



The author(s) shown below used Federal funding provided by the U.S. Department of Justice to prepare the following resource:

Document Title: Prevalence of Fentanyl and Its Analogues
in a Court-Ordered Mandatory Drug
Testing Population

Author(s): Megan Grabenauer, Ph.D., Nichole Bynum,
M.S.

Document Number: 308639

Date Received: March 2024

Award Number: 2019-R2-CX-0017

This resource has not been published by the U.S. Department of Justice. This resource is being made publicly available through the Office of Justice Programs' National Criminal Justice Reference Service.

Opinions or points of view expressed are those of the author(s) and do not necessarily reflect the official position or policies of the U.S. Department of Justice.

Prevalence of Fentanyl and Its Analogues in a Court-Ordered Mandatory Drug Testing Population

NIJ Award No. 2019-R2-CX-0017

Project Period: January 1, 2020 – December 31, 2023

Award Amount: \$239,196

Technical Summary

Submitted via Grants.gov to:
U.S. Department of Justice
Office of Justice Programs
National Institute of Justice
810 Seventh St., NW
Washington, DC 20531

Prepared By:
Megan Grabenauer, Ph.D. (PI)
Senior Research Chemist
RTI International
mgrabenauer@rti.org
919-541-7463

Nichole Bynum, M.S.
Research Forensic Scientist
RTI International

RTI International
PO Box: 12194
3040 Cornwallis Road
Research Triangle Park, NC 27709-2194
RTI Project No. 0217154

Administrative Point of Contact:
Ashley Wheeler
aswheeler@rti.org
919-990-8333

Table of Contents

PROJECT SUMMARY	1
GOALS AND OBJECTIVES	1
RESEARCH QUESTIONS	1
SUMMARY OF PROJECT DESIGN AND METHODS	2
SUMMARY OF RESULTS	6
APPLICABILITY TO CRIMINAL JUSTICE	12
PRODUCTS	13
SCHOLARLY PRODUCTS.....	13
DISSEMINATION ACTIVITIES.....	13
REFERENCE	14
APPENDIX A	15
HAIR CLASSIFICATION DESCRIPTIONS	15
APPENDIX B	16
LC-MS/MS METHOD	16
APPENDIX C	19
LOD, LLOQ, AND ULOQ RESULTS OF FENTANYL-RELATED COMPOUNDS AND OTHER COMPOUNDS.	19
APPENDIX D	20
MOST COMMON DRUGS DETECTED IN ORAL FLUID CONFIRMATION TESTING: ADULTS VS. JUVENILES.....	20
MOST COMMON DRUGS DETECTED IN HAIR CONFIRMATION TESTING: ADULTS VS. JUVENILES	21

Project Summary

Goals and Objectives

The opioid epidemic in the United States is wreaking havoc on the nation, as evidenced by the increase in law enforcement drug seizures and opioid overdose deaths. The prevalence of fentanyl and its analogs in the criminal justice system is relatively unknown although drug testing is frequently conducted in correctional settings. Court-ordered mandatory drug testing (COMDT) using hair is routinely done at large commercial laboratories but does not typically include testing for fentanyl or fentanyl-related compounds. Limited information is available on prevalence data in the COMDT population to characterize drug use patterns. The goal of the project summarized here was to provide timely, evidence-based intelligence on growing rates of drug use and patterns of use of fentanyl and fentanyl-related compounds in a criminal justice population.

Research Questions

Phase I of this project determined the prevalence of fentanyl and a selection of fentanyl-related compounds in hair specimens submitted for COMDT over 6 months. This phase of the project pursued an answer to the research question: is testing for fentanyl and fentanyl-related compounds, in addition to heroin and prescription opioids, needed in the COMDT population to improve public protection, treatment options, or probation/parole conditions?

In Phase II of this study we conducted a retrospective analysis of COMDT data from a 5-year period. Phase II of the proposed study pursued answers to three broad research questions: (1) How do drug patterns in a COMDT population compare to general workforce drug testing results? (2) How do drug patterns in a COMDT population change with inclusion of fentanyl and fentanyl-related compounds in the testing? and (3) Do oral fluid and hair provide a more

sensitive tool for identifying individuals who are using drugs to provide improved public protection, deterrence, and rehabilitation?

Summary of Project Design and Methods

In Phase I of this project researchers at RTI International (RTI) developed and validated a method to analyze fentanyl and fentanyl-related compounds in hair. RTI used the method to analyze 520 hair specimens that were collected for COMDT, which were provided by a commercial toxicology laboratory. All hair specimens were collected between November 1, 2020 and February 28, 2021. Drug-free hair was used for all calibrators, controls, and blanks. Hair specimens received in our laboratory were characterized by length, color, and curl using the classification descriptions provided in Appendix A.

