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A pilot PT scheme for external assessment of laboratory performance in testing synthetic opioid compounds in urine, plasma, and whole blood

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Abstract

A proficiency testing (PT) scheme was prepared for laboratories engaged in bioanalytical testing for synthetic opioid compounds in urine, plasma, and whole blood. Samples were prepared using compounds included in the Opioid Certified Reference Material Kit (Opioid CRM Kit) developed by the U.S. Centers for Disease Control and Prevention. Laboratories received samples during a 2-year project with each year consisting of two PT events 6 months apart. In the first year (pilot test), participants included 10 public health laboratories throughout the United States. In the second year, the group of laboratories expanded to include clinical and forensic drug testing laboratories, and 12 additional participating labs joined the program. In Year 1, overall detection percentages for the compounds present in the PT samples were 95.5% in Event 1% and 97.2% in Event 2. There were 31 apparent false positives reported in Event 1 and four apparent false positives reported in Event 2. Carryover or contamination in laboratory analytical systems were found to be the most significant causes of the false positive results, and none of the laboratories that reported false positives in Event 1 did so in Event 2. In Year 2, overall detection percentages for the compounds present in the PT samples were 89.5% in Event 3% and 94.8% in Event 4. There was one apparent false positive reported in Event 3 and three apparent false positives reported in Event 4. Improvements in drug detection between the two PT events in each year demonstrated the benefit of PT schemes in identifying and addressing potential deficiencies in laboratory systems.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Disclaimer

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CRediT authorship contribution statement

E. Dale Hart: Writing – original draft, Methodology, Formal analysis, **Nichole D. Bynum:** Writing – original draft, Methodology, Supervision, Project Visualization, **Amy Evans:** Writing – original draft, Resources, Supervision, **Kenneth D. Swanson:** Writing – review & editing. **Thomas A. Blake:** Writing – review & editing.

Keywords

Proficiency Testing; PT Scheme; Opioids; Fentanyl; Urine; Plasma; Whole blood

1. Introduction

Substance use in the United States has been an ongoing concern and will require a cooperative and aggressive approach to mitigate and control. According to the U. S. Centers for Disease Control and Prevention (CDC), approximately 500,000 people died from an overdose involving opioids between 1999 and 2019. [1] In 2020, there were more than 56,000 deaths involving synthetic opioids other than methadone in the United States. [2] Novel psychoactive substances (NPS), the most encompassing category of emerging drugs, include synthetic cannabinoids, stimulants, benzodiazepines, hallucinogens, and synthetic opioids like the fentanyl analogs. [3] In the National Forensic Laboratory Information System (NFLIS)-Drug 2017 Annual Report, carfentanil and furanyl fentanyl were listed in the top 25 most frequently identified drugs for the first time, [4] while the NFLIS-Drug 2018 Midyear Report showed acetyl fentanyl in the top 25 most frequently identified drugs. [5].

As the opioid crisis has grown, laboratories performing bioanalytical testing have expanded their testing methods to include synthetic opioids—specifically, fentanyl analogs. However, analysis of fentanyl analogs presents several challenges. [6] Structurally like fentanyl, the isomeric nature of these compounds makes chromatographic separation by gas chromatography (GC) and liquid chromatography (LC) crucial to their identification. Laboratories must be able to reliably detect analytes of interest; therefore, valid methods are imperative. The availability of proficiency testing (PT) is an important component to building confidence that laboratory results are correct.

In 2019, CDC contacted RTI International's Center for Forensic Sciences CDC to establish the Synthetic Opioid Proficiency Testing Program. This program was aimed at evaluating laboratory performance in the identification of fentanyl and fentanyl-related compounds in urine, plasma, and whole blood through simultaneous interlaboratory comparison. The program started with a pilot study that was conducted over two PT events in 2020. The first event was run in the first quarter of 2020, and the second was run in the third quarter of that same year. For each event, sample matrices were fortified with varying analyte concentrations (Tables 2 and 3). Event 1 included six urine, five plasma, and two whole-blood samples. Because many of the laboratories participating in the pilot study were still expanding detection capabilities for the analysis of fentanyl analogs, the goal of this first event was to determine the laboratories' abilities to detect half of the analytes in Table 1 at target concentrations above the typical limits of quantitation observed in forensic laboratories. Event 2 included four urine samples, six plasma, and two whole-blood samples. The goal of this event was to challenge the laboratories' abilities to detect the remaining analytes and to test their capabilities to detect some analytes at target concentrations lower than those in Event 1.

