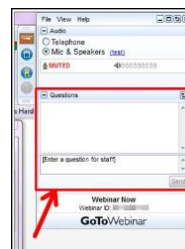




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Université Laval, Québec, Canada
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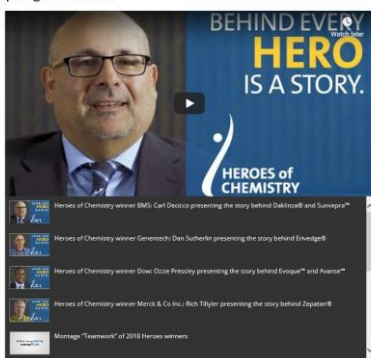
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7

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Inspiring Hero Stories



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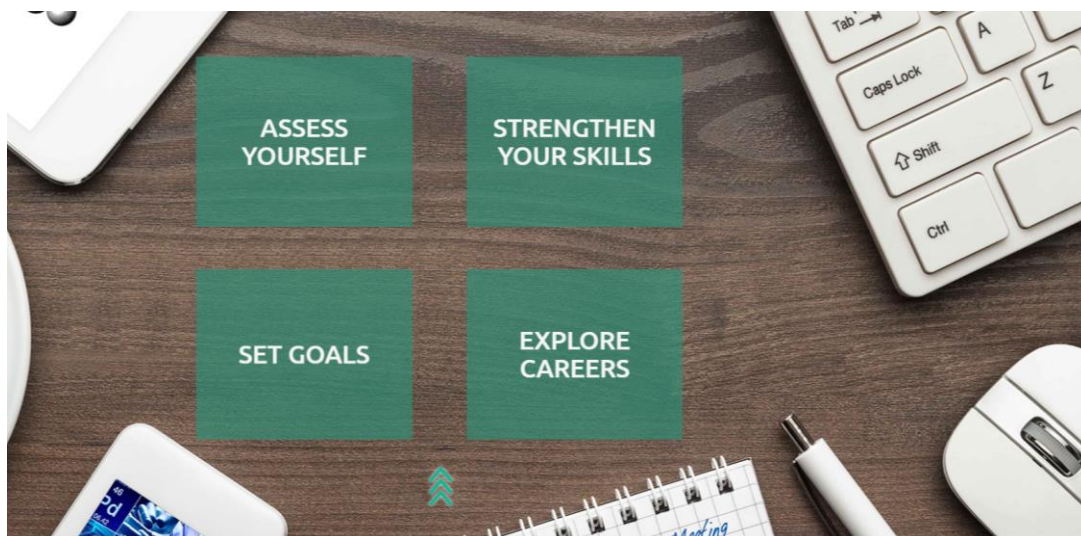
2018 Winners:



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La Dra. Luisa Whittaker-Brooks
Departamento de Química, Universidad de Utah

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11

SYNTHETIC OPIOIDS

How the CDC is Expanding National Capacity to Measure Human Exposure

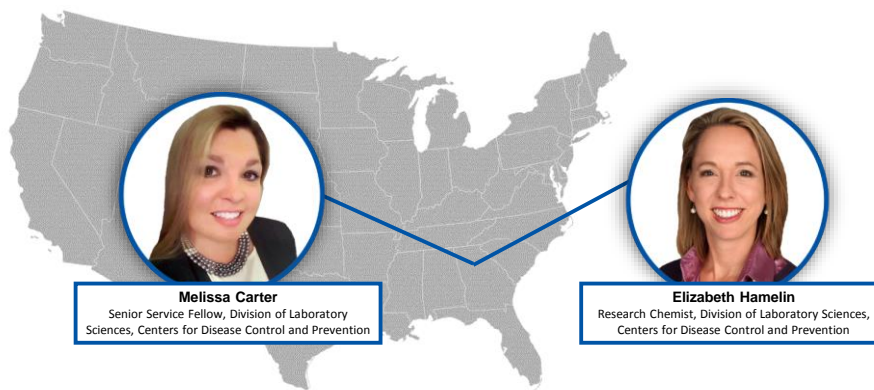
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Synthetic Opioids: How the CDC is Expanding National Capacity to Measure Human Exposure



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National Center for Environmental Health



Synthetic Opioids: How the CDC is Expanding National Capacity to Measure Human Exposure

Melissa Carter & Elizabeth Hamelin
Division of Laboratory Sciences
Centers for Disease Control and Prevention