Prior to analysis, all COMDT hair specimens were decontaminated by washing with 4 mL of water, methanol, and dichloromethane each and allowed to dry overnight. Full strands of each specimen were cut into pieces (<1 cm) using scissors then pulverized for 7 minutes with 7-8 steel balls (2.3 mm diameter). Pulverized hair specimens (25 ± 0.2 mg) were weighed into glass tubes. Methanol (1mL) was added to each specimen and the tubes were placed on a horizontal shaker overnight. After centrifugation, the supernatant was poured into a separate glass tube, evaporated to dryness with nitrogen, and reconstituted with 2% formic acid (1 mL). Specimen clean-up occurred via solid-phase extraction (Biotage Evolute Express CX) using 2% formic acid and methanol and eluted in methanol/ammonium hydroxide (95/5 v/v). Finally, the eluent was evaporated to dryness, reconstituted in ammonium formate (0.1% formic acid) and methanol (0.1% formic acid), and analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS).

Specimen analysis was performed on an Agilent 6490 triple quadrupole mass spectrometer with an electrospray source coupled to an Agilent 1290 high-performance LC system. The LC-MS/MS method was validated following the ANSI/ASB standard 036 validation protocol. Parameters validated included bias, precision, calibration model, limit of detection (LOD), lower limit of quantification (LLOQ), upper limit of quantification (ULOQ), carryover, ion suppression, and interference. The full list of analytes included in the LC-MS/MS method is shown in Table 1 and details of the method are in Appendix B.

Table 1: Fentanyl-related compounds, other compounds, and internal standards included in RTI's LC-MS/MS method

Fentanyl and Fentanyl-Related Compounds and Internal Standards	Opioids and Other Compounds and Internal Standards
4-ANPP (4-ANPP-d5)	6-Acetylmorphine (6-Acetylmorphine-d6)
para-Fluoro 4-ANPP	Benzoyllecgonine (Benzoyllecgonine-d3)
Fentanyl (Fentanyl-d5)	Buprenorphine
Acetyl fentanyl (Acetyl fentanyl-d5)	Cocaine (Cocaine-d3)
Acetyl norfentanyl (Acetyl norfentanyl-d5)	Codeine (Codeine-d3)
Benzylfentanyl	Hydrocodone (Hydrocodone-d6)
Carfentanil (Carfentanil-d5)	Methamphetamine (Methamphetamine-d9)
Chlorofentanyl	Methadone (Methadone-d9)
para-Fluorobutyryl fentanyl	Morphine (Morphine-d6)
4-Fluoroisobutyryl fentanyl	Oxycodone (Oxycodone-d6)
Fluorofentanyl	Oxymorphone (Oxymorphone-d3)
Norfentanyl (Norfentanyl-d5)	Tramadol
Phenyl fentanyl (Phenyl fentanyl-d5)	Xylazine
Phenethyl 4-ANPP	
Valeryl fentanyl (Valeryl fentanyl-d5)	

Specimens positive for fentanyl or a fentanyl-related compound were further analyzed by high resolution mass spectrometry using a LC-quadrupole-Time-of-Flight mass spectrometer (LC-QTOF-MS). The LC-QTOF-MS was a Waters G2-XS coupled to an Acquity I-Class LC. In addition to the compounds in Table 1, the LC-QTOF-MS screening method included amphetamine, bromazolam, carisoprodol, cyclobenzaprine, EDDP, etizolam, eutylone, flualprazolam, flubromazepam, flubromazolam, gabapentin, hydromorphone, levamisole,

lorazepam, LSD, MDA, MDMA, MDMB-4en-PINACA butanoic acid, meperidine, meprobamate, mitragynine, naloxone, norbuprenorphine, nordiazepam, norketamine, noroxycodone, O-desmethyltramadol, oxazepam, PCP, pregabalin, psilocin, speciociliantine, temazepam, zolpidem carboxylic acid, and zopiclone.

In Phase II of this project we received 6 years of historical COMDT test results in oral fluid and hair covering a time period of February 1, 2016 through February 28, 2022 from a commercial toxicology laboratory. Oral fluid data consisted of screening results for all specimens tested during that time period, and confirmation results for the selected specimens that underwent confirmation testing. Not all oral fluid specimens that screened positive were sent to confirmation due to differing program requirements and administrative reasons. Hair data consisted of confirmation results for all hair specimens, as the testing protocol was to send all specimens directly to confirmation testing without a screening step. The routine confirmation testing panel for hair included amphetamines, benzodiazepines, cocaine, opiates, PCP, and THC; the routine confirmation testing panel for oral fluid contained all of the hair analytes as well as buprenorphine, fentanyl, methadone, pregabalin, tapentadol, and tramadol.

The data were processed and analyzed using Stata and SAS statistical software packages. Prior to analysis, certain data cleaning activities were required. In some instances, results stemmed from multiple tests emanating from the same divided sample. To avoid double-counting, these records were identified, and duplicate records were removed from the final analysis dataset. Reported drugs were categorized by the drug classes presented in Table 2.