Year 2 of the program consisted of two PT events in 2021. As with the Year 1 pilot study, PT events in Year 2 were conducted in the first and third quarters of the year. The number

of laboratories participating in Year 2 increased from 10 to 22, with 13 laboratories joining participation and one laboratory from the first year electing not to continue. The sample compositions in the two events in the second year (Tables 4 and 5) were like those in Event 2 in the first year with two changes. One change was the addition of negative samples in each of the PT events—a negative urine sample in Event 3 and a negative plasma sample in Event 4. A second change was the inclusion of one sample in each event in which a drug was paired with its primary metabolite—fentanyl and norfentanyl (Sample #2) in Event 3 and carfentanil and norcarfentanil (Sample #1) in Event 4.

2. Materials and methods

Drug standards used in the preparation of PT samples were obtained from Cerilliant Corporation (Round Rock, Texas, USA), the manufacturer and distributer of the CDC Opioid CRM Kit. Analytical grade ethanol was purchased from Fisher Scientific (Fair Lawn, NJ). PT samples were prepared in drug-free human urine purchased from UTAK Laboratories (Valencia, California, USA) and in sheep whole blood and plasma purchased from Innovative Research (Novi, Michigan, USA). Whole blood contained sodium oxalate as an anticoagulant and sodium fluoride as a preservative. Plasma contained K2EDTA as an anticoagulant. Sheep blood products were selected to avoid any potential presence of drugs in the PT matrix.

Universal safety precautions for handling biological samples were adhered to at all times when handling blood products, and drug standards were handled by trained personnel according to internal standard operating procedures. To prepare each sample, working drug standards were first made by diluting the certified standards with ethanol. The sample matrix (200 mL) was fortified with the required working standard, and the resulting solution was mixed with a magnetic stirrer for at least 20 min. The fortified sample matrix was then aliquoted into barcode-labeled sample vials. Samples were stored frozen at − 20 °C until shipment to the participating laboratories. Three outside reference laboratories contracted by RTI confirmed analyte concentrations in each batch of PT samples using liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis. The assigned quantitative value for each analyte was based on the mean of 9 tests (3 replicates from three samples) from a single laboratory. Three reference laboratories were used to obtain quantitative test results for the most analytes possible. Samples were shipped frozen on dry ice to the PT participants.

Participation in the PT program was voluntary for each laboratory, and samples were provided at no cost to participating laboratories. PT samples were shipped to participants simultaneously with documentation establishing an expected reporting timeline. Participants were expected to follow normal operating procedures when receiving and testing the PT samples. No expectation was placed on participating laboratories for testing methodology, although most participants used an MS-based approach to detect target analytes. Upon completion of testing, the participating laboratories emailed results forms to RTI for compilation and analysis of the test results. For each event, participants were issued a summary report with data comparing their performance with other participating laboratories.

No formal remediation action was required by participating laboratories for any event during the 2-year program.

Participating laboratories received two sets of samples each year. Samples were fortified with a selection of fentanyl analogs at concentrations ranging from 5 to 80 ng/mL. Laboratories were evaluated based on their qualitative identification of the fentanyl compound(s) contained in each test sample. This evaluation was appropriate for laboratories conducting qualitative and confirmatory drug analysis in public health, clinical, and forensic applications. The first year was a pilot program consisting of 10 public health laboratories from around the United States. The second year added 13 clinical drug testing and forensic drug testing laboratories.