14



Opioid Overdose Crisis

- ❑ Opioid deaths increased six-fold from 1999 to 2017

- ❑ U.S. Drug Enforcement Administration
 - Reported that fentanyl accounted for ~75% of opioid identifications
 - Identified over 20 different fentanyl in the first half of 2018



15

T O M Kits*

Introducing the Traceable Opioid Material* Kits (TOM Kits*)

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TOM Kits* Currently Available

- **Fentanyl Analog Screening Kit (FAS Kit)**
 - <https://www.caymanchem.com/for-ensics/faskit/>
 - Includes 200 µg each of 150 fentanyl analogs
 - Provides largest collection of available fentanyl analog reference materials
- **Opioid Certified Reference Material Kit (Opioid CRM Kit)**
 - <http://www.cerilliant.com/>
 - Includes 1 mg each of 22 opioids and their matched ¹³C and ¹⁵N isotopes
 - Addresses 100% of DEA fentanyl/fentanyl-related cases in the DEA 2019 Q1 Report

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17



TOM Kits* Design

- ❑ **CDC contracted the development of Traceable Opioid Material* Kits (TOM Kits*)**
 - ✓ To promote consistency across laboratories
 - ✓ To improve surveillance efforts
 - ✓ To provide precision and accuracy using isotopically-labeled internal standards
- ❑ **The 2019 DEA Mid-Year Emerging Threat and the 2018 NFLIS Mid-Year Reports are now available**

Opioid CRM Kit Compounds	2018 Product Design				2019 Product Launch			
	DEA		NFLIS		DEA		NFLIS	
	2017 4th Quarter	2016 Annual	2019 Mid-Year	2018 Mid-Year	2017 4th Quarter	2016 Annual	2019 Mid-Year	2018 Mid-Year
	"X" if Reported	No. Cases Reported	"X" if Reported	No. Cases Reported	"X" if Reported	No. Cases Reported	"X" if Reported	No. Cases Reported
(±)-beta-hydroxythiofentanyl	--	--	--	--	--	--	--	--
3-methyl fentanyl	X	1	X	427	--	--	--	--
4-ANPP	X	23	X	8	X	100	X	891
4'-methyl acetyl fentanyl	--	--	--	--	--	--	--	--
acetyl fentanyl	X	5	X	1,669	X	183	X	2,246
acryl fentanyl	X	3	X	26	X	3	--	--
benzyl fentanyl	--	--	--	--	X	3	--	--
butyryl fentanyl	X	3	X	93	X	10	--	--
carfentanil	X	7	X	1,251	X	21	X	661
cyclopropyl fentanyl	X	8	--	--	X	3	X	852
fentanyl	X	204	X	34,199	X	1,392	X	37,140
furanyl fentanyl	X	11	X	2,273	X	17	--	--
methoxyacetyl fentanyl	X	20	--	--	X	5	X	603
norcarfentanil	--	--	--	--	--	--	--	--
norfentanyl	--	--	--	--	--	--	--	--
para-fluorobutyryl fentanyl	X	6	X	148	X	8	X	876
para-fluorofentanyl	--	--	X	5	--	--	--	--
remifentanil	--	--	--	--	--	--	--	--
U-47700	X	10	--	--	X	22	--	--
U-48800	X	3	--	--	X	1	--	--
U-49900	X	4	--	--	--	--	--	--
valeryl fentanyl	--	--	X	52	X	12	--	--
^a COUNT	14	308	11	40,151	14	1,780	7	43,269
^b TOTAL	15	311	14	40,236	16	1,783	7	46,831
^c COVERAGE	93.3%	99.0%	78.6%	99.8%	87.5%	99.8%	100.0%	92.4%

^a COUNT is the sum of the column;
^b TOTAL is obtained from the respective DEA or NFLIS reports;
^c COVERAGE equals the (COUNT/TOTAL)x100

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18



TOM Kits* Eligibility

- **U.S. Laboratories**
 - All sectors: private, public and academic
- **Have a current DEA registration**
 - Comply with respective state and local regulations
- **Submit kit requests directly to the respective vendor**
- **Available to laboratories free-of-charge**



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19

Audience Survey Question

ANSWER THE QUESTION ON BLUE SCREEN IN ONE MOMENT



20

Have you heard of the TOM* Kits before this webinar?