Table 2: Drug classes and reported drugs

Drug Classes	Reported Drugs
Amphetamines	Amphetamine, Amphetamines, MDA, MDEA, MDMA, and Methamphetamine

Benzodiazepines	Alprazolam, Benzodiazepine, Clonazepam, Diazepam, Flunitrazepam, Flurazepam, Lorazepam, Nordiazepam, Oxazepam, and Temazepam
Buprenorphine	Buprenorphine and Norbuprenorphine
Cocaine	Benzoyllecgonine, Cocaethylene, and Cocaine
Fentanyl	Fentanyl and Norfentanyl
Methadone	EDDP and Methadone
Opioids	6-MAM, Codeine, Hydrocodone, Hydromorphone, Morphine, Opiates, Oxycodone, and Oxymorphone
PCP	PCP and Phencyclidine
THC	Carboxy-THC, THC, and THC-COOH

Test results were additionally identified by the court-ordered drug testing program in which the individual was enrolled. Based on this data, records were combined into three program types: Problem-Solving Courts (e.g., “Co-Occurring Court,” “DWI Court,” “Juvenile Court,” “Veteran’s Court”), Family Court, and Probation/Parole/Pretrial. A current age measure was constructed from the reported date of birth of the participant and reported collection date. Across all confirmed records, records with unknown information on program type and age were 1.7% and 0.5%, respectively. A race determination was reported with different terminology across collection sites but were classifiable into the seven chosen race categories: White, Black, Native American, Asian, Hispanic, Other, and Unknown. The “Other” race category includes reported race values of “bi-racial,” “mixed,” “multiracial,” “o,” and “other.” Approximately one-fifth of the confirmation records were classified as an “Unknown” race. Due to the small levels of missingness for program type and age, records with unknown information were simply excluded for any analyses by those characteristics, while the unknown race was reported as its own category.

Summary of Results

Phase I

Appendix C shows the LOD, LLOQ, and ULOQ results for the analytes in Table 1. The overall positivity rate for the 520 hair specimens analyzed was 54%. The positivity rate for medium or tightly curled hair was higher (62%, n=63) than the positivity rate for straight or wavy hair (53%, n=455). The positivity rate for long or medium hair was higher (56%, n=351) than the positivity rate for short or very short hair (49%, n=168). Testing at RTI included full strands of hair, so hair of long or medium length represents a longer window of detection than hair that was short or very short. In this study hair that appeared have a natural color that was light blonde had a higher positivity rate (59% n=58) than hair that appeared to have a natural color that was medium (52% n=251), or dark (53%, n=180). Hair specimens with obvious non-natural hair colors, however, had a much higher positivity rate of 74%, n=31. Using Fisher's exact test only the higher positivity rate for non-natural vs. natural hair colors is statistically significant ($p < 0.05$). However, if the correlation holds for curly vs. straight, the effect would become significant at double the sample size ($p < 0.05$).

Table 3 shows the number of specimens in which fentanyl, fentanyl-related, and other compounds were detected (identified or quantified) during this study. Methamphetamine was the most commonly detected compound, followed by oxycodone. Fentanyl was the most commonly detected fentanyl-related compound followed by 4-ANPP. Benzylfentanyl, chlorofentanyl, phenyl fentanyl, 4-fluoroisobutyryl fentanyl, and valeryl fentanyl were not detected in any of the specimens. Acetyl norfentanyl and carfentanil were detected in one specimen each.

Table 3: Compounds detected in 520 hair specimens analyzed at RTI

Compound	# of Positive Specimens
Fentanyl and Fentanyl-related Compounds	
Benzylfentanyl	0
Chlorofentanyl	0

Phenyl fentanyl	0
4-Fluoroisobutyryl fentanyl	0
Valeryl fentanyl	0
Acetyl norfentanyl	1
Carfentanil	1
Acetyl fentanyl	6
Phenethyl-4-ANPP	6
para-Fluoro-4ANPP	8
Fluorofentanyl	17
Norfentanyl	25
4-ANPP	36
Fentanyl	77
Other Compounds	
Oxymorphone	8
Xylazine	11
Methadone	15
Morphine	18
Buprenorphine	19
Codeine	25
Tramadol	28
6-Acetylmorphine	40
Benzoylcegonine	50
Cocaine	63
Hydrocodone	67
Oxycodone	79
Methamphetamine	138

Of the 77 hair specimens that were positive for fentanyl, 36 also contained at least one fentanyl-related compound. An additional 3 specimens contained a fentanyl-related compound, but no fentanyl was detected. Fifteen of the 80 specimens for which fentanyl or a fentanyl-related compound was detected had no other analytes detected, including non-fentanyl-related compounds (e.g., morphine, 6AM, etc.). This means that these fifteen specimens (2.9%) would have been considered negative by current COMDT hair testing practices that do not include fentanyl or fentanyl-related compounds. For fentanyl in hair, the LLOQ was 5 pg/mg. For

samples with concentrations above the LLOQ, the maximum concentration was 9,245 pg/mg, average concentration was 383 pg/mg, and median concentration was 51 pg/mg.

