 ng/mL) fentanyl controls, 2 x 5mL | 04Z44-10 | **Store at:** 2 – 8°C**Unopened:** Manufacturer’s printed expiration date**Opened:** 60 dayswhen opened and stored off the system. . |

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| **Risk and Safety** |

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| **CAUTION** For in vitro diagnostic use. This product requires the handling of human specimens. Observe precautions against microbiological hazards throughout the assay procedure and follow standard procedures and guidance for the disposal of specimens. Dispose of contents and container, in accordance with local, regional, national and/or international regulation**.** Appropriate Personal Protective Equipment (PPE) must be worn according to Children’s Minnesota Laboratory policies. Current SDSs are kept on the [Children’s StarNet](https://msdsmanagement.msdsonline.com/a07dc954-23d8-42a9-b591-ef5763cdfd33/ebinder/?nas=True) page.**Alinity c:**R1 (EA) Reagents: This contains EA protein and rabbit antibodies to fentanyl, in PIPES buffer with <0.1% w/v Sodium Azide as a preservative.R2 (ED) Reagents: This contains ED peptide labeled with fentanyl and CPRG substrate in malic acid buffer with <0.1% w/v Sodium Azide as a preservative.Calibrators should be disposed of in Regulated Medical Waste (red trash). |

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| **Calibration** | **Alinity c:**

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| Assay Range: | Negative/Positive |
| Reference Material: | Immunalysis Fentanyl Urine Calibrator 1 containing 1 ng/mL of fentanyl, 1 x 5mL Immunalysis Negative Urine Calibrator, 1 x 5mL |
| Suggested Calibration Levels: |  0.0 ng/mL, 1.0 ng/mL |
| Calibration Scheme: | 2 levels, Linear |
| Calibration Frequency: | 7 Days |
| AMR | Fentanyl Urine Screen assay is an FDA-cleared/approved in vitro diagnostic assay that reports the qualitative result based on a predefined cut-off value. Verification of AMR or the cut-off value is not required by CAP or CLIA. Stated cutoff value is 1.0 ng/mL. This is a qualitative assay and values do not represent a quantitative analysis of the analyte.  |

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| **Quality Control** | **Alinity c:** * Immunalysis Fentanyl Urine Control Set containing LOW (0.5 ng/mL, Negative) and HIGH (1.5 ng/mL, Positive) fentanyl controls, 2 x 5mL

**Frequency:** Two levels each day of use**Stability:** Store unopened at 2-8 C until expiration date. Once opened, store at 2-8C for 60 days or the printed expiration date, whichever comes first. **Preparation**: This product should be treated the same as patient specimens and run in accordance with the instructions accompanying the instrument, kit, or reagent being used. **Acceptable ranges:** * High level QC must report as Positive
* Low level QC must report as Negative
* In the event of a QC failure, refer to the [Unity Real Time QC Review, General User](https://starnet.childrenshc.org/References/labsop/chem/quality/ch-2.17-unity-real-time-qc-review-general-user.pdf) and navigate to the QC Troubleshooting section.
* Do not load or release patients until QC is acceptable in Unity Real Time.
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| **Interferences** | **Alinity c:** **Hemolysis, Icterus & Lipemia (HIL) Index Values:**

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At HIL levels at or above the specified cutoff value, append the appropriate comment AFTER visually confirming presence of interfering substance: -HP for “Hemolysis present, may affect results.” -BIN for “Bilirubin Interference”-LINT for “Lipid Interference”The potential interference of unrelated drugs, endogenous substances, boric acid, pH and specific gravity in the SEFRIA™ Fentanyl Urine was assessed by spiking known amounts of each potentially interfering substances into the LOW (0.5 ng/mL) and HIGH (1.5 ng/mL) controls. **Unrelated Drugs**No interference was observed by the addition of concentrations up to the indicated amounts of the following potentially interfering substances:* 40,000 ng/mL: Cocaine, Trifluoromethylphenyl-piperazine
* 50,000 ng/mL: Lorazepam Glucuronide, MDEA, MDMA, S-(+) Amphetamine, Triazolam
* 75,000 ng/mL: 11-hydroxy-delta-9-THC, Cannabinol, (+)-MDA, 4-Bromo-2, 5, Dimethoxyphenethylamine, Desalkyflurazepam, Dextromethorphan, Diazepam, Flurazepam, Lorazepam, Lormetazepam, Maprotiline, Medezapam, Meprobamate, Methyphenidate, Midazolam, N-Desmethyltapentadol, Oxazepam glucuronide, Phentermine, Phenylpropanolamine, R,R(-)-Pseudoephedrine, Ranitidine, Ritalinic Acid, Sertraline, Theophylline, Thioridazine, Zolpidem Tartrate
* 100,000 ng/mL: 11-nor-9 carboxy THC, 1S, 2R(+)-Ephedrine, 7-Aminoclonazepam, 7-Aminoflunitrazepam, 7-Aminonitrazepam, Alprazolam, Amobarbital, Barbital, Benzoylecgonine, Bromazepam, Butabarbital, Caffeine, Cannabidiol, Carbamazepine, Carisoprodol, Chlordiazepoxide, cis-Tramadol, Clobazam, Clonazepam, Cotinine, Delta-9-THC, Demoxepam, Ecgonine, Ecgonine methyl ester, Ethyl beta-D-glucuronide, Flunitrazepam, Heroin, Hexobarbital, Ibuprofen, Ketamine, Lamotrignine, Lidocaine, LSD, Mephobarbital, Methaquolone, Naproxen, Nitrazepam, Normorphine, Norpseudoephedrine, Oxazepam, Pentobarbital, Phenobarbital, Phenylephedrine, Salicylic Acid, Secobarbital, Temazepam, Phenytoin, PMA, Propranolol
* 500,000 ng/mL: Acetaminophen