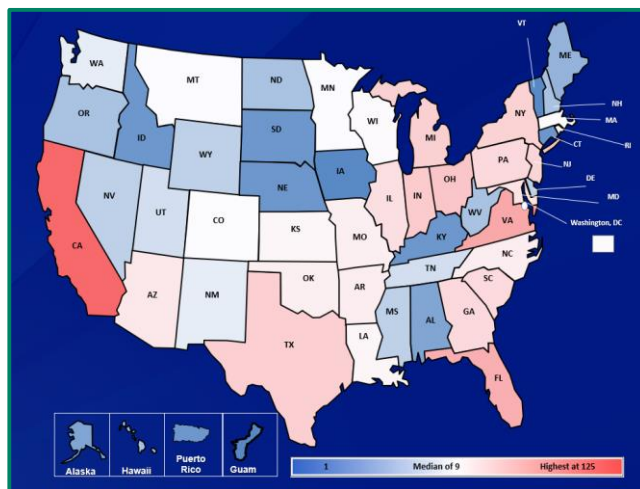
- Opioid CRM Kits only
- FAS kits only
- Both Opioids CRM and FAS kits
- None of the above

** If your answer differs greatly from the choices above tell us in the chat!*



TOM Kits* Requested as of September 11, 2019

- **FAS Kit**
 - 681 kits requested
 - 244 kits approved (Phase I)
 - 160 kits approved (Phase II)
 - 277 kits waitlisted (Phase III)
 - 344 kits shipped
- **Opioid CRM Kit**
 - 343 kits requested/approved
 - 223 kits shipped
- **1,024 TOM Kits* in total**
 - Across 50 states, 2 territories, and the District of Columbia



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21



TOM Kits* Future Projections

- **FAS Products**
 - Additional FY18 FAS Kit and FAS-V1 materials to fill waitlisted and new requests (150 compounds)
 - FAS V2 of 32 additional compounds
 - FAS V3 of 30 additional compounds
- **Opioid CRM Products**
 - Continuing distribution of FY18 Opioid CRM Kit materials
 - 22 native compounds and ¹³C and ¹⁵N isotopes
 - Opioid Polysubstance Mix Kit (OPM Kit)
 - 22 native compounds often detected in conjunction with fentanyl analogs and their deuterated isotopes

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22



Citing the Use of the TOM Kits*

Laboratories are asked to acknowledge the use of the Traceable Opioid Material* Kits in presentations, publications, reports, and other communications by using the following citation:

- ✓ “Laboratory findings were made possible, in part, by the Centers for Disease Control and Prevention’s design and support of Traceable Opioid Material* Kits. #tomkits *TRACEABLE OPIOID MATERIAL, TOM KITS, and the TOM KITS logo are marks of the U.S. Department of Health and Human Services”

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23



TOM Kits* Important Links and Resources

- **CDC’s Opioids Portal:**
 - <https://www.cdc.gov/opioids/index.html>
- **TOM Kits* CDC Website:**
 - https://www.cdc.gov/nceh/dls/erb_opioid_kits.html
- **Fentanyl Analog Screening Kit (FAS Kit)**
 - https://www.caymanchem.com/for_ensics/faskit/
- **Opioid Certified Reference Material Kit (Opioid CRM Kit)**
 - <http://www.cerilliant.com/>

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24

Laboratory Detection of Synthetic Opioids



Laboratory Testing Needs



- Immunoassay
- Receptor Assay
- GC, LC
- GC-MS, GC-MS/MS
- LC-MS, LC-MS/MS
- GC-, LC-HRMS

Diagnose & treat
Identify illegal substances
Determine cause of death
Monitor use



Targeted Mass Spectrometry Method

- Identify the synthetic opioids most commonly used
- Use the Opioid CRM Kit: 22 compounds with matched internal standards
- Our method was designed to:
 - Support surveillance in clinical samples
 - Readily incorporate new analogs as needed
 - Accommodate multiple clinical matrices

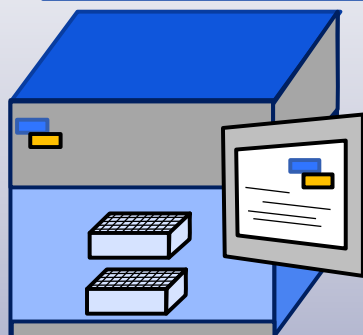
Goal: Develop a method to identify the most prevalent synthetic opioids in use

