ECHO

What Laboratory Testing Can/Can't Tell you and Updates from the Far Side

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+
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Learner Objectives

- The attendees will apply the following educational objectives
  - The technical differences used for drug screen (IA) testing
  - The technical differences between confirmation testing
  - Limitations of drug screen results and confirmation results
  - Apply skills needed to interpret complex cases involving Opiates including Fentanyl drug use
  - How to prepare for future changes in the landscape of drug use trends

Analytical Considerations Clinical/Forensic – Tiered Testing

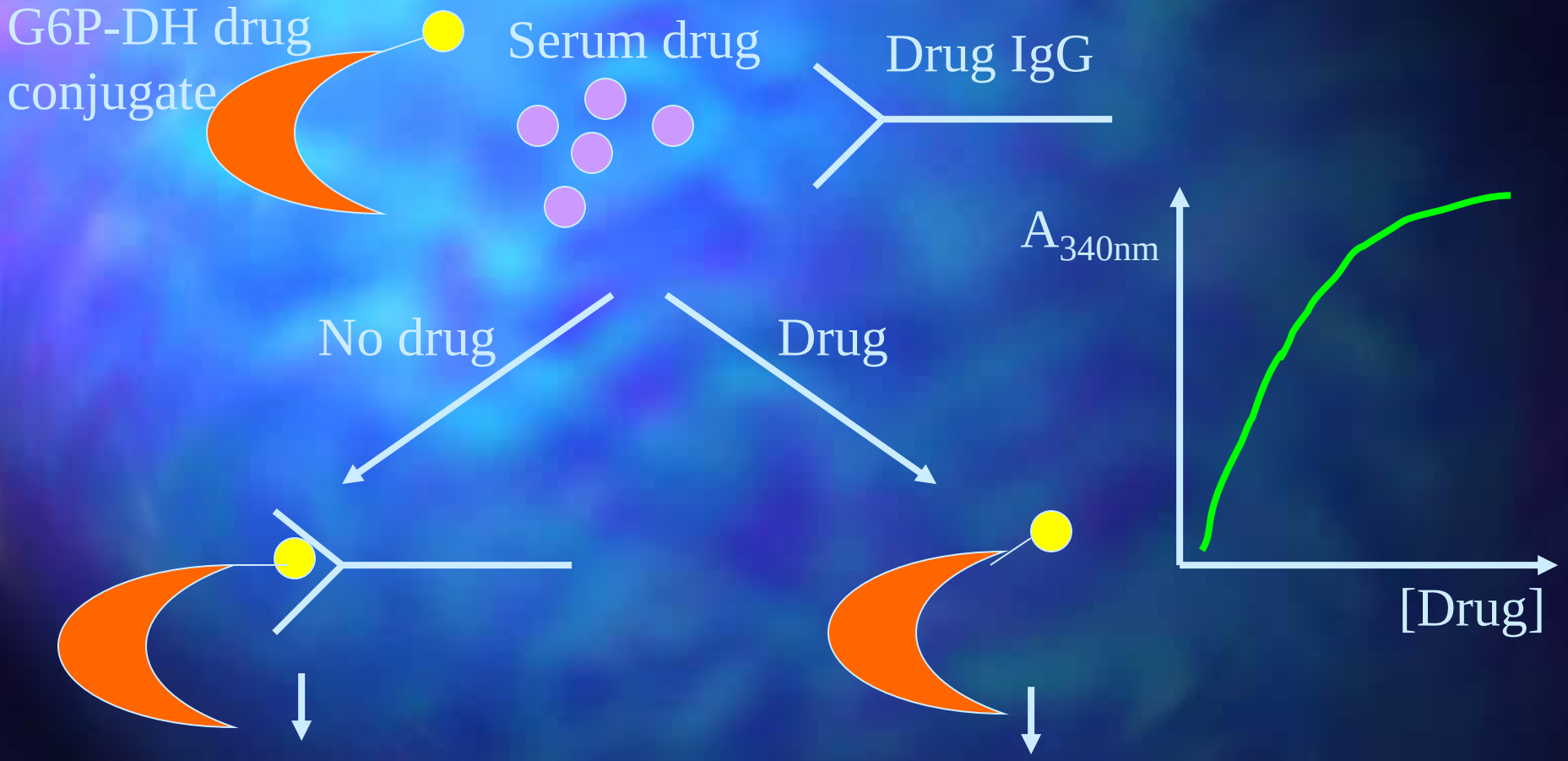
- Comprehensive testing protocol (overview)
  - Screening – Detection Techniques
    - Qualitative
    - Semi-quantitative
    - Maybe specific or may only detect families of compounds
      - Ex. Benzodiazepines
      - Ex. Benzoylecgonine
  - Confirmation - Detection Techniques
    - Identification
    - Qualitative or Quantitative
    - Technology more sensitivity and specificity for a particular drug within the drug class
      - Benzodiazepines = Class
        - Valium (Diazepam + Nordiazepam)

Testing Technologies Immunoassays

- Involve the use of immunoglobulin protein (antibodies) binding to drugs or drug metabolite (antigens)
- Sensitivity to small amounts of antigen
- Several types exist
  - **EMIT - Enzyme-multiplied immunoassay**
  - RIA – Radio immunoassay
  - FPIA - Fluorescence polarization immunoassay
  - CEDIA – Cloned enzyme donor immunoassay
  - **ELISA – Enzyme linked immunosorbent assay**