The specimens that were positive for fentanyl or a fentanyl-related compound were further screened by high resolution mass spectrometry. Specimens were screened using an in-house library developed using reference standards on the Waters Connect software. Processed data were filtered such that only those specimens that included at least one fragment ion, mass error within ± 5 mDa, and met chromatographic parameters were considered screened positive.

Table 4: Compounds detected during screening of specimens by LC-QTOF-MS

Compound	# of Specimens Screened Positive
Fentanyl and Fentanyl-related Compounds	
Acetyl fentanyl	2
Fluorofentanyl	3
Norfentanyl	3
4-ANPP	3
Fentanyl	24
Other Compounds	
Hydromorphone	1
7-aminoclonazepam	1
Norbuprenorphine	1
Meperidine	1
Mitragynine	2
Speciociliane	2
Xylazine	2
Codeine	2
Levamisole	3
Noroxycodone	3
Morphine	4
EDDP	4
Amphetamine	6
Methadone	8
Tramadol	8
6-Acetylmorphine	8
Benzoylcegonine	20

Cocaine	24
Hydrocodone	5
Oxycodone	7
Methamphetamine	38

Phase II

RTI received test results from a commercial toxicology laboratory for 959,237 oral fluid and 62,528 hair specimens that were analyzed for misused substances representing a random, national COMDT sampling from 2016 to 2022. The overall screening positivity rate in oral fluid was 34%, whereas the overall hair positivity rate was 57%. These COMDT positivity rates are up to 5 times higher than oral fluid and hair in a U.S. workforce population during a similar time period (10.0% and 10.4%, respectively) as published in the Quest Drug Testing Index (Quest Diagnostics, 2018).

The commercial toxicology laboratory completed confirmation testing on 96,826 oral fluid specimens and all hair specimens. A summary of testing outcomes by program type, age group, gender, and race is shown in Table 5.

Table 5: Testing outcomes for oral fluid and hair confirmations

	Oral Fluid Confirmations				Hair Confirmations			
	Positive		Negative		Positive		Negative	
	Total	Percent	Total	Percent	Total	Percent	Total	Percent
Total	69,073	100.00	27,753	100.00	35,486	100.00	27,042	100.00
Program Type								
Problem Solving Courts	8,912	12.90	4,150	14.95	1,842	5.19	2,318	8.57
Family Court	57,178	82.78	15,584	56.15	30,807	86.81	21,524	79.59
Probation/Parole/Pretrial	2,983	4.32	8,019	28.89	2,837	7.99	3,200	11.83
Age Group								
Juvenile (10-17)	487	0.71	398	1.43	571	1.61	693	2.56
Adults (18+)	67,756	98.09	27,187	97.96	33,597	94.68	25,642	94.82
Ages <10 or unknown	830	1.20	168	0.61	1,318	3.71	707	2.61
Gender								
Unknown	0	0.00	0	0.00	1	0.00	0	0.00
Male	41,370	59.89	14,214	51.22	23,389	65.91	17,394	64.32
Female	27,703	40.11	13,539	48.78	12,096	34.09	9,648	35.68
Race								
White	40,375	58.45	16,670	60.07	20,868	58.81	17,376	64.26
Black	8,059	11.67	3,506	12.63	6,631	18.69	3,085	11.41
Native American	573	0.83	229	0.83	235	0.66	195	0.72
Asian	102	0.15	110	0.40	64	0.18	76	0.28
Hispanic	1,205	1.74	1,257	4.53	1,950	5.50	1,867	6.90
Other	1,112	1.61	372	1.34	180	0.51	123	0.45
Unknown	17,647	25.55	5,609	20.21	5,558	15.66	4,320	15.97

The top five most frequently identified drugs in confirmation testing were THC (64%), amphetamines, (26%), buprenorphine (15%), cocaine (7%), and fentanyl (6%) in oral fluid, and amphetamines (59%), cocaine (30%), THC (28%), opioids (27%) and benzodiazepines (3%) in hair, see Figure 1.