The target concentration range of 5–80 ng/mL for fentanyl and norfentanyl in urine was within reported concentrations when patients are administered 25 μg/h to 100 μg/h of fentanyl for chronic pain management. [7] In plasma and whole blood, fentanyl and norfentanyl at target concentrations of 5–20 ng/mL were consistent with either long-term high-dose fentanyl administration or overdose cases. [8] Because most of the fentanyl analogs included in this PT scheme are illicit drugs and not approved for human use, reported concentrations for those analytes are generally limited to postmortem overdose cases. Target concentrations for these analytes in all three samples matrices were consistent with those reported in accidental overdose cases. [9,10].

The fentanyl analogs used in the PT program were sourced from the Opioid Certified Reference Material Kit (Opioid CRM Kit) developed by the CDC as part of the Traceable Opioid Material Kits® product line. [11] The kit included 22 fentanyl-related compounds (Table 1).

In the data analysis, a reported result for a target analyte was labeled as a false negative if the testing laboratory reported the capability to test a target analyte but did not identify the target analyte when intentionally spiked. In some cases, a participating laboratory did not test any sample of one or more of the provided matrices (urine, plasma, or whole blood); however, those results were not considered false negative. A reported result for a target analyte was labeled as an apparent false positive if the reported analyte was not intentionally spiked into the fortified sample.

3. Results

In Event 1 (Table 2), reference test results agreed well with the target concentrations for the analytes. Nine of the 10 participating laboratories reported testing for the urine and plasma samples and six of 10 laboratories reported testing for the whole-blood samples. In total, 111 of 130 (85.4%) sample matrix challenges were tested.

All participating laboratories detected the analytes present in eight of the 13 samples with seven of the laboratories detecting all analytes. One false negative was reported in each of three urine samples (Samples 1, 2, and 5), one of the plasma samples (Sample 10), and one of the whole-blood samples (Sample 12). Of the false negatives, one laboratory missed three analytes and another two laboratories missed one each. The overall detection percentage

across all samples was 95.5% (106 of 111 challenges). In addition to the target analytes, there were 31 unexpected compounds reported in nine of the 13 samples. Six of the 10 laboratories reported at least one unexpected analyte. Two laboratories accounted for 16 of the 31 additional analytes reported. All reports of the presence of para-fluorofentanyl (Samples 7, 8, 9, 10, 11, and 13) were from one laboratory. A second laboratory reported 10 of the unexpected analytes in Sample 7 and Sample 9. Of the remaining four laboratories that reported unexpected analytes, the number of reports ranged from one to six compounds.

In Event 2 (Table 3), there was good agreement between the reference laboratory results and the target concentrations. Nine of the 10 participating laboratories reported testing the urine samples, all 10 of the laboratories reported testing the plasma samples (one laboratory did not test the analyte in Sample #8), and eight of the 10 laboratories reported testing for the whole-blood samples. In total, 111 of 120 (92.5%) of the sample matrix challenges were tested, an increase in testing of the total number of samples by the same 10 laboratories as in Event 1.

All laboratories detected the analytes present in nine of the 12 samples. Nine of the ten laboratories detected the analytes present in all 12 samples. One false negative was reported in one of the plasma samples (Sample 10) and in each of the whole blood samples (Samples 11 and 12). All three false negatives were from one laboratory. The overall detection percentage across all samples was 97.3% (108 of 111 challenges). Four unexpected analytes were reported in Event 2 by two laboratories, 87% fewer compounds than the 31 reported in Event 1. None of the six laboratories that reported unexpected analytes in Event 1 did so in Event 2.