27



Sample Preparation

Supported Liquid Extraction



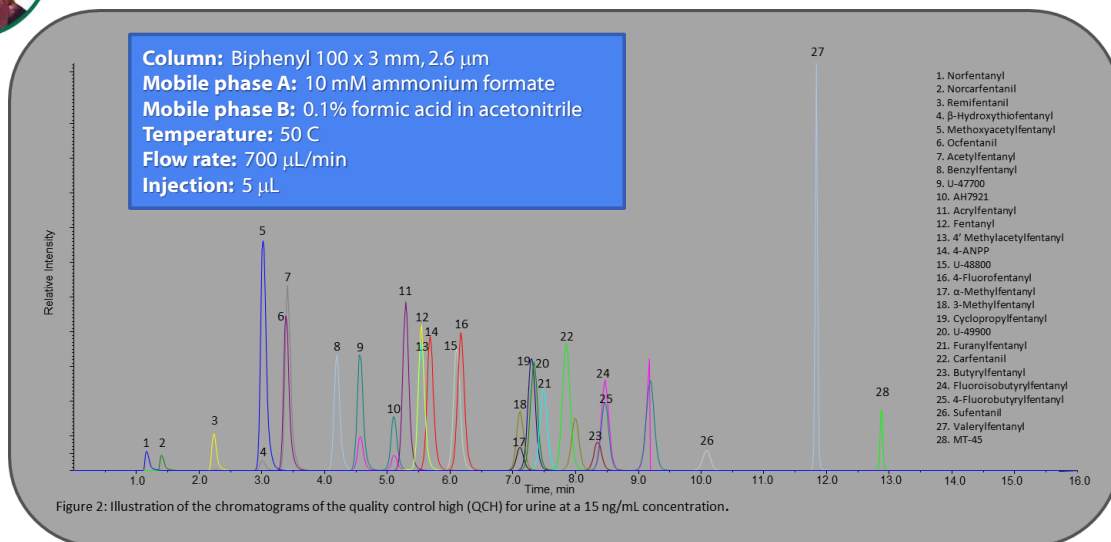
- Load: 200 μ L of sample + 200 μ L diluent
- Elute: 2 x 900 μ L dichloromethane
- Dry and reconstitute: 100 μ L of mobile phase

- Designed for fentanyl analogs
- Applicable for urine and plasma samples

28



Chromatography



29



Mass Spectrometry

- Two transitions per compound
- One transition per internal standard
- Sensitivity from 0.025 - 0.75 ng/mL
- Quality Controls established at
 - 150x LOD (QCL)
 - 15 ng/mL (QCH)

Targeted Fentanyl	Urine		Plasma	
	LOD	QCL	LOD	QCL
β -Hydroxythiofentanyl	0.075	0.1125	0.500	0.7500
Methoxyacetyl fentanyl	0.025	0.0375	0.025	0.0375
Ocfentanil	0.025	0.0375	0.025	0.0375
Acetyl fentanyl	0.025	0.0375	0.025	0.0375
U-47700	0.050	0.0750	0.025	0.0375
AH7921	0.025	0.0375	0.025	0.0375
Acrylfentanyl	0.025	0.0375	0.025	0.0375
Fentanyl	0.050	0.0750	0.025	0.0375
4-Fluorofentanyl	0.025	0.0375	0.050	0.0750
α -Methylfentanyl	0.250	0.3750	0.500	0.7500
Cyclopropylfentanyl	0.050	0.0750	0.050	0.0750
Furanylfentanyl	0.025	0.0375	0.025	0.0375
Carfentanil	0.050	0.0750	0.025	0.0375
β -Methylfentanyl	0.075	0.1125	0.050	0.0750
Butyrylfentanyl	0.050	0.0750	0.100	0.1500
Fluoroisobutyrylfentanyl	0.050	0.0750	0.050	0.0750
Sufentanil	0.075	0.1125	0.100	0.1500
MT-45	0.075	0.1125	0.075	0.1125
Valerylfentanyl	0.025	0.0375	0.025	0.0375
4-Fluorobutyrylfentanyl	0.025	0.0375	0.025	0.0375
Norfentanyl	0.025	0.0375	0.100	0.1500
Norcarfentanil	0.025	0.0375	0.100	0.1500
U-48800	0.050	0.0750	0.050	0.0750
U-49900	0.025	0.0375	0.025	0.0375
4-ANPP	0.100	0.1500	0.050	0.0750
Benzylfentanyl	0.025	0.0375	0.025	0.0375
4' methylacetyl fentanyl	0.025	0.0375	0.025	0.0375
Remifentanil	0.025	0.0375	0.025	0.0375

