|  |  |  |  |
| --- | --- | --- | --- |
| EFFECTIVE DATE: | 01/03/25 | APPROVAL: | 11/07/2024 |
| APPROVED BY: | Patrice Y. Ohouo, Ph.D.  Main Laboratory Director | **PROCEDURE NO.:** | **CHM.01022** |

|  |
| --- |
| Procedure |
| This procedure covers the Fentanyl Urine Enzyme Immunoassay Procedure for Drugs of Abuse Screening on the Beckman Coulter AU5800 analyzers. |
| 1. *PURPOSE*    1. This procedure provides instruction for performing the qualitative analysis of Fentanyl in human urine on Beckman Coulter AU analyzers. 2. *BACKGROUND*    1. Fentanyl is an extremely fast acting synthetic opioid related to the phenylpiperidines. It is available in injectable as well as transdermal formulations. The analgesic effects of fentanyl is similar to those of morphine and other opioids: it interacts predominantly with the opioid mu-receptor. These mu-binding sites are discretely distributed in the human brain, spinal cord, and other tissue.    2. Fentanyl is approximately 80% to 85% protein bound. In plasma, the protein binding capacity of fentanyl decreases with increasing ionization of the drug. Alterations in pH may affect its distribution between plasma and the central nervous system (CNS). The average volume of distribution for fentanyl is 6 L/kg (range 3-8).    3. In humans, the drug appears to be metabolized primarily by oxidative N-dealkylation to norfentanyl and other inactive metabolites that do not contribute materially to the observed activity of the drug. Within 72 hours of intravenous (IV) administration, approximately 75% of the dose is excreted in urine, mostly as metabolites with <10% representing unchanged drug.    4. In clinical settings, fentanyl exerts its principal pharmacologic effects on the CNS. In addition to analgesia, alterations in mood (euphoria, dysphoria) and drowsiness commonly occur. Because the biological effects of fentanyl are similar to those of heroin and other opioids, fentanyl has become a popular drug of abuse. 3. *PRINCIPLE*    1. This assay uses a Fentanyl specific antibody. The assay is based on the competition of Fentanyl labeled enzyme glucose-6-phosphate dehydrogenase (G6PDH) and the free drug in the urine sample for the fixed amount of antibody binding sites. In the absence of the free drug in the sample, the antibody binds the drug enzyme conjugate and enzyme activity is inhibited. This creates a dose response relationship between drug concentration in the urine and enzyme activity. The enzyme G6PDH activity is determined at 340 nm spectrophotometrically by the conversion of NAD to NADH. 4. *INTENDED USE*    1. The Immunalysis Fentanyl Urine Enzyme Immunoassay is intended for the qualitative determination of Fentanyl in human urine with a cutoff of 1 ng/mL. CleanSlate utilizes the 1 ng/mL cutoff. This assay is calibrated against Fentanyl.    2. The Immunalysis Fentanyl Urine Enzyme Immunoassay Kit provides only a preliminary analytical test result. A more specific alternate chemical method must be used 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 applied to any drug of abuse test result, particularly when preliminary positive results are used. 5. *SCOPE*    1. Fentanyl is a synthetic narcotic analgesic of high potency and short duration of action. Although 200 times more potent than Morphine, Fentanyl has a high safety margin. The drug is available as a citrate salt in an injectable solution containing 50 g/mL. It is also available as a transdermal patch containing 2.5 – 10 mg Fentanyl and provides a dose of 25 – 100 g/hr for 72 hours for management of chronic pain. While Fentanyl has all the properties of Morphine, it is structurally different and therefore cannot be detected by screening tests for Morphine and related opiates. Because of the potency of the drug, concentrations encountered in biological fluids are in the sub nanogram range.    2. The Immunalysis Fentanyl Urine Enzyme Immunoassay Kit is a sensitive in-vitro test used for the detection of Fentanyl in human urine samples. 6. *Definitions*    1. N/A 7. *Responsibilities*    1. Only trained personnel are authorized to perform this procedure. Qualified personnel are responsible for the proper execution of this procedure. Ultimately, it is the responsibility of the Laboratory Director to ensure the proficiency of laboratory personnel performing this test.    2. Training is documented in the training file of each qualified staff member.    3. All patient information is handled in a manner that is compliant with HIPAA guidelines. Refer to <http://www.hhs.gov/ocr.hipaa/>. And also to Clean Slate’s HIPAA Policy, <https://cleanslatecenters.training.reliaslearning.com> or equivalent.    4. Under the direction of the Laboratory Director, the direct review of all quality control, equipment maintenance and reporting of patient results is performed by the technical supervisor and/or the lab manager. 8. *SAFETY*    1. Standard Precautions       1. Care should be taken and personal protective equipment is required when handling material of human origin. All biological specimens should be considered potentially infectious.       