Immunalysis Fentanyl Assay on Beckman AU and DxC Systems

Purpose

This procedure provides instructions to perform Immunalysis SEFRIA Fentanyl Urine testing on Beckman AU and DxC systems. It includes instructions for processing, storing, and testing specimens.

Principle

The Immunalysis SEFRIA Fentanyl test on the Beckman AU and DxC systems is a modified FDA-cleared, high complexity, qualitative assay to be used for analysis of fentanyl in human urine. This test is an enzyme immunoassay with a cutoff of 2.0 ng/mL.

All manufacturer's instructions are followed except the following modifications:

- **For AU Systems:**
  - Cutoff changed from 1.0 ng/mL to 2.0 ng/mL fentanyl
- **For DxC Systems:**
  - Test performed on DxC System
  - Cutoff changed from 1.0 ng/mL to 2.0 ng/mL fentanyl

Scope

This procedure is intended for Clinical Laboratory Scientists and Medical Laboratory Technicians.

Safety Precautions

All staff members performing these procedures must adhere to regional and local workplace safety policies. These will include but may not be limited to:

- Equipment safety, proper body mechanics, sharps exposure
- Proper use of gloves/personal protective equipment while performing these procedures
- Exposure to body fluids
- Proper handling of regular and biohazardous waste
- Handling of regular and infectious waste.
- Proper cleaning of work area
- Proper handwashing
- Proper storage and disposal of chemical hazardous waste

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Policy	The list below states the policies followed by the organization. • Do not use expired materials. • Tests must be performed by testing personnel who have been trained and found competent to perform the procedure. • The laboratory participates in the following CAP proficiency testing survey: UDS • The laboratory does not interchange or mix reagents from different lots
Specimen Sources	The following specimen source is acceptable for testing: • Urine
Specimen Collection and Processing	Specimen collection and processing instructions include: • Follow your laboratory's procedure for collecting and preparing urine • Use standard urine collection containers without preservatives • Centrifuge urine to remove gross particulate matter • Samples should be well mixed before performing the assay.
Specimen Storage and Stability	Patient specimens can be stored up to 6 months refrigerated (2-8°C)
Specimen Rejection	The followings specimens will be rejected: • Specimens improperly labeled • Specimens collected in the wrong container type • Specimens not meeting storage and stability requirements • Specimens with insufficient volume

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Equipment

- Beckman DxC or AU analyzer

Reagents / Materials

The following contains the list of reagents/materials required.

Description	Manufacturer	CAT #	OneLink	Storage
SEFRIA Fentanyl Urine Reagent	Immunalysis	618UR-0025	10873995	• Unopened: Until printed expiration at 2-8°C • Opened: 28 days at 2-8°C (when stored on-board the analyzer)
Negative Calibrator	Immunalysis	NEG-600-5	10873968	• Unopened: Until printed expiration at 2-8°C • Opened: 60 days at 2-8°C
Fentanyl Urine Calibrator 1 (1 ng/mL)	Immunalysis	10019-5	10873966	
Fentanyl Urine Calibrator 2 (2 ng/mL)	Immunalysis	10020-5	10812996	
Fentanyl Urine Calibrator 3 (4 ng/mL)	Immunalysis	10021-5	10873967	
For AU only: Beckman AU Reagent Bottle 30 mL	Beckman Coulter	63094	10339816	*See SEFRIA Fentanyl Urine Reagent*
For DxC only: Beckman DxC UDR Cartridge	Beckman Coulter	442835 (12 pack) 443729 (2 pack)	*Ordered through Beckman e-store*	*See SEFRIA Fentanyl Urine Reagent*

Note: All opened reagents/calibrators must be labeled with new expiration date.

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Reagent Preparation

Follow the steps below to prepare a reagent cartridge to load on the analyzer:

If using a...	Then ...
Beckman AU	

Step	Action
1	Prepare the R1 reagent. • Pour the contents of the **EA** reagent into an empty 30-mL reagent container. • Label with reagent name, lot number, expiration date and the date prepared
2	Prepare the R2 reagent. • Pour the contents of the **ED** reagent into an empty 30-mL reagent container. • Label with reagent name, lot number, expiration date and the date prepared
3	From the Home Screen, select **Reagent Management.**
4	Click on the **Details** tab and use the drop-down menu to change the specimen **Type** to **URINE**.
5	On the **Details** screen, scroll down to find the dedicated reagent position for Urine Fentanyl reagent.
6	Click on the assigned reagent position for R1 and then select **Edit.**
7	If this is a new reagent lot, enter the last 4 digits of the reagent kit lot number, and assign any 4-digit bottle number (e.g. 0001).
8	If the reagent lot is the same but a new bottle is loaded, assign a new 4-digit bottle number (e.g. 0002) so the instrument knows that it is a new reagent and the onboard stability resets back to 28 days.
9	Load R1 reagent.
10	Repeat steps 6 to 9 for the **R2** reagent.

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Reagent Preparation, continued

If using a...	Then ...
Beckman DxC	

Step	Action
1	Remove all screw caps from the new UDR cartridge.
2	Carefully pipette or transfer sufficient volume of EA to Compartment A and ED to Compartment B of UDR cartridge.
3	Remove any liquid adhering to the chimneys of the cartridge and any bubbles on the surface of the reagent to prevent level sensing issues.
4	Select **Rgts/Cal** from the menu bar. 
5	Page down to locate the position on the CC carousel where reagents will be placed.
6	Select the position to load the reagent cartridge.
7	Select **Load F1.**
8	Place cartridge in the load position in front of the barcode reader beam.
9	Enter the following information: a) Reagent Name b) Lot number (without letter) c) Serial Number: **1** d) Expiration Date Select the tab key on the keyboard to save the entries.
10	Verify the reagent information is displayed on the DXC monitor.
11	Move the cartridge onto the reagent carousel.
12	Close the reagent carousel door.

