TRAINING UPDATE

Lab Location:	WOMC	**Date Distributed:**	4/11/23
Department:	Core Lab - Chemistry	**Due Date:**	4/25/23

DESCRIPTION OF PROCEDURE

Name of NEW procedures:

Title: WOMC.C 2008 Urine ARK Fentanyl II by Dimension Vista® System

Description of change(s):

- Fentanyl testing will be implemented at SGMC and WOMC on April 25th.
- Read the attached Fentanyl SOP for your site and take the MTS training quiz.
- Ensure that you receive hands on training, **complete the training documentation, and return the document to your supervisor** before performing test.

This SOP will be implemented on April 25, 2023

Document your compliance with this training update by taking the quiz in the MTS system.

Technical SOP

Title	**ARK Fentanyl II Assay by Dimension Vista Analyzer**
Prepared by	Ashkan Chini Date: 04/03/2023
Owner	Robert SanLuis Date:

Laboratory Approval	**Local Effective Date:**	
Print Name and Title	**Signature**	**Date**
Refer to the electronic signature page for approval and approval dates.		

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1. TEST INFORMATION

Assay	Method/Instrument	Test Code
Fentanyl II	Dimension Vista Analyzer	UFENT

Synonyms/Abbreviations
Fentanyl II

Department
Chemistry

2. ANALYTICAL PRINCIPLE

The ARK Fentanyl II Assay is an immunoassay intended for the qualitative detection of fentanyl in human urine at a cutoff concentration of 1.0 ng/mL. The ARK Fentanyl II Assay is a homogeneous enzyme immunoassay technique used for the analysis of a specific compound in human urine. The assay is based on competition between drug in the specimen and drug labeled with recombinant glucose-6-phosphate dehydrogenase for antibody binding sites. As the latter binds antibody, enzyme activity decreases. In the presence of drug from the specimen, enzyme activity increases and is directly related to the drug concentration. Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NADH in the presence of glucose-6-phosphate (G6P), resulting in an absorbance change that is measured spectrophotometrically. Endogenous G6PDH does not interfere because the coenzyme NAD functions only with the bacterial enzyme used in the assay.

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Specimen Collection and/or Timing	Freshly voided urine specimens should be used for testing.
Special Collection Procedures	No additives or preservatives are needed. Adulteration of the urine specimen may cause erroneous results. If adulteration is suspected, obtain a fresh specimen. Urine specimens should be handled and treated as if they are potentially infected. Preferred method is the Urine Collection Kit with specimen transferred to Urine Chemistry Collection Tube (yellow top).
Other	If Urine Collection Kit is not used, submit to Laboratory within 2 hours of collection.

3.2 Specimen Type & Handling

Criteria	
Type -Preferred **-Other Acceptable**	Urine None
Collection Container	Urine Collection Kit or sterile container
Volume - Optimum **- Minimum**	15 mL 2 mL
Transport Container and Temperature	Urine Chemistry Collection Tube (yellow top) or container at room temperature.
Stability & Storage Requirements	Room Temperature: 24 hours
	Refrigerated: 7 days
	Frozen: 6 months
Timing Considerations	Deliver specimens to laboratory immediately.
Unacceptable Specimens & Actions to Take	Specimens that are unlabeled, improperly labeled, or those that do not meet the stated criteria are unacceptable. Samples in Urine Analysis Preservative Tube are NOT acceptable. Request a recollection and credit the test with the appropriate LIS English text code for "test not performed" message. Examples: Quantity not sufficient-QNS; Wrong collection-UNAC. Document the request for recollection in the LIS.
Compromising Physical Characteristics	Turbidity: Centrifuge turbid samples before analysis. Frozen: Thaw and mix thoroughly prior to analysis. Avoid repeat freeze/thaw cycles.
Other Considerations	The pH range for urine specimens is 4.0 – 11.0 To protect the integrity of the sample, do not induce foaming. Obtain another sample for testing if adulteration of the sample is suspected. Adulteration of urine specimens can affect the test result. No additives or preservatives are required. Do not use Boric Acid as a preservative. Plastic transfer pipettes should NOT be used for delivering patient specimens. Before placing on system, ensure samples are free of: • Bubbles or foam • Particulate matter

NOTE: Labeling requirements for all reagents, calibrators and controls include: (1) Open date, (2) Substance name, (3) Lot number, (4) Date of preparation, (5) Expiration date, (6) Initials of tech, and (7) Any special storage instructions. Check all for visible signs of degradation. When placed onboard the analyzer, the instrument captures the date / time loaded and calculates and tracks the opened expiration.