30



Method Performance

- Blinded plate spiked above and below reportable limit
- All analytes were detected above reportable limit
- 94% of analytes were detected 50% below reportable limit
- May be able to reduce reportable limit

Percent Analyte Detected at Various Spiking Levels				
Analyte	2x QC Low	1.5x QC Low	0.50x QC Low	0.25x QC Low
β -Hydroxythiofentanyl	100%	100%	100%	100%
Methoxyacetylfentanyl	100%	100%	100%	100%
Ocfentanil	100%	100%	100%	100%
Acetylfentanyl	100%	100%	100%	24%
U-47700	100%	100%	96%	40%
AH7921	100%	100%	96%	60%
Acrylfentanyl	100%	100%	100%	96%
Fentanyl	100%	100%	100%	100%
4-Fluorofentanyl	100%	100%	100%	100%
α -Methylfentanyl	100%	100%	100%	100%
Cyclopropylfentanyl	100%	100%	96%	32%
Furanylfentanyl	100%	100%	36%	0%
Carfentanil	100%	100%	100%	96%
3-Methylfentanyl	100%	100%	96%	16%
Butyrylfentanyl	100%	100%	100%	84%
Fluoroisobutyrylfentanyl	100%	100%	100%	64%
Sufentanil	100%	100%	100%	100%
MT45	100%	100%	100%	60%

31



Method Evaluation

Results from the 2019 College of American Pathologists Proficiency Test (CAP PT)

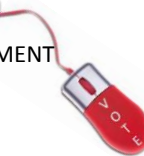
Analyte	Plasma MS/MS method
U-47700	Detected
4-Fluorofentanyl	Detected*
4-ANPP	Not measured‡
α -Methylfentanyl	Detected
Furanylfentanyl	Detected
Acetylfentanyl	Detected
Acrylfentanyl	Detected
Carfentanil	Detected

*RT differences confirm positional isomer with Fentanyl Analog Screening (FAS) Kit, ‡ Analyte was not in method at time of analysis.

32

Audience Survey Question

ANSWER THE QUESTION ON BLUE SCREEN IN ONE MOMENT



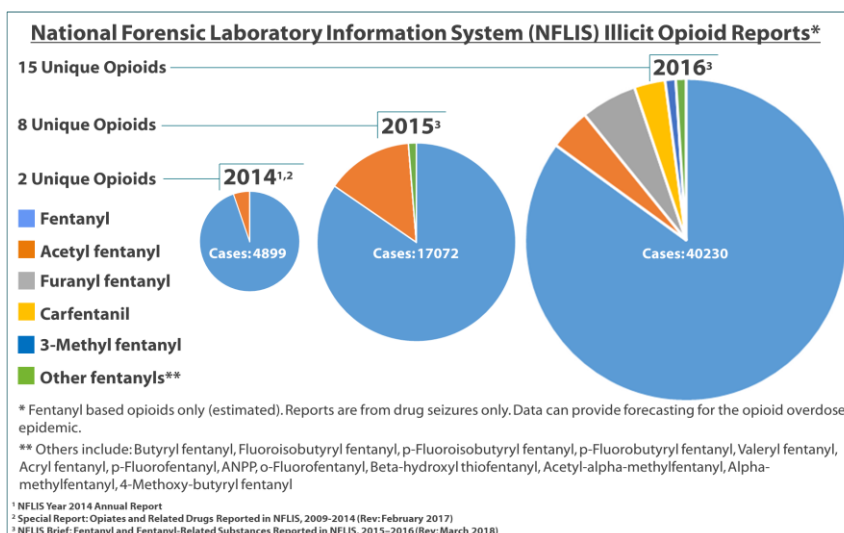
Do you analyze for synthetic opioids in your laboratory?

- With immunoassay
- With mass spectrometry
- With both immunoassay and mass spectrometry
- None of the above
- Question not applicable to me

** If your answer differs greatly from the choices above tell us in the chat!*



What about other synthetic opioids?