In Event 3 (Table 4), reference laboratory results for the sample analytes were generally higher than the target concentrations. Eleven additional laboratories participated in the event while one of the previous participants dropped out resulting in a total of twenty participating laboratories. For each matrix, 19 laboratories tested for urine, 15 for plasma, and 12 for blood. Because Sample 2 contained two analytes, fentanyl and norfentanyl, and Sample 1 contained blank urine, the number of potential analytical challenges was 260. In total, 201 of 260 (77.3%) of the analytical challenges were tested, a significant decrease compared with Event 2. This decrease in testing rate was due to the addition of several urine-only testing laboratories, which did not test the plasma and whole-blood samples. Drug detection percentages in Event 3 (Year 2) were lower than in Event 2 (Year 1). The overall detection percentage for Event 3 was 90.0% (181 of 201 challenges), including the negative sample. False negatives (20) were reported in Samples 3–8 and 10–12. Previous laboratory participants reported three false negatives, whereas new participating laboratories reported 17 false negatives. Ten laboratories detected all analytes within the matrices that they tested, while ten other laboratories reported at least one false negative. Labs that detected all analytes tested included six of the original participants and four of the new laboratories. Labs reporting false negatives included three of the original participants and seven of the new laboratories. The three original participants reported one false negative each, while the new laboratories' false negatives ranged from one to five.

Only one additional analyte was reported in Event 3: ± cis-3-methyl fentanyl in Sample 10.

In Event 4 (Table 5), two laboratories were added raising the total number of participants to 22 laboratories. Reference test results for the analytes were generally higher than the target concentrations. In total, 205 of 286 (71.7%) of the analytical challenges were tested, a decrease in testing of the total number of samples compared with Event 3. This decrease in testing rate was due to the addition of laboratories that did not test some matrices or analytes. The overall detection percentage was 96.6% (198 of 205 challenges) and included correctly identifying Sample 5 as “negative.” This is an improvement from Event 3 and similar performance to the two events in Year 1 of the PT program. This improvement was likely due to increased experience among the new laboratory participants. There was a marked decrease in false negative reports from twenty in Event 3 to six in Event 4. Seventeen of the participating laboratories detected all analytes for which they tested. This included eight of the original nine laboratories and nine of the thirteen new laboratories. False negative results were reported by one of the original laboratories and four of the new laboratories. One of the new laboratories tested for fentanyl and norfentanyl in urine-only. Since those analytes were not included in the urine samples, that laboratory was counted as “not testing” for the analytes in the urine samples.

There were three unexpected analytes reported in Event 4: Butyryl fentanyl in Sample 2, 4-ANPP in Sample 5, and ortho-fluorofentanyl in Sample 8. Note that Sample 5 was a blank plasma sample.

4. Discussion

The results observed in the two events from Year 1 of the PT scheme showed that the participating laboratories were proficient at detecting the synthetic opioids included in the scheme. Overall, the detection percentages in Events 1 and 2 (95.5% and 97.2%, respectively) were very good. The laboratories also increased the number of matrix challenges as all laboratories tested the plasma samples and two additional laboratories tested the blood samples. This increased the sample testing rate from 85.4% to 90.8% during Year 1.

The most notable observation in Event 1 was the relatively high number of apparent false positives. There are several likely causes for the apparent false positives reported in Event 1:

1. Possible presence of trace amounts of degradants or synthetic intermediates in the Certified Reference Materials detected by laboratories with very low limits of detection.
2. Artifacts produced as part of the analytical process at the laboratory.
3. Laboratory system contamination.
4. Carryover in the laboratories’ analytical procedures or instruments.

Four of the apparent false positives can be explained by trace contaminants in the certified standards as reported by the manufacturer. The two instances of norfentanyl reported in Sample 1 ((±)-beta-hydroxythiofentanyl) and the 4-ANPP reported in Sample 7 (acetyl fentanyl) and Sample 13 (furanyl fentanyl) are consistent with those compounds being reported as present on the manufacturer’s certificates of analysis for the standards used

to prepare those respective PT samples. However, none of the remaining apparent false positives can be explained by their presence in the standards.

Although the PT program did not include a formal remedial action process requiring laboratories to discuss their corrective actions with us, we did contact some of the laboratories that detected analytes that were not spiked into the PT samples. This was done after the completion of Event 2. What we found was that the most common reported causes of the apparent false positives were analytical system contamination or carryover. Laboratories addressed what they found in Event 1 by either checking their analytical systems or by reevaluating their reporting limits.