EMIT

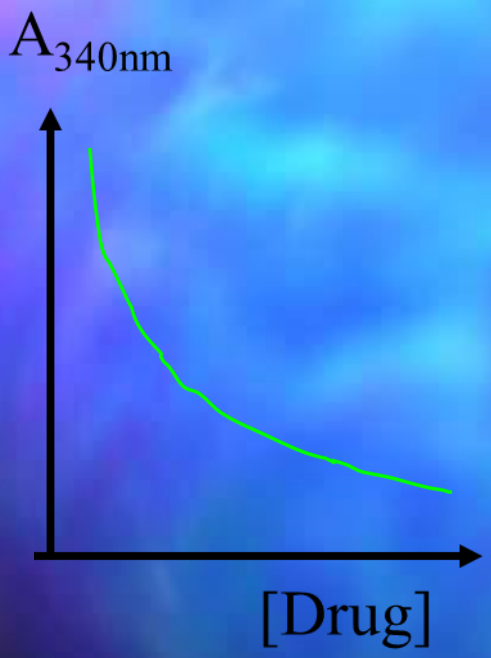
(**E**nzyme-**M**ultiplied **I**mmunoassay **T**echnique)



Binding of IgG to E-D conjugate inhibits enzyme activity

Unbound E-D conjugate has more activity

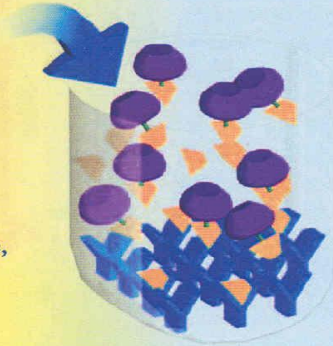
ELISA



MICRO-PLATE HETEROGENEOUS ENZYME IMMUNOASSAY (EIA)

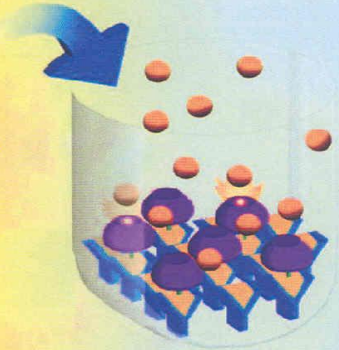
OTI's Micro-Plate assays are rapid, non-isotopic EIA's possessing high sensitivity and specificity. These assays are competitive type immunoassays and are performed either manually or through the use of automated Micro-Plate instruments.

- Combine sample and enzyme-drug conjugate to microtiter plate coated with drug-specific antibodies.
- Incubate 30 minutes, then wash.



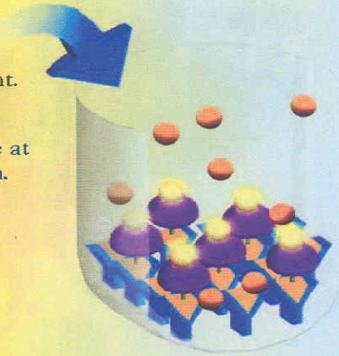
Free drug and enzyme conjugate compete for binding sites on antibody.

- Add substrate.
- Incubate 30 minutes in the dark.



Enzyme begins to react on substrate.

- Add stopping reagent.
- Measure absorbance at 450 nm and 630 nm.



Amount of drug present is inversely proportional to the amount of signal produced.

Clinical Toxicology Principles

- The great majority of drug morbidity and mortality will occur within 4 hrs of patient's admission-thus emergency shipments of specimen out will mostly be of no use in the acute management of the overdosed patient
- More importantly, there is only a small group of drug assays which will affect management.
  - Inclusion of other assays will make the clinician feel better, but not the patient. Even this small group of drugs will be prohibitive in cost and time, if all are performed indiscriminately.
- The best strategy is to use the clinical history, signs and symptoms to select from the list of drugs which have treatments dictated by drug levels.

In-house Tox Screen

- Clinical ER
  - QUALITATIVE DRUG **URINE SCREEN** that detect the following 10 drug classes:
  - *Amphetamine, Methamphetamine, Antidepressants, Barbiturates, Benzodiazepines, Cocaine, Cannabinoids Methadone, Opioids, Tricyclic.*
  - However, be aware that these screens are not all inclusive and have false positives and false negatives.
  - SERUM QUALITATIVE Tricyclic screen as well.
- Drugs/poisons for which laboratory assay is advisable in an OD situation include:
  - **Salicylate, Acetaminophen, Ethanol, Methanol, Ethylene Glycol, Tricyclic antidepressants, Digoxin, Theophylline, Barbiturate, Iron, Lithium**.