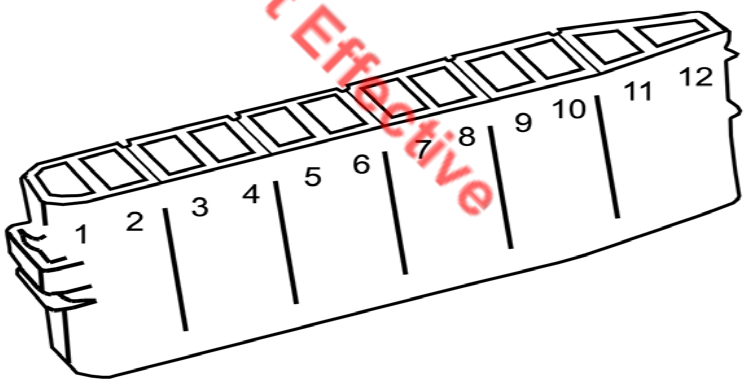
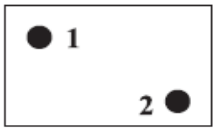
4. REAGENTS

The package insert for a new lot of kits must be reviewed for any changes before the kit is used. A current Package Insert is included as a Related Document.

4.1 Reagent Summary

Reagents	Supplier & Catalog Number
Fentanyl II Assay	ARK Diagnostics, Cat. No. 5069-0001-00
EMPTY Flex Reagent Cartridge	Siemens Dimension Vista, Cat. No. 10445218

4.2 Reagent Preparation and Storage

Reagent	**Antibody/Substrate Reagent 1 & Enzyme Reagent 2**
Container	Plastic vial
Storage	Store at 2-8° C
Stability	When not in use, reagents must be stored at 2–8°C, upright and with screw caps tightly closed. If stored as directed, reagents are stable until the expiration date printed on the label. **Pre-filled Empty Flex Reagent:** Onboard 24 hours
Preparation	Reagent is liquid and ready to use. The wells of an empty Flex Reagent Cartridge are identified as shown below.  The Flex well must be vented before filling with reagent. To vent a Flex well, puncture the film at a corner of the well (see picture below). Care must be taken to minimize the size of the vent; do not tear the film.  Transfer reagents into empty reagent packs according to the table below. Try to avoid bubbles as much as possible. Label the

	reagent packs with reagent name, lot number, date prepared, and expiration date.

Reagent	Wells	Volume per Well	Tests per Well
Reagent 1 Antibody / Substrate	11 and 12	0.8 mL	10
Reagent 2 Enzyme	1 and 2	0.8 mL	10

4.3 Loading Pre-filled Flex Reagent Cartridge on Dimension Vista

Note: Load one Empty flex on board at a time. Since there is no way to differentiate between empty flexes on board, operator must load one empty flex and identify it before loading the next empty flex.

Load the flex on board. From the Home Page go to **Set Up – Inventory – Reagents** – Select "**Empty**" – Identify the method and lot number – **Finish**

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
Fentanyl Calibrator A (Negative)	ARK Diagnostics, Cat. No. 5031-0002-01
Fentanyl Calibrator B (Cutoff)	ARK Diagnostics, Cat. No. 5031-0002-02

5.2 Calibrator Preparation and Storage

Calibrator	Fentanyl Calibrators A & B
Preparation	The calibrators are provided ready to use. Mix each level by gentle inversion before dispensing.
Storage/Stability	• Store at 2-8° C • If stored as directed, calibrators are stable until the expiration date printed on the label.