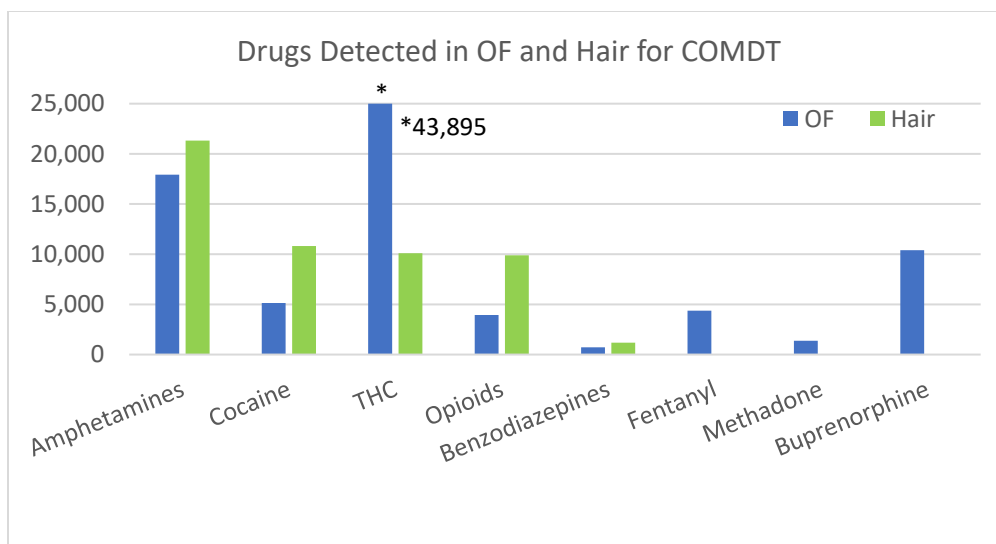


Figure 1: Most Common Drugs Detected in OF and Hair Confirmation Testing

Appendix D contains the most commonly identified drugs for each matrix by age and gender. In oral fluid testing juveniles had a higher positivity rate for THC while adults had higher positivity rates for amphetamines, buprenorphine, and fentanyl. In hair testing juveniles also had a higher positivity rate for THC, while adults had higher positivity rates for cocaine and opioids. Fentanyl was only tested for in oral fluid specimens. Of the 69,073 oral fluid specimens that confirmed positive for any drug, 4,367 (6.3%) were positive for fentanyl. The substances most often found in combination with fentanyl were amphetamines, THC, and cocaine. 1,492 specimens (2.2%) were positive only for fentanyl, no other drugs were found, meaning that these specimens would have been considered negative samples without the inclusion of fentanyl in the testing panel. The percent of positive tests that were positive only for fentanyl was 1.9% in 2020, 2.1% in 2021, and 3.3% in 2022, with the increase from 2021 to 2022 being statistically significant ($p < 0.01$). This is in good agreement with the results from RTI's in-house testing of hair specimens from Phase I (collected in late 2020 through early 2021) in which 2.9% of specimens were positive only for fentanyl or a fentanyl-related compound.

Applicability to Criminal Justice

This project provides actionable information from numerous, geographically diverse U.S. jurisdictions. To the best of our knowledge, it is the first large-scale drug prevalence study in a COMDT population and provides a view of drug combinations in this population, specifically fentanyl and fentanyl-related compounds in combination with other substances.

Many laboratories may not test for fentanyl or fentanyl-related compounds depending on the type of case. Looking at a cohort of individuals in the criminal justice system, RTI International was able to provide the prevalence of fentanyl and fentanyl-related compounds in this subset. In both the 6-year historical oral fluid dataset and the hair specimens tested at RTI, the positivity rate for specimens containing fentanyl or a fentanyl-related compound and no other substances was around 3%. These results will provide the policy makers, drug testing laboratories, and other stakeholders with data on which to base decisions as to whether to add fentanyl and fentanyl-related compounds to their routine testing batteries for drug testing within the criminal justice system. This research can also advance use of hair testing for fentanyl-related compounds by confirming the fentanyl-related compounds' presence and detection in this matrix and opens the possibility of additional research to further address both the detection of unique fentanyl-related compound metabolites in hair and the potential of external contamination, which are beyond the scope of this project.

Narcotic analgesics are analyzed in cases of drug-facilitated crimes such as in suspected sexual assaults. Often, victims do not report to the police within 72 hours, and the delay reduces the chance of detecting the presence of drugs in the victim's urine specimens. Knowledge of

drug exposure during the timing of a criminal act could be used as evidence in the investigation and prosecution of the offense. Using hair testing more routinely to identify fentanyl and fentanyl-related compounds could potentially increase this window of detection in suspected drug-facilitated sexual assault cases.

Products

Scholarly Products

A manuscript intended for publication in a peer-reviewed journal is currently under development. This project produced 6-year historical data for hair and oral fluid drug testing and targeted LC-MS/MS testing for a selection of drugs of interest in 6 months of hair specimens previously collected for COMDT. The historical testing data and results from 6 months of hair specimens have been archived at the National Archive of Criminal Justice Data (NACJD) for this award (2019-R2-CX-0017).

Dissemination Activities

Results from this project were presented in an oral presentation at the 2023 SOFT Annual Meeting and have been accepted for a poster presentation at the 2024 AAFS Annual Meeting as well as an oral presentation at the NIJ R&D Symposium at the 2024 AAFS Annual Meeting. In addition, ongoing progress and results of this project have been shared informally with SAMHSA's drug testing advisory board and the National Laboratory Certification Program.