Analytic Performance of a Diagnostic Test

	ACTUAL POSITIVE	ACTUAL NEGATIVE	TOTALS
TEST POSITIVE	**80** True Positives	**25** **False Positives**	**105** Test Positives
TEST NEGATIVE	**20** **False Negatives**	**75** True Negatives	**95** Test Negatives
TOTALS	**100** Actual Positives	**100** Actual Negatives	**200**

Sensitivity/Specificity

- **Sensitivity:**
  - If a person has a drug, how often will the test be positive (true positive rate)?
  - ***Sensitivity= true positives/(true positive + false negative)***
- **Specificity:**
  - If a person does not have the drug how often will the test be negative (true negative rate)?
  - ***Specificity=true negatives/(true negative + false positives)***
- **Predictive value for a positive result (PV+):**
  - PV+ asks " If the test result is positive what is the probability that the patient actually has the drug?‟
  - ***PV+= true positive/(true positive + false positive)***
- **Predictive value for a negative result (PV-):**
  - PV- asks " If f the test result is negative what is the probability that the patient does not have drug?‟
  - ***PV-= true negatives/(true negatives +false negatives)***

Cross Reactivity

Table 2. Cross-Reactivities of Common Drugs in an Amphetamine/ Methamphetamine Immunoassay

Compound	% Reactivity
d-Amphetamine	100
d-Methamphetamine	100
l-Methamphetamine	50
Phentermine	50
l-Amphetamine	16.7
l-Ephedrine	0.55
Tyramine	0.5
Phenylpropanolamine	0.3
Pseudoephedrine (100 mg/L)	0.15
Pseudoephedrine (1000 mg/L)	0.08

Table 1. Cross-Reactants in Immunoassays

Immunoassay	Common Cross-Reacting Substances
Amphetamine/ methamphetamine	MDA, MDMA, chloroquine, ephedrine, pseudoephedrine, phenylpropanolamine, tyramine, phentermine, phenmetrazine, fenfluramine, ranitidine
Benzodiazepines	Chlorpromazine
Benzoylecgonine/(Cocaine)	Ecgonine, ecgonine methyl ester, cocaine
Cannabinoids/(THC metabolites)	Ketoprofen, tolmetin, naproxen, ibuprofen, acetylsalicylic acid
LSD	Ergotamine, tricyclic antidepressants, verapamil, sertraline, fentanyl
Morphine	Codeine, dihydrocodeine, thebaine, hydrocodone, dihydromorphine, hydromorphone, oxycodone, oxymorphone, meperidine, norcodeine
PCP	TCP, diphenhydramine, dextromethorphan

Cross Reactivity

Ex. l-ephedrine (0.55%) cross rxn

- Note: Potential [Ephedrine]
  - ~10,000 – 200,000 ng/mL vs. [Amphetamine] ~<5000 ng/mL
- So: At the higher end of l-ephedrine the apparent [amphetamine] ~1100 ng/mL which is > 1000 ng/mL cutoff
- SO: Need GC/MS or LCMSMS confirmation to rule out cross reactions

Cross Reactivity

- Lower Cross Reactivity may not be the same for the stereoisomers of drugs
  - Ex. l – amphetamine vs. d – amphetamine
- Antiserum produced from the immunogen creates an antibody that recognizes the phenyl portion of the of methamphetamine hapten.
  - Ex. Compare methamphetamine and phentermine more similar to d-methamph. vs the l – amphetamine
- Concentration dependent cross reactivity
  - Ex. 0.15%/0.08% Pseudoephedrine at 100/1000 ug/mL
- False positive due to cross reactivity
  - Ex. Phentermine 50% cross reactivity
  - Urine with 5000 ng/mL will screen "+"
    - Anorectic meds: Adipex or Ionamin

Cross Reactive Tox Lab vs Clinical

Analyte	Conc (ng/mL)	% Cross-Reactivity
(+) Amphetamine	500	100.00
(-) Amphetamine	100,000	0.5
(±) Amphetamine	1,300	38.5
MDA	1,500	33.3
PMA	2,000	25.0
Tyramine	100,000	0.5
MDMA	500,000	0.1
MDEA	100,000	0.5
Phenylpropanolamine	500,000	0.1
Phentermine	1,000,000	0.05
(+) Methamphetamine	1,000,000	0.05
(-) Methamphetamine	1,000,000	N.D.
(+) Ephedrine	1,000,000	N.D.
(-) Ephedrine	1,000,000	N.D.
(+) Pseudoephedrine	1,000,000	N.D.
(-) Pseudoephedrine	1,000,000	N.D.
Phenylephrine	1,000,000	N.D.
Diphenhydramine	1,000,000	N.D.
Fenfluramine	1,000,000	N.D.

Analyte	Conc (ng/mL)	% Cross-Reactivity
(+) Amphetamine	500	100.00
(-) Amphetamine	Y	AMP
(±) Amphetamine	Y	AMP
Bupropion	Y/?	MAMP/AMP
Fenfluramine	Y	MAMP/AMP
MDA	Y	AMP
MDMA	Y	MAMP
MDEA	Y	MAMP
Phenylpropanolamine	N	N/A
Phentermine	Y	AMP
(+) Methamphetamine	Y	MAMP
(-) Methamphetamine	Y	MAMP
(+) Ephedrine	Y	MAMP
(-) Ephedrine	Y	MAMP
(+) Pseudoephedrine	N	N/A
(-) Pseudoephedrine	N	N/A
Phenylephrine	Y	MAMP
Diphenhydramine	N	N/A

WHS – Tox Cutoffs = Specificity

WHS – Clinical Cutoffs MedTox - Immunochromatographic (Urine)