In Event 2, the number of apparent false positives was reduced to four, an 87% decrease compared with Event 1. None of the six laboratories that reported unexpected analytes in Event 1 did so again in Event 2. This implies that actions taken by those laboratories to address errors in Event 1 were successful.

In Year 2, 13 additional laboratories were added to the program, and one laboratory that participated in Year 1 opted out of the second year. Several of the new laboratories only tested the urine samples, and many did not test all the analytes included in the PT scheme. To account for these changes, testing rates were calculated based on the number of analytical challenges, accounting for both the matrices and the analytes that were tested. Therefore, testing rate across the three matrices was reduced to 77.3% in Event 3% and 71.7% in Event 4. The decreased detection percentage in Event 3 of 90.0% versus 97.2% in Event 2 was likely due to the addition of new laboratories with less experience detecting the analytes added to the samples. The detection percentage of 96.6% in Event 4 was a marked improvement from Event 3 and might be attributed to remedial actions taken by the new laboratories in response to their performance in Event 3.

In contrast to Year 1, the number of apparent false positives reported in Year 2 was significantly reduced—from 35 in Year 1 to four in Year 2. This was probably the most significant improvement in laboratory performance of the 2 years of the program. The fact that the six laboratories that reported false positives in Event 1 did not do so in Event 2 is indicative of the benefit provided by PT schemes in identifying laboratory deficiencies. It also illustrates how quickly laboratories can respond once they are aware of systemic issues in their analytical processes.

An additional significant observation was the improvement in false negative results between Event 3 and Event 4. Although false negative test results were relatively low in Year 1 (five in Event 1 and three in Event 2), the number increased to 20 in Event 3 (Year 2) before declining to six in Event 4. The magnitude of false negatives in Event 3 (10%) would translate to a very high number of missed identifications in the field. The improvement in this measure in Event 4 shows the benefit of PT schemes in improving all aspects of laboratory performance.

5. Conclusion

A 2-year PT scheme consisting of 22 fentanyl analogs was conducted to assess laboratory performance in the detection of the compounds in urine, plasma, and whole blood. The pilot test demonstrated the feasibility of the PT activity. The completion of 2 years of the PT scheme with four PT events demonstrated that the participating laboratories were proficient in the detection and identification of all analytes in at least one of three matrices. We also noted that some laboratories expanded their testing by adding the sample matrices to their testing panels. Improvement in laboratory performance was observed as a reduction in apparent false positives between the first and second PT events and a continuing low false positive rate in the third and fourth PT events. Overall, the results of this PT scheme showed the benefits of the use of performance testing schemes in the overall quality assurance programs for bioanalytical laboratories.

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Table 1

Fentanyl and Fentanyl Analogs used in the Preparation of PT samples.

Fentanyl and Fentanyl Analogs		
4-ANPP	Fentanyl	para-Fluorobutyryl fentanyl (p-FBF)
4’-methyl acetyl fentanyl HCl	para-Fluorofentanyl	Remifentanil HCl
Acetyl fentanyl	Furanyl fentanyl HCl (FU-F)	U-47700
Acryl fentanyl HCl	(±)-beta-Hydroxythiofentanyl HCl	U-48800 HCl
Benzylfentanyl HCl	Methoxyacetyl fentanyl HCl	U-49900
Butyryl fentanyl	(±)-cis-3-Methyl fentanyl HCl	Valeryl fentanyl HCl
Carfentanil oxalate	Norcarfentanil oxalate	
Cyclopropyl fentanyl	Norfentanyl oxalate	

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Table 2

PT Sample Results for Event 1.