5.3 Calibration Parameter

Criteria	Special Notations
Reference Material	Fentanyl Calibrators A & B
Assay Range	See Package Insert for specific assay ranges.
Suggested Calibration Level	See Reagent Package Insert for lot specific assigned values in ng/mL

Frequency	• When changing lot numbers of primary reagent packs. • When indicated by quality control results. • After major maintenance or service. • Every 14 days for any one lot. At the end of the onboard stability interval, replace the reagent pack on the system with a new reagent pack. Recalibration is not required, unless the lot calibration interval is exceeded.
Calibration Scheme	See Package Insert for specific calibration scheme.
Procedure	• Fill 2 sample cups with 250 uL of each calibrator.

5.4 Tolerance Limits

IF……	THEN……
If result fall within assay-specific specification, and QC values are within acceptable limits,	proceed with analysis
If result falls outside assay-specific specification, or QC values are out of Acceptable limits,	troubleshoot the assay and/or instrument and repeat calibration

6. QUALITY CONTROL

6.1 Controls Used

Controls	Supplier and Catalog Number
Fentanyl Control, Low & High	ARK Diagnostics, Cat. no. 5031-0003-00

6.2 Control Preparation and Storage

Control	Fentanyl Control, Low & High
Preparation	The controls are ready for use. No preparation is required. • Aliquot 2 mL into screw top vials • Print labels from the Vista and label each vial • Immediately load onto the instrument for 2 – 8 ºC storage
Storage/Stability	• Store at 2 – 8 ºC • When stored at 2 – 8 ºC the controls are stable opened or unopened in the original container until the expiration date printed on the vial. • On board Dimension Vista, QC vials will remain stable for 7 days.

6.3 Frequency

Analyze all levels of QC material after every calibration and each day of testing (notated on the QC frequency sheets posted on the instruments).

Refer to the Siemens Dimension Vista QC Schedule and in the Siemens Dimension Vista Quick Reference Guide.

6.4 Tolerance Limits and Criteria for Acceptable QC

Step	Action
1	Acceptable ranges for QC are programmed into the instrument's Quality Control software system and Unity Real Time, and may be posted near the instrument for use during computer downtime.
2	**Run Rejection Criteria** • Anytime the established parameters are exceeded, the run is considered out of control (failed) and patient results must not be reported. • The technologist must follow the procedure in the Laboratory QC Program to resolve the problem.
3	**Corrective Action**: • All rejected runs must be effectively addressed through corrective action. Steps taken in response to QC failures must be documented. Patient samples in failed analytical runs must be reanalyzed according to the Laboratory QC Program. Supervisors may override rejection of partial or complete runs only with detailed documentation and criteria for overrides that are approved by the Medical Director. Consult corrective action guidelines in Laboratory QC Program. Follow corrective action guidelines in the Laboratory QC Program. • Corrective action documentation must follow the Laboratory Quality Control Program.
4	**Review of QC** • QC must be reviewed weekly by the Group Lead or designee and monthly by the Supervisor/Manager or designee. • If the SD and/or CV are greater than established ranges, investigate the cause for the imprecision and document implementation of corrective actions.

6.5 Documentation

- QC tolerance limits are programmed into the instrument and Unity Real Time; it calculates cumulative mean, SD and CV and stores all information for easy retrieval.
- Quality control records are reviewed daily at the bench, weekly by the Group Lead or designee, and monthly by the Supervisor/Manager or designee.

- Refer to complete policies and procedures for QC documentation and for record retention requirements in the Laboratory QC Program.

6.6 Quality Assurance Program

- Each new lot number of reagent or new shipment of the same lot of reagent must be tested with external control materials and previously analyzed samples. Performance of the new lot must be equivalent to the previous lot; utilize published TEA for acceptability criteria.
- Training must be successfully completed and documented prior to performing this test. This procedure must be incorporated into the departmental competency assessment program.
- The laboratory participates in CAP proficiency testing. All proficiency testing materials must be treated in the same manner as patient samples.
- Monthly QC must be presented to the Medical Director or designee for review and signature.
- Consult the Laboratory QC Program for complete details.

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

Siemens Dimension Vista Analyzer

7.2 Equipment

- Refrigerator capable of sustaining 2–8°C.
- Freezer capable of sustaining range not to exceed -20 to -50°C.
- Centrifuge

7.3 Supplies

- System Fluids
- Assorted calibrated pipettes (MLA or equivalent) and disposable tips

8. PROCEDURE

ARK Fentanyl II Assay is required to perform this test.