AMP Amphetamine (d-Amphetamine)	500 ng/mL	OPI Opiates (Morphine)	100ng/mL or 2000 ng/mL
BAR Barbiturates (Butalbital)	200 ng/mL		
BZO Benzodiazepines (Nordiazepam)	150 ng/mL	OXY Oxycodone (Oxycodone)	100 ng/mL
BUP Buprenorphine (Buprenorphine)	10 ng/mL	PCP Phencyclidine (Phencyclidine)	25 ng/mL
COC Cocaine (Benzoylecgonine)	150 ng/mL	PPX Propoxyphene (Norpropoxyphene)	300 ng/mL
MAMP Methamphetamine (d-Methamphetamine)	500 ng/mL	THC Cannabinoids (11-nor-9-carboxy-△9-THC)	50 ng/mL
MTD Methadone (Methadone)	200 ng/mL	TCA Tricyclic Antidepressants (Desipramine)	300 ng/mL

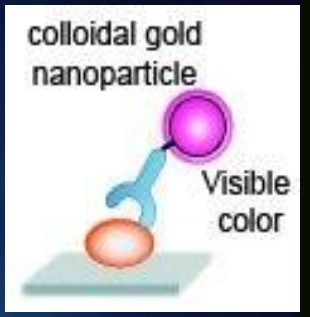
WHS – Clinical Cutoffs = Sensitivity

WHS – Toxicology Laboratory Cutoffs Screening (Urine)

Drug Class	Cut Off ng/mL	Drug Class	Cut Off ng/mL	Drug Class	Cut Off ng/mL
Amphet/Metham	500	Opiate	300	Meperidine (Demerol)	200
MDMA	500	PCP	25	Ultram (Tramadol)	200
Barbiturates	200	Cannab (THCCOOH)	20	Fentanyl+NorFent	2
Benzodiazepines	200	Methadone + EDDP	300	Bup/Norbup	5
Cocaine (BZE)	150	Propox+Metab	300	Syn THC	10
ETOH	20 mg/dL	ETG	500	Carisop+Meprob (Soma)	100

WHS – Tox Cutoffs = Sensitivity

Immunochromatographic Assay (ICA)



- ICA also known as lateral flow immunoassay
  - Rapid test immunoassay detects the presence (or absence) of a target analyte in sample (matrix)
  - No need for specialized and costly equipment.
- Capture antibodies are immobilized as a cross line on a porous hydrophilic materials.
- Analytes in buffer are then added on one side of the test stripe, driven by the lateral capillary force.
- The analytes flow through over the capture antibody line and captured by antibody.
  - Sandwich format positive = colored line indication
  - Competitive format = analyte (drug) competes with labeled antibody conjugate
    - Positive = no color line
- Captured analytes accumulated, the complex could be revealed by the nanoparticle labels (usually colloidal gold) and viewed directly by naked eyes.

- PRO: Rapid, easy to use, low cost, safe, and visible w/o equipment (but can be implemented) – Use as POC
- CON: Low sensitivity/specificity, limited drug panel, limited stable life and storage condition dependent

WHS – Cups Cutoffs (ng/mL) Immunochromatographic (Urine)

AMP Amphetamine (d-Amphetamine)	500	OPI Opiates (Morphine)	1000 (300) 2000
BAR Barbiturates (Butalbital)	(300)200		
BZO Benzodiazepines (Nordiazepam)	(150) 200 ng/mL	OXY Oxycodone (Oxycodone)	*100*
BUP Buprenorphine (Buprenorphine)	10 ng/mL	PCP Phencyclidine (Phencyclidine)	25
COC Cocaine (Benzoylecgonine)	(100)150 ng/mL	PPX Propoxyphene (Norpropoxyphene)	300
MAMP Methamphetamine (d-Methamphetamine)	500 ng/mL	THC Cannabinoids (11-nor-9-carboxy-△9-THC)	(25)50
MTD Methadone (Methadone)	200 (300)	TCA Tricyclic Antidepressants (Desipramine)	300

WHS – Clinical and Forensic Cutoffs = Sensitivity; GREEN = Clin/Forensic Cutoffs are the same

CUPS (POCT UDS) vs Auto analyzers

- Cup/IA cross rxn with Parent vs Metab
  - FEN LOD = 50 ng/mL; FEN x 350 @ 14%
    - NFEN = 50 @ 100%
  - FEN LOD = 100 ng/mL; FEN x 750 @ 13%
    - NFEN = 100 @ 100%
- Cup/IA cross with other meds
- FEN LOD = 200 ng/mL; FEN x 200
  - True LOD 2 ng/mL
  - Buspirone = False "+" for FEN
- Auto Analyzer IA platform
  - FEN LOD = 2 ng/mL @ 100% BUT NF ≠ 0%