Presentation Completed:

Prevalence of substance use in US court-ordered mandatory drug testing of oral fluid and hair. Jeri D. Roper-Miller, Megan Grabenauer, Nicholas J. Richardson, David C. Heller. Oral presentation at SOFT October 2023 in Denver, CO.

Abstracts Accepted:

Drug Positivity Rates in a Court-Ordered Mandatory Drug Testing Population. Katherine Bollinger, Megan Grabenauer, Nichole D. Bynum, Jeri Roper-Miller, Nicholas J. Richardson, David C. Heller. Oral presentation accepted for the NIJ R&D symposium at AAFS February 2024 in Denver, CO.

Retrospective analysis of results from US court-ordered mandatory drug testing of oral fluid and hair and the impacts of expanding the testing scope to include fentanyl and fentanyl related compounds. Jeri D. Roper-Miller, Megan Grabenauer, Nichole D. Bynum, Nicholas J. Richardson, David C. Heller. Poster presentation accepted at AAFS February 2024 in Denver, CO.

Reference

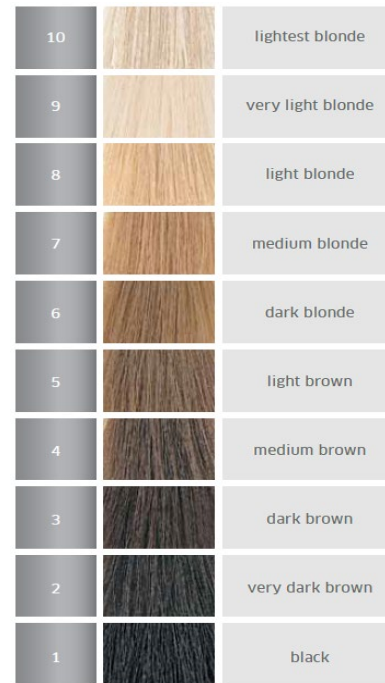
Quest Diagnostics. Drug Testing Index, Spring 2018. [QuestDiagnostics.com/DTI](https://www.questdiagnostics.com/DTI)

Appendix A

Hair Classification Descriptions

Color (1-9)

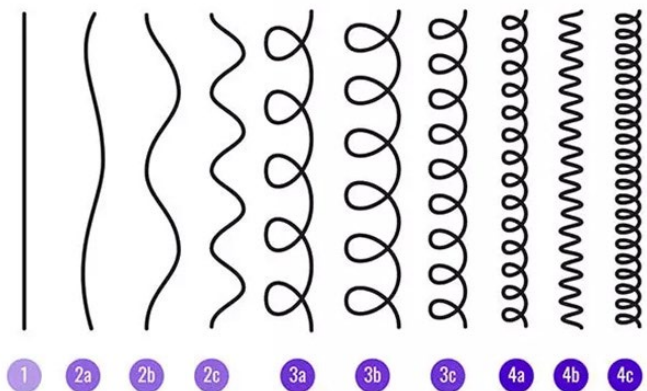
	Detailed Classification	Project Classification
1	Black	1 - Black
2	Very dark brown	2 Med to Dark Brown
3	Dark brown	
4	Medium brown	
5	Light brown	3 Light Brown
6	Dark blonde	4 Med to Dark Blonde
7	Medium blonde	
8	Light blonde	5 Light Blonde
9	Very light blonde	
10	Lightest blond	
11	Grey or white	6 Grey or white
12	Salt and Pepper	7 Salt and Pepper
13	Red	8 Red
14	Non-natural color	9 Non-natural color



Curl (S,W,M,T)

Hair Curl Type	Project Classification
1	S - Straight
2a	W - Wavy
2b	
2c	
3a	M - Med curl
3b	
3c	
4a	T - Tight curl
4b	
4c	

HAIR TYPES



Length

Length	Project Classification
0-1 inch	Very short
1-3 inches	Short
3-8 inches	Med
Over 8 inches	Long

Appendix B

LC-MS/MS Method

All data were acquired with fragmentation voltage of 380 V and cell acceleration voltage of 4 V. Chromatography was completed using an Agilent Poroshell 120 SB-C18 column (2.1 × 100 mm, 2.7 μm). Mobile phase A consisted of 5 mM ammonium formate in water with 0.1% formic acid, mobile phase B consisted of methanol with 0.1% formic acid. The injection volume was 20 μL, and the flow rate was 0.40 mL/min. The mobile phase composition was initially 5% B and increased to 25% A over 3 min. After holding for 0.5 min, mobile phase B increased to 50%, held for 0.5 min, then increased to 70%. After holding for 1 min the composition returned to 95% A and equilibrated for 2.5 min. The assay monitored two transitions for each analyte, except for para-fluoro-4ANPP and internal standards. Ion transitions are summarized in Table B1.