Notes:

- Avoid cross-contamination of EA and ED reagents. Do not switch the screw caps between the EA and ED reagents.
- The ED Reagent should be yellow-orange in color. A dark red or purple-red color indicates that the reagent has been contaminated and must be discarded.

Quality Control Frequency

Fentanyl Calibrator 1 (1 ng/mL) and Calibrator 3 (4 ng/mL) are required to be run as Quality Controls (QC):

- Daily, prior to testing samples
- To verify instrument operation
- After performing a calibration (calibration verification)

Note: Calibrators 1 and 3 are used as QC because separate control products are not available for a fentanyl cut-off of 2 ng/mL. These calibrator levels are not used to calibrate the method.

Quality Control Procedure

Follow the steps below to perform quality control:

Step	Action
1	Follow instrument quality control procedure to program the quality control tests.
2	Run Fentanyl Calibrator 1 and 3 as quality controls. Ensure contents of the calibrator bottles are mixed before each use by gently inverting the bottles 3-4 times. Note: The calibrators are ready for use and may be used directly from the refrigerator.
3	Document and evaluate results on the appropriate log. Do not perform patient testing until QC results are acceptable.

Quality Control Troubleshooting

Perform the steps below as needed to troubleshoot out-of-range quality control results.

Step	Action
1	• Review previous QC results for shifts/trends • Verify acceptable control range is correct
2	Repeat test using the same QC bottle
3	Repeat test using a fresh QC bottle
4	Repeat test using a fresh reagent cartridge
5	Recalibrate the test
6	If quality control results are still unacceptable after performing all these steps, see Troubleshooting section
7	Document all corrective actions taken on appropriate log

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Patient Test Procedure

Follow the steps below to perform a patient test.

Step	Action
1	Don appropriate PPE. Verify patient identifiers.
2	Verify daily quality control was performed and results were acceptable for patient testing.
3	Verify the correct order is placed for the sample.
4	Centrifuge urine to remove gross particulate matter
5	Analyze sample following analyzer patient testing procedure

Results Reporting

Interpret and report results using the following table:

If result is...	And…	Then report result as...
< 2.0 or "OIR LOW" (on the DxC)	Any patient	Negative
≥ 2.0	Patient is < 14 years old OR *Patient is a new mother	Preliminary Positive. Pending Confirmation **Note:** OPIOID CONFIRMATION [254043] will automatically be reflexed after result verification. Send sample to RRL for confirmatory testing.
	Patient is ≥ 14 years old AND *Patient is not a new mother	POSITIVE. Confirmation upon request only.

*Patients from L&D will be assumed to be originating from a new mother

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Procedural Notes

- The Immunalysis SEFRIA™ Fentanyl Urine Enzyme Immunoassay provides only a preliminary analytical test result. A more specific alternate chemical method must be used in order 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.

Reference Range

Negative

Troubleshooting

If unexpected/questionable results are obtained or if an analyzer issue is suspected:

- Contact Immunalysis Technical Support 1-877-441-7440 or Beckman Coulter Technical Support 1-800-854-3633
- Notify a Lead/Manager
- Document all issues and corrective actions
- Do not perform patient testing until issue is resolved

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Calibration

Calibration is required:

- At least weekly
- If results for the QC solutions are outside acceptable limits and the system cannot be corrected to bring control values into the acceptable range
- If QC results show an unusual trend or shift
- If the instrument has been serviced, after major preventive maintenance, or change of a critical instrument component

To perform a calibration/recalibration:

Step	Action
1	Follow instrument calibration procedure to program the fentanyl calibration.
2	Calibrate the test using the Negative and 2 ng/mL Fentanyl calibrators. Ensure contents of the calibrator bottles are mixed before each use by gently inverting the bottles 3-4 times. Note: The calibrators are ready for use and may be used directly from the refrigerator.
3	Upon successful calibration, follow Quality Control Procedure to verify calibration.
4	Document and evaluate results on the appropriate log. Do not perform patient testing until calibration is verified.

Cut-Off Verification Frequency

The analytic performance around the cut-off value was established initially and is reverified:

- At least every 6 months
- After replacement of major instrument components
- After major service to the instrument
- When QC materials reflect an unusual trend or shift or are outside of the laboratory's acceptable limits, and other means of assessing and correcting unacceptable control values fail to identify and correct the problem.

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Cut-Off Verification Procedure

Follow the steps below to verify the 2 ng/mL fentanyl cut-off value:

Step	Action
1	Use matrix-appropriate materials near the cut-off value (one positive and one negative).
2	Run each verification material in triplicate following the patient testing procedure.
3	The analytic performance around the cut-off value is verified if all runs are concordant with expected results.
4	If results are unacceptable, repeat verification procedure with fresh verification material. If repeat is still unacceptable, prepare a new reagent cartridge and/or recalibrate the assay and review patient results for any potential impact.
5	See Troubleshooting section for additional steps. Do not perform patient testing until cut-off values are verified.

Limitations

The following list describes known limitations of the Immunalysis SEFRIA Fentanyl test:

- The assay is designed and validated for use with human urine only.
- 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.
- Adulteration of urine specimens can cause erroneous results.

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Interfering Substances	• No known interfering substances • Refer to product insert for list of potential interferents and concentrations tested. Interference with other substances not listed have not been evaluated and can cause false results.
Non-Controlled Documents	The following non-controlled documents support this policy: • College of American Pathologists, All Common and Chemistry Testing Checklist • Immunalysis SEFRIA Fentanyl Urine Enzyme Immunoassay Package Insert • Immunalysis Fentanyl Urine Calibrators and Negative Calibrator Package Inserts
Author(s)	Chemistry Working Group