**Endogenous Substances, pH and Specific Gravity** No interference was observed by the addition of the following compounds, up to the concentrations listed below:

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| Analyte | Concentration |
| Acetone | 1.0 g/dL |
| Ascorbic Acid | 0.56 g/dL |
| Bilrubin | 2.0 mg/dL |
| Boric Acid | 1.0% w/v |
| Creatinine | 0.5 g/dL |
| Ethanol | 1.0 g/dL |
| Galactose | 10 mg/dL |
| γ-Globulin | 0.5 g/dL |
| Glucose | 2.0 g/dL |
| Hemoglobin | 0.5 g/dL |
| Human Serum Albumin | 0.5 g/dL |
| Oxalic Acid | 0.1 g/dL |
| Riboflavin | 7.5 mg/dL |
| Sodium Azide | 1.0% w/v |
| Sodium Chloride | 6.0 g/dL |
| Sodium Flouoride | 1.0% w/v |
| Urea | 2.0 g/dL |
| pH | 3.0-11.0 |
| Specific Gravity | 1.000-1.030 |

Interferences from medication or endogenous substances may affect results.For accurate results, highly turbid specimens should be centrifuged before analysis. |
| **Reference Value** |

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Reference value: Negative (Results < 1.0 ng/mL) |
| **Critical Value** | Critical Value: Positive (Results > 1.0 ng/mL). Any positive result is considered a critical value and must be called according to the Critical Value Policy. |
| **Limitations** | The instrument reporting system contains flags and comments to provide the user with information regarding instrument processing errors, instrument status information and potential errors in results. Refer to the [Alinity](https://starnet.childrenshc.org/References/labsop/chem/operator/alinity-ci-series-operations-manual.pdf) Operator’s Guides for the meaning of report flags and comments, and instructions for addressing them. Do not report results until a report containing flags and/or comments is resolved.The assay is designed for use with human urine only. Only drugs listed in the “Unrelated Drugs” section have been evaluated. Interference with other substances have not been evaluated and can cause false results.Interpretation of results must take into account that urine concentrations can vary extensively with fluid intake and other biological variables. Immunoassays that produce a single result in the presence of a drug and its metabolites cannot fully quantitate the concentration of individual components. Clinical consideration and professional judgment should be applied to any test result, particularly with a preliminary positive result. |
| **Dilutions** | Do not dilute |
| **Result Reporting** | **Alinity c:** * **CAUTION:** Instrument numerical values are not indicative of fentanyl concentration, never share or report measured numerical results.
* Results < 1.0 ng/mL will be reported as Negative (NEG)
	+ A negative result does not indicate drug-free sample. Rather, the drug in question may be at concentration less than the detectable cutoff for a positive result. For cutoff values, see AMR section. If a positive drug is suspected despite negative finding, the urine should be sent to MedTox for analysis by mass spectrometry.
* Results > 1.0 ng/mL will be reported as Positive (POS)
	+ A positive screen is a presumptive result only. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectroscopy (GC/MS) is the preferred confirmatory method.
	+ Confirmatory testing, when needed, requires the provider order the specific drug confirmatory test from Medtox. Medtox requires a minimum sample volume of 3 mL

SunQuest Function **OEM**: 1. Results are sent automatically to SunQuest as POS or NEG. Every positive result will have the following comment automatically appended by SunQuest: “Unconfirmed result. Confirmatory testing is recommended. Unconfirmed screening results must not be used for non-medical purposes such as employment or legal testing.”2. Accept (A), Modify (M), or Reject (R) according to Resulting Reporting in Chemistry procedureSunQuest Function **MEM**: Result using SunQuest Worksheet ABUS. Type “POS” or “NEG” at each prompt. NOTE: Do not type “POSITIVE” or “P” as the correct comment will not append. |
| **Specimen Storage** | Promptly stopper tested specimen and store upright in a specimen rack. Every 8 hours remove specimens to refrigerator/freezer storage. Samples are retained 7 days in specimen storage freezer. |
| **References** | 1. R.C. Baselt. Disposition of Toxic Drugs and Chemicals is Man. 8th Edition, p 616-619
2. M.C. Milone. “Laboratory testing for prescription opioids.” J. Med. Toxicology. (2012), vol 8, p408-416
3. Jacobs & DeMott Laboratory Test Handbook, Lexi-Comp, Inc., Hudson, OH, 5th Edition, 2001
4. Immunalysis SEFRIA™ Fentanyl Urine Package Insert, Immunalysis Corporation, Pomona, CA 31767, May 2023.
5. Immunalysis SEFRIA™ Fentanyl Urine Control Set Package Insert, Immunalysis Corporation, Pomona, CA 31767, September 2021.
6. Immunalysis SEFRIA™ Negative Calibrator Package Insert, Immunalysis Corporation, Pomona, CA 31767, October 2021.
7. Immunalysis SEFRIA™ Fentanyl Urine Calibrator 1 Package Insert, Immunalysis Corporation, Pomona, CA 31767, August 2023.
8. Alinity ci‑series Operations Manual, Abbott Laboratories Diagnostics Division, Abbott Park, IL 60064, April 2018.
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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | Matt Johnson | December 19, 2023 | New Procedure for Abbott analyzers |