Matrix: Urine						
Sample Number	**Analytes**	**Target Concentration (ng/mL)**	**Reference Test Results (ng/mL)***	**Positive Results**	**Negative Results**	**Not Tested**
1	(±)-beta-Hydroxythiofentanyl	80	85.3	8 (88.9%)	1	1
2	4’-Methyl acetyl fentanyl	40	42.9	8 (88.9%)	1	1
3	Carfentanil	8	Positive	9 (100%)	0	1
4	Fentanyl	40	44	9 (100%)	0	1
5	Norcarfentanil	8	9.19	8 (88.9%)	1	1
6	U-48800	40	42.5	9 (100%)	0	1
Matrix: Plasma Sample Number	**Analytes**	**Target Concentration (ng/mL)**	**Reference Test Results (ng/mL)***	**Positive Results**	**Negative Results**	**Not Tested**
7	Acetyl fentanyl	20	20.8	9 (100%)	0	1
8	Cyclopropyl fentanyl	20	21.9	9 (100%)	0	1
9	Fentanyl	20	20.7	9 (100%)	0	1
10	Norfentanyl	5	6	8 (88.9%)	1	1
11	U-49900	20	22.7	9 (100%)	0	1
Matrix: Whole Blood						
Sample Number	**Analytes**	**Target Concentration (ng/mL)**	**Reference Test Results (ng/mL)***	**Positive Results**	**Negative Results**	**Not Tested**
12	4-ANPP	20	21.1	5 (83.3%)	1	4
13	Furanyl fentanyl	20	21.8	6 (100%)	0	4
Additional Analytes Reported						
Sample Number	**Analytes Reported (Number of Laboratories)**					
1	Norfentanyl (2) and Methoxyacetyl fentanyl (1)					
2	Fentanyl (1)					
4	Norfentanyl (2), 4’-Methyl acetyl fentanyl (1), and Acetyl fentanyl (1)					
7	para-Fluorofentanyl (1), Benzyl fentanyl (1), Methoxyacetylfentanyl (1), 4-ANPP (1), U-49900 (1), and 4’-Methylacetylfentanyl (1)					
8	para-Fluorofentanyl (1), Acetyl fentanyl (1), Valeryl fentanyl (1), and Butyryl fentanyl (1)					
9	para-Fluorofentanyl (1), Acetyl fentanyl (2), Carfentanil (1), Furanyl fentanyl (1), U-47700 (1), beta-Hydroxythiofentanyl (1), Norfentanyl (1), and U-49900 (1)					
10	para-Fluorofentanyl (1)					
11	para-Fluorofentanyl (1)					
13	para-Fluorofentanyl (1) and 4-ANPP (1)					

* Reference testing was subcontracted to three qualified laboratories that provided results directly to RTI. Quantitative results are based on nine replicate tests at one laboratory.

Table 3

PT Sample Results for Event 2.

Matrix: Urine						
Sample Number	**Analytes**	**Target Concentration (ng/mL)**	**Reference Test Results (ng/mL)***	**Positive Results**	**Negative Results**	**Not Tested**
1	Benzylfentanyl	40	Positive	9 (100%)	0	1
2	Methoxyacetyl fentanyl	40	Positive	9 (100%)	0	1
3	para-Fluorofentanyl	40	44.9	9 (100%)	0	1
4	Remifentanil	40	Positive	9 (100%)	0	1
Matrix: Plasma						
Sample Number	**Analytes**	**Target Concentration (ng/mL)**	**Reference Test Results (ng/mL)***	**Positive Results**	**Negative Results**	**Not Tested**
5	cis-3-Methylfentanyl	5	5.9	10 (100%)	0	0
6	4-ANPP	5	5.9	10 (100%)	0	0
7	Acryl Fentanyl	5	6.7	10 (100%)	0	0
8	Butyryl fentanyl	5	5.7	9 (100%)	0	1
9	U-47700	10	10.6	10 (100%)	0	0
10	Valeryl fentanyl	5	5.3	9 (90%)	1	0
Matrix: Whole Blood						
Sample Number	**Analytes**	**Target Concentration (ng/mL)**	**Reference Test Results (ng/mL)***	**Positive Results**	**Negative Results**	**Not Tested**
11	Fentanyl	5	5.7	7 (87.5%)	1	2
12	para-Fluorobutyryl fentanyl	5	5.8	7 (87.5%)	1	2
Additional Analytes Reported						
Sample Number	**Analytes Reported (Number of Laboratories)**					
2	4-ANPP (1)					
4	Norcarfentanil (1)					
11	para-Fluorobutyryl fentanyl (1)					
12	Fentanyl (1)					

Table 4

PT Sample Results for Event 3.