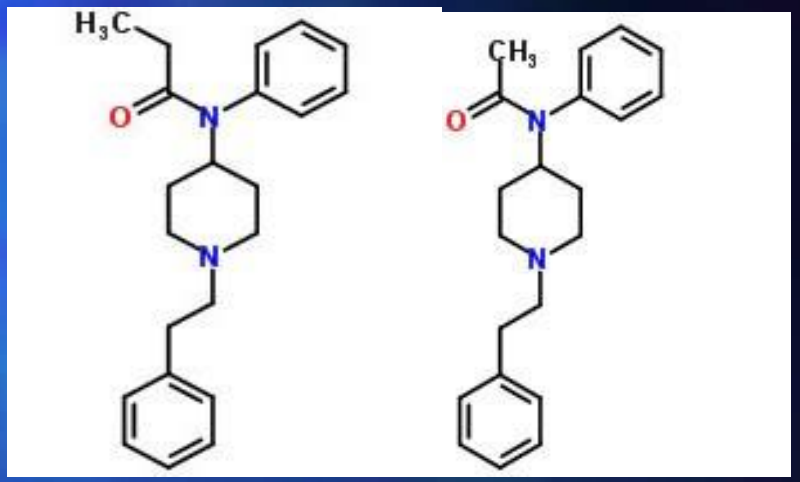
Addiction – Gender Issues

- Both Genders are susceptible to this addiction illness
- But there are differences with the female gender worth noting
- Prescribing
  - Opioid use disorder rises higher for females 54% vs males 46%
  - This elevated prescription use may be a result of higher risk to pain experience/sensitivity
  - Co prescribing benzodiazepines 11% (F) vs. 7.7% (M)
  - Drug-Drug interaction = greater risk
  - Greater risk of OD 583(F) vs 404 (M) 1999-2016
  - Treatment success is lower for women vs men
    - Women less likely to be in the criminal justice system but more likely to be unemployed, experience partner violence, and have child-care

“Lights Out” reprisal role?

- RI - 2013
  - Real-time Outbreak and Disease Surveillance System - spike in OD fatalities March through May
  - 14 individuals YOA range 19 – 57
    - More not reported – incomplete ER records
    - Cases were clustered in close proximity
- PA
  - 50 deaths
- 13 Other states quickly report similar fatalities
- CDC issues a warning based on the RI cases
  - Recommend large doses of Naloxone should be available for care

Acetyl Fentanyl 2013



Fentanyl Acetylfentanyl

- RI Laboratory Clues
  - EIA Drugs Screen – Fentanyl "+"
  - Drug Confirmation - Negative (GC/MS)
    - Unknown peak consistent with ACF
- Drug scenes and Hx
  - Consistent with opiate users and addiction
- DEA
  - Provides a qualitative drug to verify ACF

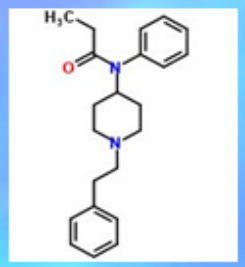
Chicago - Fast Forward 2016

- **2016** Cook County Medical Examiner - **273** Fentanyl Fatalities
  - Under estimate
  - Dennis A. Wichern, DEA Chicago *special agent*
- **2015** Cook County Medical Examiner - **102** Fentanyl Fatalities
- **74** heroin OD reported in **72 hours** between Tuesday and Friday afternoon (Chicago Tribune – **October 3 2015**)
- Is this new?????

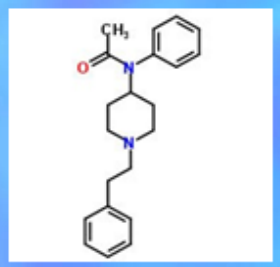
April 2005 – March 2007
Non Pharmaceutical Fentanyl (NPF)

- "**Lights Out**" – Heroin laced with Fentanyl **1013** NPF related deaths **'05 – '07**
- The **NPF** Epidemic in **2006**, in Wayne County, Michigan, fentanyl contributed to **195** (32.4%) of **602** deaths resulting from drug use
  - (C. Schmidt, MD, Wayne County Medical Examiner's Office, personal communication, 2007).
  - DEA began regulating access to **N-phenethyl-4-piperidone (NPP)**, a chemical used to make illicit NPF
- One gram of pure fentanyl can be cut into approximately 7,000 doses for street sale
  - 81 % male 55/4% Caucasian, 39.8% African American, 4.2 % Hispanic
- An earlier epidemic in the 1980s resulted in at least 110 fatal overdoses caused by 10 different fentanyl analogs (*3*).
  - Chicago, Detroit, and Philadelphia see the greatest report fatalites
- NPF-related deaths were reported in suburban and rural areas of Illinois, Michigan, Pennsylvania, Kentucky, Maine, Maryland, Massachusetts, New Hampshire, Ohio, and Virginia during the same period.