Fentanyl is performed on the Siemens Dimension Vista Analyzer after the method is calibrated and Quality Controls are acceptable.

NOTE: For all procedures involving specimens, buttoned lab coats, gloves, and face protection are required minimum personal protective equipment. Report all accidents to your supervisor.

8.1	Instrument Set-up Protocol
1.	Perform any required instrument maintenance.
2.	Ensure that the instrument has sufficient primary and ancillary reagents.
3.	Check status of cuvettes and tips. Check waste levels. Fill or empty as appropriate.
4.	Check calibration status and re-calibrate as needed.

8.2	Specimen Testing
1.	For QC placement and frequency, refer to the Dimension Vista® QC Schedule in the Laboratory QC Program.
2.	Follow the instructions, outlined in the Dimension Vista® Operator's Manual
3.	The instrument reporting system contains error messages to warn the user of specific malfunctions. Results followed by such error messages should be held for follow-up. Refer to the Dimension Vista® system manual "Error messages" section for troubleshooting.
4.	Follow protocol in Section 10.5 "Repeat criteria and resulting" for samples with results above or below the Analytical Measurement Range (AMR). Investigate any failed delta result and repeat, if necessary.
5.	Append the appropriate English text code qualifier messages to any samples requiring a comment regarding sample quality and/or any other pertinent factors.

NOTE: In the event that the test system becomes inoperable, notify supervision or designee for further direction. Patient specimens must be stored in a manner that maintains the integrity of the specimen.

9. CALCULATIONS

None

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

The cutoff analyte value for the Fentanyl is 1.0 ng/mL, however, cutoff normalized value and POS or blank-a factor is calculated to adjust the cutoff calibrator to 1000.

Positive Results: Positive samples are ≥ 1000 and appear with a POS.

Negative Results: Negative samples are < 1000 and appear with a blank.

10.2 Rounding

N/A

10.3 Units of Measure

N/A

10.4 Clinically Reportable Range (CRR)

N/A

10.5 Review Patient Data

Each result is reviewed for error messages. Refer to the Dimension Vista system manual "Error messages" section for troubleshooting. Resolve any problems noted before issuing patient reports.

10.6 Repeat Criteria and Resulting

Specimens that give an "Abnormal Reaction" message must be repeated.

If the result is Positive **and** the patient's location is L&D (Labor and Delivery), DI will flag the result to be held for repeat. Repeat the test, if possible run repeat on another analyzer:

- If repeat is positive, report as POSITIVE
- If repeat is negative, run QC to verify instrument performance. If QC is within range on the same reagent lot as used for patient run, rerun patient sample a third time. Report result that duplicated (2 of 3 results).

Message	Code
Verified by repeat analysis	Append –REP to the result.

11. EXPECTED VALUES

11.1 Reference Ranges

None Detected

11.2 Critical Values

None Established

11.3 Standard Required Messages

The following comment is automatically added to the report by the LIS when a urine Fentanyl test is ordered:

"This is a screening assay. Fentanyl is detected in concentrations at or above 1.0 ng/mL.".

The following comment is automatically added to the report by the LIS when a urine drug screen is ordered:

"The drug of abuse panel is a screening assay. It detects the following drugs of abuse in concentrations at or above the concentrations listed below.

Phencyclidine	25 ng/mL
Benzodiazepines	200 ng/mL
Cocaine	300 ng/mL
Amphetamines	1000 ng/mL
THC	50 ng/mL
Opiates	300 ng/mL
Barbiturates	200 ng/mL
Methadone	300 ng/mL
Buprenorphine	5 ng/mL
Fentanyl	1.0 ng/mL

The ingestion of certain herbal or plant products containing Ephedra or its metabolites may cause false positive amphetamine/metamphetamine results.

This test is for medical screening purposes ONLY. For confirmation a separate order for Gas Chromatography by Mass Spectrophotometry (GCMS) is required."