Table B1- Ion transitions for all analytes and internal standards

Compound Name	Precursor Ion	Product Ion	Collision Energy (V)
Acetyl fentanyl	323.21	188.1	25
Acetyl fentanyl	323.21	105.1	41
Acetyl fentanyl-d5	328.2	105	49
6-Acetylmorphine	328.16	211.1	40
6-Acetylmorphine	328.16	165.2	45
6-Acetylmorphine-d6	334.19	165	49
Acetyl norfentanyl	219.1	84	21
Acetyl norfentanyl	219.1	55.1	45
Acetyl norfentanyl-d5	224.1	84	21
4-ANPP	281.2	188.2	17
4-ANPP	281.2	105.2	33
4-ANPP-d5	286.2	105.2	33
Benzoylcegonine	290.14	168.2	17
Benzoylcegonine	290.14	105	33
Benzoylcegonine-d8	298.19	171.1	21
Benzylfentanyl	323.21	174.1	25
Benzylfentanyl	323.21	91.1	53
Buprenorphine	468.31	396.1	49
Buprenorphine	468.31	55.1	60
Carfentanyl	395.24	335.3	17
Carfentanyl	395.24	113	41
Carfentanyl-d5	400.2	113	41
Chlorofentanyl	371.19	188.1	25
Chlorofentanyl	371.19	105.2	60
Cocaine	304.16	182.2	21
Cocaine	304.16	82	37

Cocaine-d3	307.18	185.1	21
Codeine	300.16	165	49
Codeine	300.16	152.2	60
Codeine-d6	306.2	165	57
Fentanyl	337.23	187.9	25
Fentanyl	337.23	105.1	49
Fentanyl-d5	342.26	188.1	21
Fluorofentanyl	355.22	188.1	29
Fluorofentanyl	355.22	105.1	49
4-Fluoroisobutyryl fentanyl	369.24	188.1	25
4-Fluoroisobutyryl fentanyl	369.24	105.1	53
Hydrocodone	300.16	199.1	30
Hydrocodone	300.16	128.1	70
Hydrocodone-d6	306.2	202.1	33
Methadone	310.22	265.2	13
Methadone	310.22	105	25
Methadone-d9	319.1	105	25
Methamphetamine	150.13	119.1	9
Methamphetamine	150.13	91	21
Methamphetamine-d9	159.19	93.1	21
Morphine	286.15	165.1	45
Morphine	286.15	151.9	68
Morphine-d6	292.18	181.1	49
Norfentanyl	233.17	84.1	21
Norfentanyl	233.17	55.1	45
Norfentanyl-d5	238.2	84.1	21
Oxycodone	316.16	298.2	17
Oxycodone	316.16	256.2	29
Oxycodone d6	322.19	304.2	21
Oxymorphone	302.14	284.1	14
Oxymorphone	302.14	227	30
Oxymorphone-d3	305.2	287.1	14
para-fluoro-4ANPP	299.2	105.1	41
Phenethyl 4-ANPP	385.27	105.1	52
Phenethyl 4-ANPP	385.27	187.9	21
Phenyl fentanyl-d5	390.2	105.1	49
Phenyl fentanyl	385.23	188.1	25
Phenyl fentanyl	385.23	105	45
Tramadol	264.2	58.2	29
Tramadol	264.2	42	60
Valeryl fentanyl	365.26	188.1	29

Valeryl fentanyl	365.26	105	49
Valeryl fentanyl-d5	370.2	188.1	29
Xylazine	221.1	164.1	31
Xylazine	221.1	89.9	25

Appendix C

LOD, LLOQ, and ULOQ results of fentanyl-related compounds and other compounds.

Compound	ULOQ (pg/mg)	LOD (pg/mg)	LLOQ (pg/mg)
Morphine	60,000	15	25
Oxymorphone	60,000	3	5
Codeine	60,000	5	5
Oxycodone	60,000	5	5
Hydrocodone	60,000	3	5
6-Acetylmorphine	10,000	5	5
Methamphetamine	60,000	500	2,000
Benzoylcegonine	60,000	100	2,000
Xylazine	60,000	15	2,000
Tramadol	60,000	100	2,000
Cocaine	60,000	100	100
Methadone	60,000	100	100
Buprenorphine	NA	5	Qualitative
Acetyl norfentanyl	5-2,000	1	5
norfentanyl	5-2,000	5	5
Acetyl fentanyl	5-2,000	5	5
4-ANPP	5-2,000	1	5
para-Fluoro-4ANPP*	NA	1	Qualitative
Benzylfentanyl	5-2000	1	5
Fentanyl	5-2,000	1	5
Fluorofentanyl	1-2,000	1	1
Carfentanil	1-2,000	1	1
Phenyl fentanyl	1-2,000	1	1
4-Fluoroisobutyryl fentanyl	5-2,000	1	5
Chlorofentanyl	5-2,000	5	5
Valeryl fentanyl	5-2,000	1	5