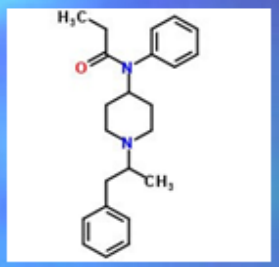
Fentanyl Analogues – Origins



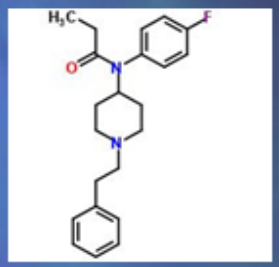
Fentanyl
CSID 3228; 1960



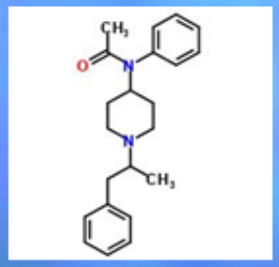
Acetylfentanyl
CSID 459388; 2013



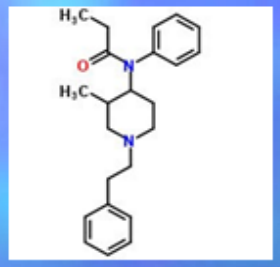
α-Methylfentanyl
CSID 56081; 1979



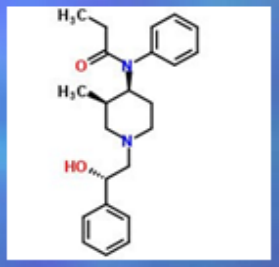
Parafluorofentanyl
CSID 56096; 1980s



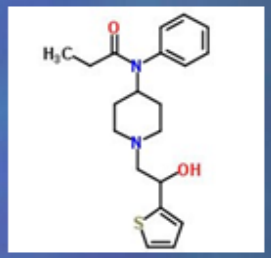
α-Methylacetylfentanyl
CSID 56102; 1984



3-Methylfentanyl
CSID 55844; 1984



Ohmefentanyl
CSID 8649506; 1985

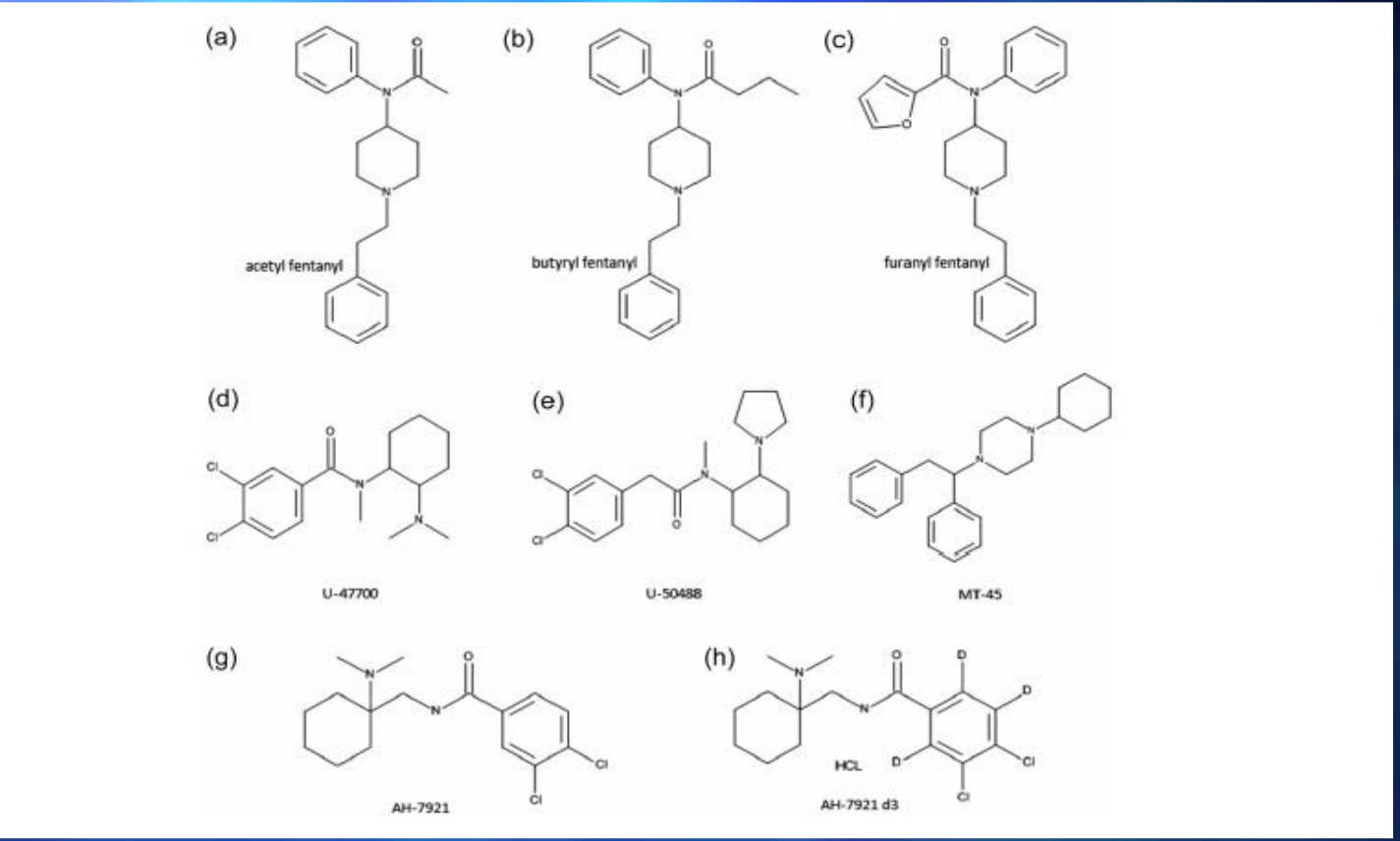


β-Hydroxythiofentanyl
CSID 21106268; 1985

Figure 1 Fentanyl and some Analogues (6)
CSID = ChemSpider ID; Year = Year introduced

Acetyl Fentanyl *"New Heroin" Leads to Opiate Fatalities By Michael A. Wagner, PhD, Jeffery H. Moran, PhD, Amy L. Patton, MS, and Laurie Ogilvie, MS. March 2014 - CFTN*

Designer Opiates (NPS - new psychoactive substances)