Slide by Mike Mojica



High Resolution Mass Spectrometry Method

- Identify as many synthetic opioids as possible
- Use the FAS Kit containing 150 compounds
- Develop database with library spectra
- Use untargeted data collection

Designed to

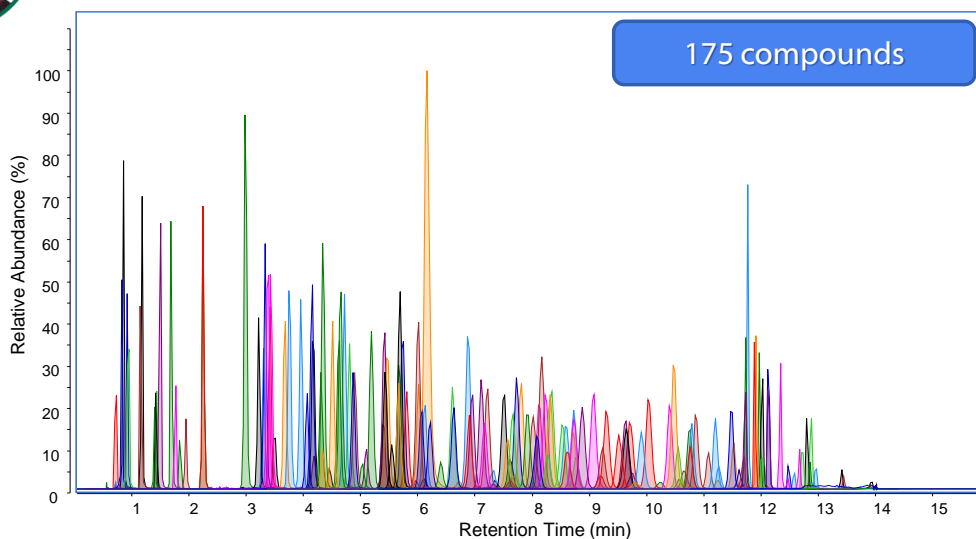
- Rapidly add new analogs to library and database
- Flexible for multiple matrices

Goal: Develop a method to identify emerging synthetic opioids using library matching

35

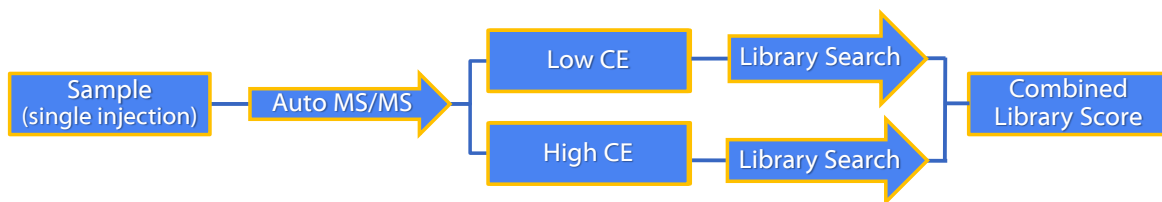


HRMS Synthetic Opioids Library





HRMS Data Collection

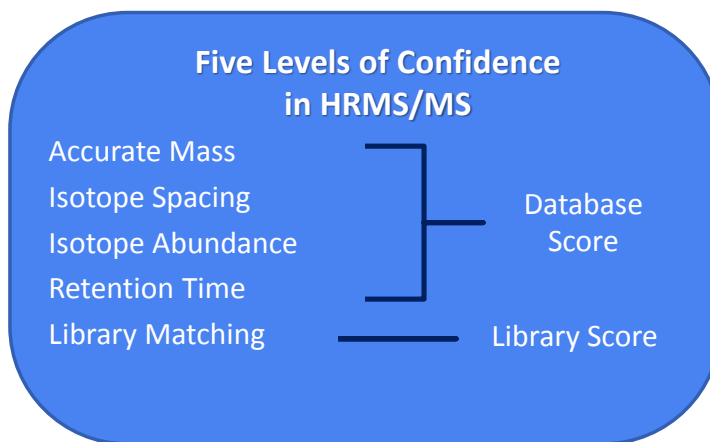


- In Auto MS/MS, compounds are isolated in an untargeted manner, based upon abundance
- Two collision energy (CE) levels allow for a combined library yielding increased confidence of a match

37



Positive Identification



38



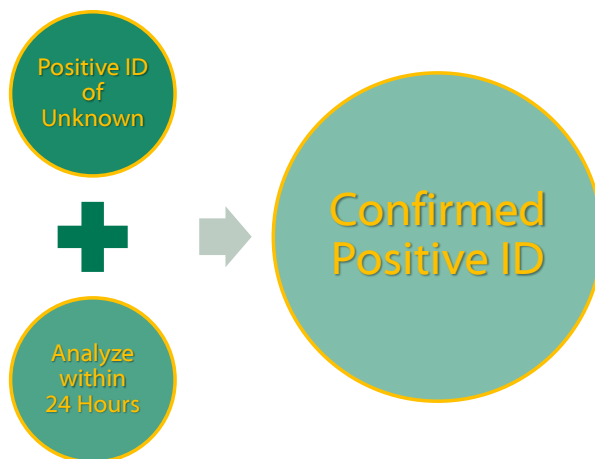
Confirmatory Standard Analysis

Fentanyl Analog Screening Kit

- Manufactured and distributed by Cayman Chemical
- Includes 200 micrograms each of 120+ fentanyl analog analytical reference materials
- Provides largest collection of available fentanyl analog reference materials