Matrix: Urine						
Sample Number	**Analytes**	**Target Concentration (ng/mL)**	**Reference Test Results (ng/mL)***	**Positive Results**	**Negative Results**	**Not Tested**
1	Blank	Neg	Neg	0	19 (100%)	1
2	Fentanyl	20	22.7	19 (100%)	0	1
	Norfentanyl	40	47.7	19 (100%)	0	1
3	4-ANPP	20	34.6	16 (89.5%)	2	2
4	U-49900	30	Positive	13 (77.7%)	5	2
Matrix: Plasma						
Sample Number	**Analytes**	**Target Concentration (ng/mL)**	**Reference Test Results (ng/mL)***	**Positive Results**	**Negative Results**	**Not Tested**
5	U-48800	8	11.2	13 (86.7%)	2	5
6	Norcarfentanil	5	Pos	13 (86.7%)	2	5
7	Remifentanil	8	Pos	13 (86.7%)	2	5
8	Methoxyacetyl fentanyl	8	8.7	14 (93.3%)	1	5
Matrix: Whole Blood						
Sample Number	**Analytes**	**Target Concentration (ng/mL)**	**Reference Test Results (ng/mL)***	**Positive Results**	**Negative Results**	**Not Tested**
9	Carfentanil	5	5.9	12 (100%)	0	8
10	4’-methyl Acetyl fentanyl	5	Pos	9 (75%)	3	8
11	para-Fluorobutyryl fentanyl	5	5.3	11 (91.7%)	1	8
12	beta-Hydroxythiofentanyl	5	Pos	10 (83.3%)	2	8
Additional Analytes Reported						
Sample Number	**Analytes Reported (Number of Laboratories)**					
10	± cis-3-Methyl fentanyl (1)					

Table 5

PT Sample Results for Event 4.

Matrix: Urine						
Sample Number	**Analytes**	**Target Concentration (ng/mL)**	**Reference Test Results (ng/mL)**	**Positive Results**	**Negative Results**	**Not Tested**
1	Carfentanil	5	Pos	18 (100%)	0	4
	Norcarfentanil	5	6.2	11 (78.6%)	3	8
2	Cyclopropyl fentanyl	20	26.2	19 (100%)	0	3
3	Acetyl fentanyl	20	24.2	18 (100%)	0	4
4	Acryl fentanyl	20	23.7	19 (100%)	0	3
Matrix: Plasma						
Sample Number	**Analytes**	**Target Concentration (ng/mL)**	**Reference Test Results (ng/mL)***	**Positive Results**	**Negative Results**	**Not Tested**
5	Blank	Neg	Neg	1	15 (93.8%)	6
6	Furanyl fentanyl	8	8.4	16 (100%)	0	6
7	Benzylfentanyl	8	Pos	14 (100%)	0	8
8	para-Fluorofentanyl	8	9.6	15 (93.8%)	1	6
Matrix: Whole Blood						
Sample Number	**Analytes**	**Target Concentration (ng/mL)**	**Reference Test Results (ng/mL)***	**Positive Results**	**Negative Results**	**Not Tested**
9	± cis-3-Methyl fentanyl	5	6.1	12 (92.3%)	1	9
10	U-47700	5	5.8	14 (100%)	0	8
11	Butyryl fentanyl	5	6.0	13 (92.9%)	1	8
12	Fentanyl	10	9.4	14 (100%)	0	8
Additional Analytes Reported						
Sample Number	**Analytes Reported (Number of Laboratories)**					
2	Butyryl fentanyl (1)					
5	4-ANPP (1)					
8	ortho-Fluorofentanyl (1)					