*monitored for presence of peaks only

Appendix D

Most Common Drugs Detected in Oral Fluid Confirmation Testing: Adults vs. Juveniles

Adults (Ages 18 or Older)			Adults Males (Ages 18+)			Adult Females (Ages 18+)		
Drug	Total	Percent of Positive Tests	Drug	Total	Percent of Positive Tests	Drug	Total	Percent of Positive Tests
THC	42,886	63.29	THC	25,038	61.40	THC	17,848	66.16
Amphetamines	17,638	26.03	Amphetamines	11,260	27.61	Amphetamines	6,377	23.64
Cocaine	5,030	7.42	Buprenorphine	6,968	17.09	Buprenorphine	3,361	12.46
Buprenorphine	10,329	15.24	Cocaine	2,979	7.31	Cocaine	2,051	7.60
Opioids	3,895	5.75	Fentanyl	2,667	6.54	Fentanyl	1,656	6.14
Fentanyl	4,323	6.38	Opioids	2,350	5.76	Opioids	1,545	5.73
Benzodiazepines	713	1.05	Methadone	873	2.14	Methadone	499	1.85
PCP	22	0.03	Benzodiazepines	490	1.20	Benzodiazepines	223	0.83
Methadone	1,372	2.02	PCP	3	0.01	PCP	19	0.07

Juveniles (Ages 10 -17)			Juvenile Males (Ages 10 -17)			Juvenile Females (Ages 10 -17)		
Drug	Total	Percent of Positive Tests	Drug	Total	Percent of Positive Tests	Drug	Total	Percent of Positive Tests
THC	394	80.90	THC	153	85.00	THC	241	78.50
Amphetamines	83	17.04	Amphetamines	25	13.89	Amphetamines	58	18.89
Cocaine	30	6.16	Cocaine	9	5.00	Cocaine	21	6.84
Buprenorphine	2	0.41	Opioids	2	1.11	Opioids	3	0.98
Opioids	5	1.03	Buprenorphine	2	1.11	Fentanyl	0	0.00
Fentanyl	1	0.21	Fentanyl	1	0.56	PCP	0	0.00
Benzodiazepines	0	0.00	PCP	0	0.00	Benzodiazepines	0	0.00
PCP	0	0.00	Benzodiazepines	0	0.00	Methadone	0	0.00
Methadone	0	0.00	Methadone	0	0.00	Buprenorphine	0	0.00

Note: Percentages may not add to 100% due to polydrug use.

Most Common Drugs Detected in Hair Confirmation Testing: Adults vs. Juveniles

	Adults (Ages 18 or Older)			Adults Males (Ages 18+)			Adult Females (Ages 18+)	
Drug	Total	Percent of Positive Tests	Drug	Total	Percent of Positive Tests	Drug	Total	Percent of Positive Tests
Amphetamines	20,030	58.16	Amphetamines	12,818	57.19	Amphetamines	6,804	60.84
Cocaine	10,375	30.13	Cocaine	6,743	30.09	Cocaine	3,382	30.24
THC	9,494	27.57	Opioids	6,413	28.61	THC	3,001	26.84
Opioids	9,494	27.57	THC	6,222	27.76	Opioids	2,882	25.77
Benzodiazepines	1,172	3.40	Benzodiazepines	865	3.86	Benzodiazepines	305	2.73
PCP	30	0.09	PCP	19	0.08	PCP	9	0.08
Fentanyl	8	0.02	Fentanyl	5	0.02	Fentanyl	3	0.03
Methadone	6	0.02	Methadone	4	0.02	Buprenorphine	3	0.03
Buprenorphine	5	0.01	Buprenorphine	2	0.01	Methadone	2	0.02

	Juveniles (Ages 10 -17)			Juvenile Males (Ages 10 -17)			Juvenile Females (Ages 10 -17)	
Drug	Total	Percent of Positive Tests	Drug	Total	Percent of Positive Tests	Drug	Total	Percent of Positive Tests
Amphetamines	315	54.22	THC	146	48.99	Amphetamines	180	65.93
THC	261	44.92	Amphetamines	133	44.63	THC	107	39.19
Cocaine	133	22.89	Cocaine	72	24.16	Cocaine	59	21.61
Opioids	55	9.47	Opioids	32	10.74	Opioids	23	8.42
Benzodiazepines	2	0.34	PCP	1	0.34	Benzodiazepines	1	0.37
PCP	1	0.17	Benzodiazepines	1	0.34	Fentanyl	0	0.00
Fentanyl	0	0.00	Fentanyl	0	0.00	PCP	0	0.00
Methadone	0	0.00	Methadone	0	0.00	Methadone	0	0.00
Buprenorphine	0	0.00	Buprenorphine	0	0.00	Buprenorphine	0	0.00

Note: Percentages may not add to 100% due to polydrug use.