12. CLINICAL SIGNIFICANCE

Fentanyl is a synthetic opioid narcotic analgesic similar to morphine. Fentanyl is 50-100 times more potent than morphine. It is prescribed for patients with chronic pain and is used to manage pain after surgery or for treatment of breakthrough pain in cancer patients. Fentanyl is prescribed in various forms: by injection, transdermal patch, and orally. Fentanyl such as the transdermal system can be abused in a manner similar to other opioid agonists, legal or illicit. All patients receiving opioids should be routinely monitored for signs of misuse, abuse and addiction. Fentanyl has high potency and short duration of action, and it is abused for its intense euphoric effects. It is very dangerous when substituted illicitly for other opioids because of its potency and overdoses can lead to respiratory depression and death.
The ARK Fentanyl II Assay detects fentanyl in human urine. The test is not intended to differentiate between drugs of abuse and prescription use of fentanyl. There are no uniformly recognized drug levels for fentanyl in urine. The primary metabolism of fentanyl leads to the time-dependent urinary excretion of fentanyl and norfentanyl. The half-life of fentanyl may range 3 - 12 hours. Fentanyl is exclusively metabolized by N-dealkylation and hydroxylation. More than 90% of the dose is eliminated as norfentanyl and hydroxylated metabolites. Less than 7% of the dose is excreted unchanged in the urine.

13. PROCEDURE NOTES

- **FDA Status:** FDA Approved/Modified

- **Validated Test Modifications:** Specimen stabilities have been modified from the package insert based on in-house stability studies performed at Quest Diagnostics.

The instrument reporting system contains error messages to warn the operator of specific malfunctions. Any report slip containing such error messages should be held for follow-up.

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

Qualitative Assay: cutoff concentration of 1.0 ng/mL, however, cutoff normalized value and POS or blank-a factor is calculated to adjust the cutoff calibrator to 1000.

14.2 Precision

Human Urine (ng/mL)	Relative % Cutoff	# of Results	Results
0	-100	160	160 Negative
0.25	-75	160	160 Negative
0.50	-50	160	160 Negative
0.75	-25	160	160 Negative
1.00	Cutoff	160	84 Negative, 76 Positive
1.25	+25	160	160 Positive
1.50	+50	160	160 Positive
1.75	+75	160	160 Positive
2.00	+100	160	160 Positive

14.3 Interfering Substances

Urine samples with specific gravity values from 1.002 to 1.030 and pH values ranging from 3.0 to 11.0 were tested in the presence of the two levels of fentanyl at ± 50% of the cutoff concentration. No interference was observed when tested with the ARK Fentanyl II Assay.

14.4 Clinical Sensitivity/Specificity/Predictive Values

Not available

15. SAFETY

Refer to your local and corporate safety manuals and Safety Data Sheet (SDS) for detailed information on safety practices and procedures and a complete description of hazards.

ARK Fentanyl II products contain ≤0.09% sodium azide. As a precaution, affected plumbing including instrumentation should be flushed adequately with water to mitigate the potential accumulation of explosive metal azides. No special handling is required regarding other assay components.

16. RELATED DOCUMENTS

1. Dimension Vista Operating, QC, Calibration and Maintenance procedure
2. Laboratory Quality Control Program
3. QC Schedule for Siemens Dimension Vista
4. Laboratory Safety Manual
5. Safety Data Sheets (SDS)
6. Quest Diagnostics Records Management Procedure
7. Dimension Vista System Error Messages Chart
8. Centrifuge Use, Maintenance and Function Checks (Lab policy)
9. Specimen Acceptability Requirements (Lab policy)
10. Repeat Testing Requirement (Lab policy)
11. Current package insert of ARK Fentanyl II Assay

17. REFERENCES

1. Package Insert, Fentanyl II Assay, ARK Diagnostics Inc., 05/2021.
2. Package Insert, Fentanyl Calibrator, ARK Diagnostics Inc., 03/2020.
3. Package Insert, Fentanyl Control, ARK Diagnostics Inc., 03/2020.
4. Package Insert, Fentanyl II Assay Siemens Dimension Vista Analyzer, ARK Diagnostics Inc., 08/2020.

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval

19. ADDENDA

None