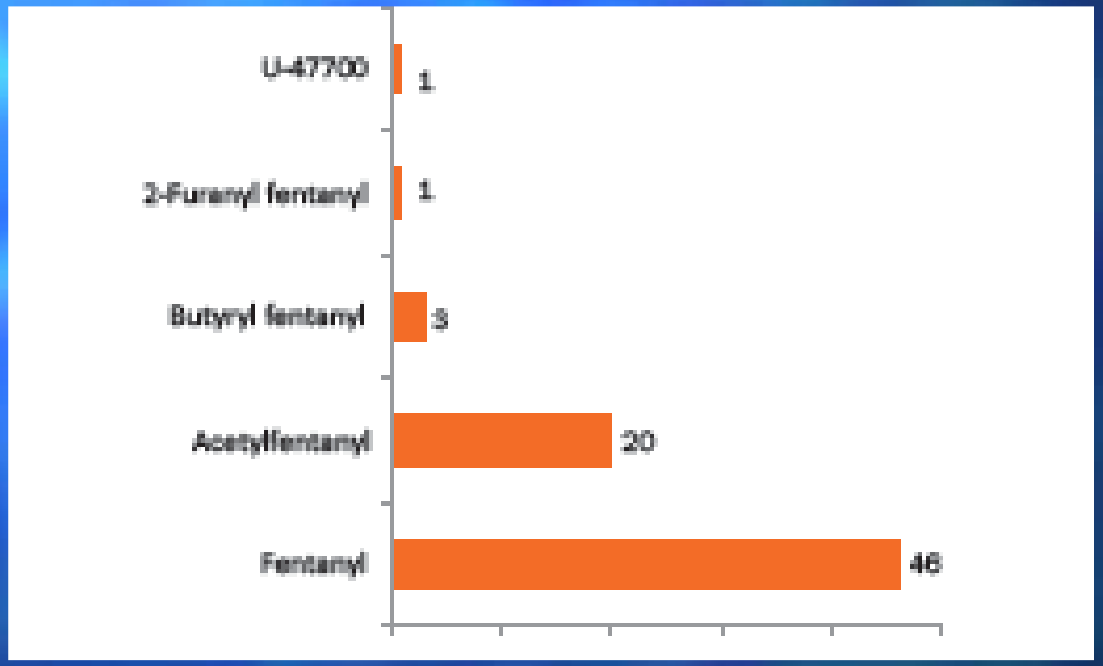


Analysis of Novel Synthetic U-47700, U-50488, Furanyl Fentanyl by LCMSMS PM, JAT Sept 1 2016 BK Logan et al.

Designer Opiate Test Panel – Fentanyl Analogues

- 2-Furanylfentanyl; 4-ANPP; Acryl Fentanyl;
- Butyrylfentanyl;
- Carfentanil; Cyclopropylfentanyl; cis-3-Methylfentanyl
- Isobutyrylfentanyl
- meta-Methylmethoxyacetylfentanyl, Methoxyacetylfentanyl
- ortho-Fluorofentanyl
- para-Fluorobutyrylfentanyl; para-Fluorofentanyl; para-Fluoroisobutyrylfentanyl; para-Methylmethoxyacetylfentanyl
- trans-3-Methylfentanyl, THF-F
- U-47700; U-49900; U-51754;
- Valeryl Fentanyl

Opioids/Analgesic – 65% ID first Quarter CY 2016



Emerging Threat Report CY 2016

National Estimates

Table 1 shows that from January 2015 through December 2016, a total of 57,155 fentanyl and fentanyl-related substance reports were identified by State and local forensic laboratories in the United States.

Table 1 National Annual Estimates of Fentanyl and Fentanyl-Related Substances Reported in NFLIS, 2015–2016[1]

Fentanyl and Fentanyl-Related Substances	2015		2016		Total	
	Number	Percent	Number	Percent	Number	Percent
Fentanyl	14,440	84.59%	34,204	85.33%	48,644	85.11%
Acetyl fentanyl	2,412	14.13%	1,669	4.16%	4,080	7.14%
Furanyl fentanyl	0	0.00%	2,273	5.67%	2,273	3.98%
Carfentanil	0	0.00%	1,100	2.74%	1,100	1.92%
3-Methylfentanyl	1	0.01%	427	1.07%	428	0.75%
Butyryl fentanyl	205	1.20%	93	0.23%	298	0.52%
Fluoroisobutyryl fentanyl	0	0.00%	82	0.20%	82	0.14%
p-Fluoroisobutyryl fentanyl	0	0.00%	76	0.19%	76	0.13%
p-Fluorobutyryl fentanyl	2	0.01%	72	0.18%	74	0.13%
Valeryl fentanyl	0	0.00%	52	0.13%	52	0.09%
Acryl fentanyl	0	0.00%	26	0.06%	26	0.05%
p-Fluorofentanyl	8	0.05%	5	0.01%	13	0.02%
o-Fluorofentanyl	0	0.00%	3	0.01%	3	0.01%
Beta-hydroxythiofentanyl	3	0.02%	0	0.00%	3	0.01%
ANPP	0	0.00%	1	0.00%	1	0.00%
Acetyl-alpha-methylfentanyl	1	0.01%	0	0.00%	1	0.00%
Alpha-methylfentanyl	0	0.00%	1	0.00%	1	0.00%
4-Methoxy-butyryl fentanyl	0	0.00%	*	*	*	*
Total[2]	**17,071**	**100.00%**	**40,083**	**100.00%**	**57,155**	**100.00%**

ANPP=4-Anilino-N-phenethyl-4-piperidine

[1] Includes drugs submitted to laboratories from January 1, 2015, through December 31, 2016, that were analyzed within three months of the calendar year reporting period.