CDC

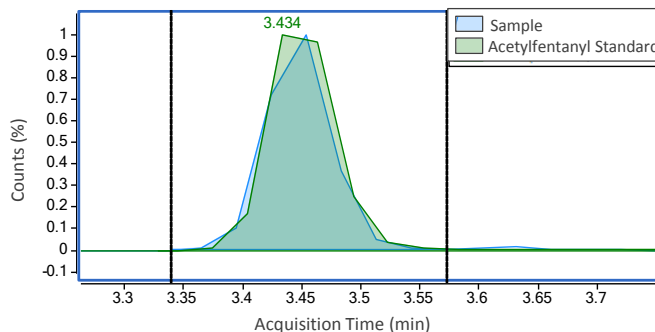
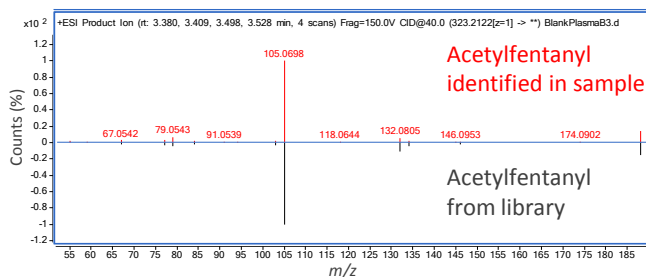


39



Method Application

- Acetylfentanyl identified in purchased sample
- Identified with
 - Database score of 98.00
 - Library score of 97.96
- Reference standard analysis confirmed identification



40



Method Performance

Urine Matrix	Positive Rate (%)					
	4 ng/mL (n=24)	3 ng/mL (n=25)	2 ng/mL (n=20)	1 ng/mL (n=17)	0.5 ng/mL (n=17)	0 ng/mL (n=50)
Acetylfentanyl	100%	100%	100%	100%	96%	0%
Carfentanil	100%	100%	100%	100%	100%	0%
Cyclopropylfentanyl	100%	100%	100%	100%	100%	0%
Fentanyl	100%	100%	100%	100%	100%	0%
Fluoroisobutyrylfentanyl	100%	100%	100%	100%	100%	0%
Furanylfentanyl	100%	100%	100%	100%	100%	0%
Methoxyacetylfentanyl	100%	100%	100%	100%	100%	0%
Not Optimized for Sample Prep/Chromatography						
4-ANPP	100%	100%	100%	68%	20%	0%
Naloxone	100%	100%	100%	96%	80%	0%
Norfentanyl	94%	100%	100%	44%	20%	0%

Plasma Matrix	Positive Rate (%)					
	4 ng/mL (n=25)	3 ng/mL (n=25)	2 ng/mL (n=20)	1 ng/mL (n=17)	0.5 ng/mL (n=17)	0 ng/mL (n=50)
Acetylfentanyl	100%	100%	100%	100%	100%	2%
Carfentanil	100%	100%	100%	100%	100%	0%
Cyclopropylfentanyl	100%	100%	100%	100%	64%	0%
Fentanyl	100%	100%	100%	100%	100%	2%
Fluoroisobutyrylfentanyl	100%	100%	100%	100%	100%	2%
Furanylfentanyl	100%	100%	100%	100%	96%	0%
Methoxyacetylfentanyl	100%	100%	100%	100%	100%	0%
Not Optimized for Sample Prep/Chromatography						
4-ANPP	94%	100%	100%	80%	4%	0%
Naloxone	100%	100%	100%	60%	12%	0%
Norfentanyl	82%	82%	95%	88%	56%	2%

Blinded samples spiked from 0.5 - 4 ng/mL to evaluate detection

41



Proficiency Testing

Results from the 2019 College of American Pathologists Proficiency Test (CAP PT)