2. For up-to-date recommendations on handling biological specimens refer to the CDC website: <http://cdc.gov/ncidod/dhqp/pdf/guidelines/Isolation2007.pdf> or CLSI document M29-A3, Protection of Laboratory Workers from Occupationally Acquired Infections. Clinical and Laboratory Standards Institute; Approved Guidelines and or Refer to Clean Slate’s safety policy, [https://cleanslatecenters.training.reliaslearning.com](https://cleanslatecenters.training.reliaslearning.com/) or equivalent    2. Computer and Web Portal       1. Passwords must be assigned only to authorized personnel.       2. To ensure HIPAA compliance, it is recommended that the computer, printer and printouts be located away from the visibility and access of unauthorized individuals. 9. *SPECIMEN REQUIREMENTS*    1. Urine samples should be collected in industry standard urine collection containers.    2. Ensure that the samples are free of gross debris. Highly turbid specimens should be centrifuged before analysis.    3. Adulteration of urine specimens can cause erroneous results.    4. Samples should be well mixed before assay.    5. Urine specimens stored at ambient temperature up to 30°C and up to 14 days and at 2-8°C for up to 6 months may be used; or, follow guiding laboratory stability studies.    6. Repeated freezing and thawing should be avoided.    7. HANDLE ALL URINE SPECIMENS AS IF THEY ARE POTENTIALLY INFECTIOUS.    8. Criteria for Unacceptable Specimens       1. Unlabeled specimens- there must be two ID link between the test order and the specimen container. Unlabeled specimens cannot be accepted.       2. All specimens are examined for correct identification when accessioned and processed or rejected if it does not have two matching patient identifiers.       3. Insufficient quantity or urine specimen containers that are “empty” or have improper storage cannot be accepted. 10. *MATERIALS & EQUIPMENT*     1. AU2700/AU5800 Beckman Coulter Analyzers.     2. Immunalysis Fentanyl Urine Enzyme Immunoassay (Cat# 618UR-0500).     3. Immunalysis Fentanyl Control Set containing LOW and HIGH Controls (Cat # 3009-5).     4. Immunalysis Fentanyl Calibrator 0 ng/mL (Cat # NEG-600-5), and Calibrator 1 ng/mL of Fentanyl (Cat # 10019-5).     5. AU routine cleaning solutions.     6. AU2700/AU5800 Sample Racks and Containers. 11. *REAGENTS*      1. Preparation        1. The Immunalysis Fentanyl Urine EIA Kit reagents are provided ready for use and may be used directly from the refrigerator. No preparation is required.     2. Storage and Stability        1. Opened reagents are stable for 28 days when stored in the refrigerator compartment of the analyzer.        2. Unopened reagents are stable until the expiration date stated on the label if stored in the refrigerator at 2-8°C. Reagent bottles should remain closed when not in use with screw caps tightly closed.        3. Do NOT use reagents past their expiration date. 12. *CALIBRATIONS & CONTROLS*     1. Calibration        1. The frequency of calibration is once a week.        2. The calibrators are ready to use and do NOT require reconstitution. Mix the contents of the vial gently by inverting the vial 3-4 times.        3. For qualitative analysis, use the 0 ng/mL and 1ng/mL calibrators.        4. Recalibration of this test is required when any of these conditions exist:           1. An observed drift in QC values.           2. A change of bottle/Lot number.           3. Major preventative maintenance was performed on the analyzer.           4. A critical part was replaced.        5. Storage and Stability of Calibrators:           1. Store Calibrators at 2-8°C. DO NOT FREEZE. Store control vials tightly capped when not in use.           2. Stability of Controls is the expiration date printed on each vial. DO NOT use any control after its expiration date.     2. Controls        1. During operation of the Beckman Coulter AU analyzers, at least two levels of quality control material will be tested at a minimum of once a day (LOW & HIGH QC).        2. In addition, controls should be performed:           1. After calibration.           2. With each new lot of reagent.           3. After specific maintenance or troubleshooting steps described in the appropriate Beckman Coulter AU analyzer User Guide.        3. New Quality control material is poured fresh each day. The controls are ready to use and do NOT require reconstitution. Mix the contents of the vial gently by inverting the vial 3-4 times.        4. Storage and Stability of Controls:           1. Store Controls at 2-8°C. DO NOT FREEZE. Store control vials tightly capped when not in use.           2. Stability of Controls is the expiration date printed on each vial. DO NOT use any control after its expiration date. 13. *PROCEDURE(S)*     1. Specimen Receipt: The test(s) have been previously ordered at the point of collection through the EMR and populated into LabDaq. Specimens are received into the main lab already labeled.        1. Specimens are scanned into LabDaq and received.        2. Specimens are placed into sample racks.     2. Specimen Processing:        1. Sample rack is placed on the rack feeder of the AU2700/AU5800 by the operator.        2. The operator presses Start.        3. The rack is moved to the barcode reader where sample programming is determined.        4. The rack is moved to the sample aspiration position.        5. The reagent probe, working with the reagent syringe, delivers R1 reagent into a cuvette.        