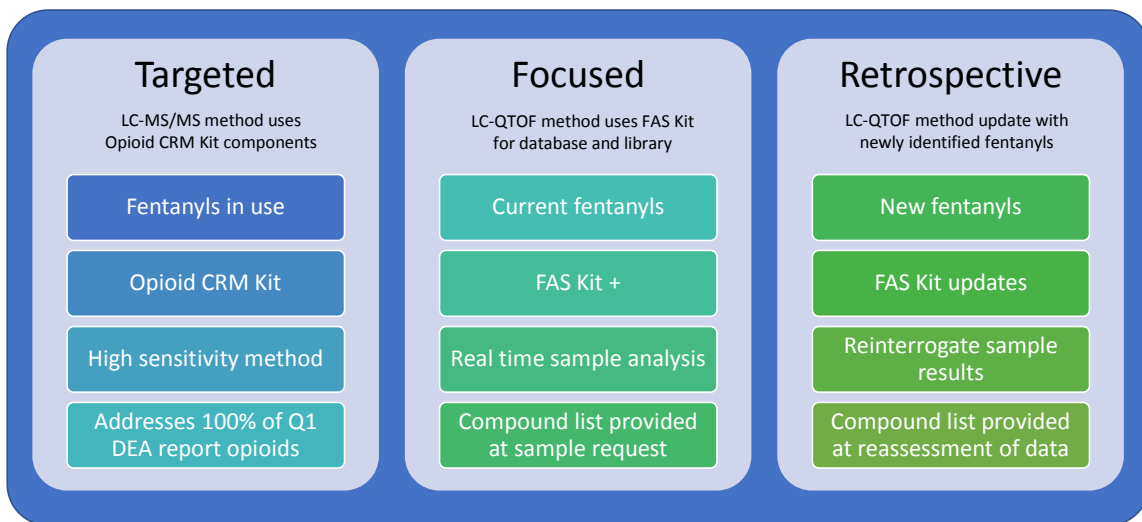
Analyte	Plasma MS/MS method	Plasma HRMS method
U-47700	Detected	Detected
4-Fluorofentanyl	Detected*	Detected
4-ANPP	Not measured‡	Detected
α-Methylfentanyl	Detected	Detected
Furanylfentanyl	Detected	Detected
Acetylfentanyl	Detected	Detected
Acrylfentanyl	Detected	Detected
Carfentanil	Detected	Detected

*RT differences confirm positional isomer with Fentanyl Analog Screening (FAS) Kit, ‡ Analyte was not in method at time of analysis.

42



Synthetic Opioids Detection



43

Key Messages

from today's ACS Webinar



Webinar Key Points

- ✓ More information and helpful links to support response to the opioid overdose crisis can be found on the CDC's Opioids Portal at:
 - <https://www.cdc.gov/opioids/index.html>
- ✓ Traceable Opioid Material* Kits (TOM Kits*) cover 100% of the compounds reported on the 2019 - 1st Quarter DEA Emerging Threat Report and are available, free-of-charge to all U.S. laboratories with a DEA registration. More information on how to request a kit can be found at:
 - https://www.cdc.gov/nceh/dls/erb_opioid_kits.html
- ✓ Laboratory detection of synthetic opioids can be improved and expanded using the TOM Kits* for mass spectrometric detection using both targeted and untargeted methods.

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45

For more information, contact NCEH
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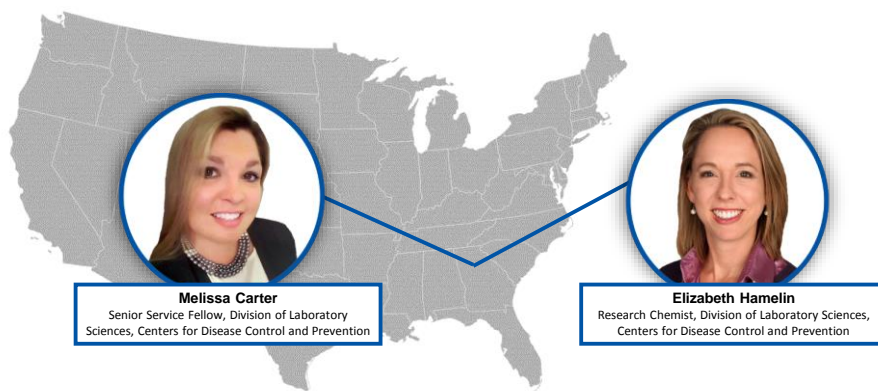

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54