6. An R1 mix bar mixes the reagent in the cuvette.        7. The photometer starts taking readings,        8. The sample probe, working with the sample syringe, aspirates and dispenses sample into the cuvette in the cuvette wheel.        9. A sample mix bar mixes the sample and the reagent. The photometer continues to take readings.        10. If required, the reagent probe, working with the reagent syringe, delivers the R2 reagent into the cuvette.        11. An R2 mix bar mixes the reaction mixture.        12. The photometer continues to take reaction readings.        13. The cuvette is washed, rinsed and dried by the wash nozzle probes using water/wash solution.        14. When the sample is no longer needed the rack is moved to the rack collection area.        15. All tests performed in a cuvette on the AU2700/AU5800 will have a total of 28 read points (represented by P0 through P27). The readings will be used in calculating a result that is specific to that particular assay. 14. *REFERENCE INTERVAL OF PATIENT RESULTS*     1. Linearity        1. The Fentanyl Urine Enzyme Immunoassay is for qualitative analysis only.        2. The assay is calibrated at the cutoff (1ng/mL), using the 0 ng/mL and 1ng/mL calibrators.        3. Samples will be reported as Positive/Negative.     2. Critical Values        1. N/A 15. *CALCULATIONS*     1. N/A 16. *INTREPTATION OF RESULTS*     1. Interpreting Results        1. Positive Fentanyl is any test resulting above or equal to the 1 ng/mL cut-off level. Negative Fentanyl is test resulting below 1 ng/mL cut-off level.        2. The qualitative analysis, use the 1 ng/mL calibrator as a cutoff level to distinguish “positive” and “negative” specimens.        3. Flagged test results: The system generates flags when the system encounters a condition that can affect the result. This condition can range from minor warnings to severe errors that require attention immediately. Analysts will review each flag, investigate to identify the root cause, and perform the corrective action. Results with an unresolved or unexpected flag will not be reported. When in doubt, always consider repeating the sample analysis, and diluting or condensing the sample if necessary. Please refer to chapter 7 of the AU5800 chemistry analyzer instruction for use (AU5800 Chemistry Analyzer IFU A98352AC.pdf ), which contains a list of all flags in priority order, suggestions of their cause, and corrective action to take. The priority determines what flags you see if a result generates multiple flags. A maximum of four flags can display. 17. *REPORTING*     1. Report Transmission        1. Patient test results uploaded into LABDAQ are reviewed by lab technicians and released for transmission into EMR chart via interface; results within the normal are transmitted to EMR via Auto-verification. 18. *LIMITATIONS*     1. The assay is designed for use in human urine ONLY.     2. Boric Acid is NOT recommended as a preservative for urine.     3. A urine sample with 6 g/dL of Sodium Chloride spiked with 4 ng/mL of Fentanyl will screen negative.     4. Other substances and factors not listed may interfere with the test and cause false results.     5. 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. 19. *TROUBLESHOOTING*     1. Notify Laboratory Manager.     2. See the Beckman Coulter AU/2700/AU5800 Operators’ Manual or go online to the following link: <https://www.beckmancoulter.com/en/products/chemistry/au5800#/training>        * AU Calculated Parameter Configuration        * AU Routine Cleaning Solutions        * AU2700/AU5800 Competency Checklist        * AU2700/AU5800 Sample Racks and Containers        * AU2700/AU5800 Every Other and Weekly Efficiency Job Aid        * AU2700/AU5800 Every Other Week and Weekly Maintenance Job Aids        * AU2700/AU5800 In-Lab Training Manual        * AU2700/AU5800 Job Aid Booklet        * AU2700/AU5800 Monthly Maintenance        * AU2700/AU5800 Test Configuration        * AU2700/AU5800 Quick Start Guide        * AU2700/AU5800 Sample Processing Overview        * AU2700AU5800 Software Overview        * AU Systemic Approach to Troubleshooting        * AU2700/AU5800 Daily Startup        * Tips for Working with your Service Representative     3. For AU5800 SN # 2018124306: Call Beckman Technical Support (800)-223-0130, (System ID 57095589).     4. For AU5800 SN # 2012090372: Call Horiba Technical Support (800)-813-4691. 20. *Performance Characteristics*     1. Refer to the immunalysis Fentanyl Urine Enzyme Immunoassay Insert for Fentanyl Performance Characteristics and Validations Studies conducted by CleanSlate.   Table. 20.1: Qualitative Cross-Reactivity with Related Drugs |

|  |  |
| --- | --- |
| REFERENCES: | * Refer to Beckman Coulter Operating Procedure, CHEM.SOP-001 * AU5800 chemistry analyzer instruction for use * Clean Slate’s HIPAA Policy * Clean Slate’s, Safety Policy * Immunalysis Fentanyl Urine Enzyme Immunoassay Insert. * CAP Laboratory General Checklist. * R.C. Baselt and R. H. Cravey. Disposition of Toxic Drugs and Chemicals in Man. 8th edition, 450-453. * L.S. Goodman and A. Gilman. The Pharmaceutical Basis of Therapeutics. 4th edition, 258. |
| REVISION HISTORY: | N/A |

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Patrice Y. Ohouo, M.S., Ph.D., NRCC-TC

Main Laboratory Director