[2] Numbers and percentages may not sum to totals because of rounding.

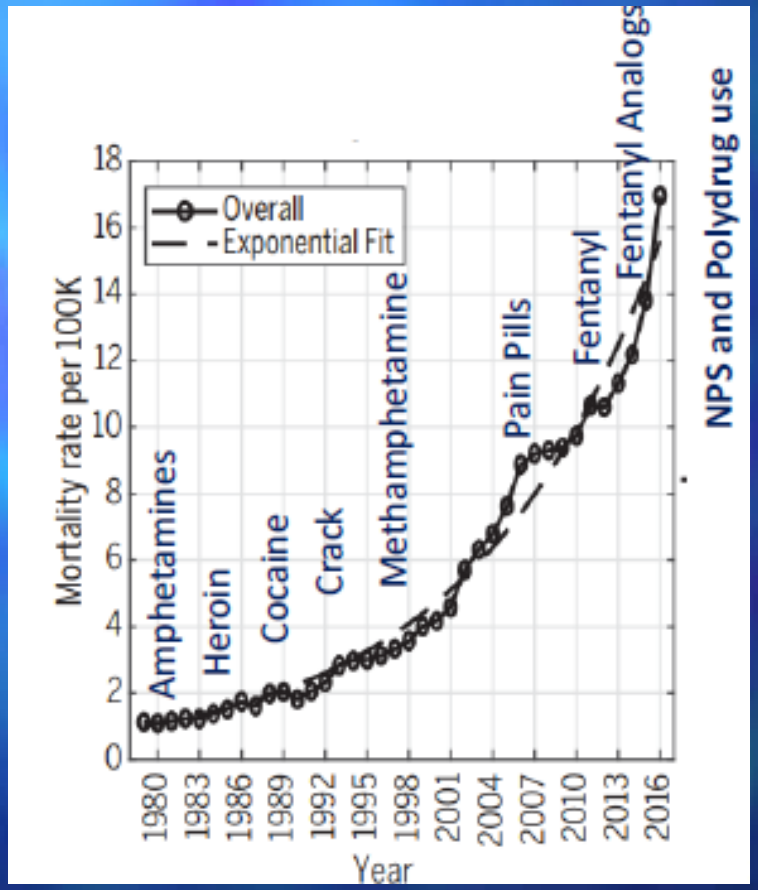
** The estimate for this drug does not meet the standards of precision and reliability.*

Fentanyl Analogues - Screening/Confirming

EMIT	Conc	% CRxn
Fentanyl	2 ng/mL	100
Acetyl Fentanyl	4 ng/mL	53
Desproprionyl	25 ng/mL	8
Carfentanyl	4 ng/mL	50
Sufentanyl	75 ng/mL	3
Butyryl	1.9 ng/mL	111
Cis-Methyl	95 ng/mL	2.3
TransMethyl	3.7 ng/mL	57
4-Methoxybutyl	3.0 ng/mL	77
Valeryl Fentanyl	2.5 ng/mL	80
Isobutyryl	2.7 ng/mL	74
Para-fluorobutyrl	2.2 ng/mL	100
Furanyl Fentanyl	2.8 ng/mL	75

Changing Dynamics of the Drug Overdose Epidemic in the United States, 1979-2016

Science. 2018 September 21; 361(6408): . doi:10.1126/science.aau1184



Data does not include Synthetic Cannabinoids

Figure adaptations CFSRE – B. Logan 2021

40 Fentanyl Analogues

2,5-Dimethylfentanyl 3-Allylfentanyl 3-Methylbutyrfentanyl 3-Methylfentanyl
3-Methylthiofentanyl 4-Fluorobutyrfentanyl p-chloroisobutyrfentanyl
p-fluoroisobutyrfentanyl 4-Fluorofentanyl 4-Methoxybutyrfentanyl 4-Phenylfentanyl
Acrylfentanyl α-Methylacetylfentanyl α-Methylbutyrfentanyl α-Methylfentanyl
α-Methylthiofentanyl Acetylfentanyl Alfentanyl Benzylfentanyl β-Hydroxyfentanyl
β-Hydroxythiofentanyl β-Methylfentanyl Butyrfentanyl Brifentanyl Carfentanyl
Cyclopentylfentanyl Isobutyrfentanyl Furanylfentanyl Furanylethylfentanyl Lofentanyl
N-Methylcarfentanyl Methoxyacetylfentanyl Mirfentanyl Ocfentanyl Ohmefentanyl
R-30490 Remifentanyl Sufentanil Thenylfentanyl Thiofentanyl Trefentanyl Valerylfentanyl

Intelligence Drug Use Forums

International Journal of Drug Policy 98 (2021) 103393

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journal homepage: www.elsevier.com/locate/drugpo

Research paper

Online surveillance of novel psychoactive substances (NPS): Monitoring Reddit discussions as a predictor of increased NPS-related exposures

Elan Barenholtz[a,1], Alex J. Krotulski[b,1,*], Paul Morris[a], Nicole D. Fitzgerald[c], Austin Le[d], Donna M. Papsun[e], Barry K. Logan[b,e], William E. Hahn[a], Bruce A. Goldberger[f], Linda B. Cottler[c], Joseph J. Palamar[d]

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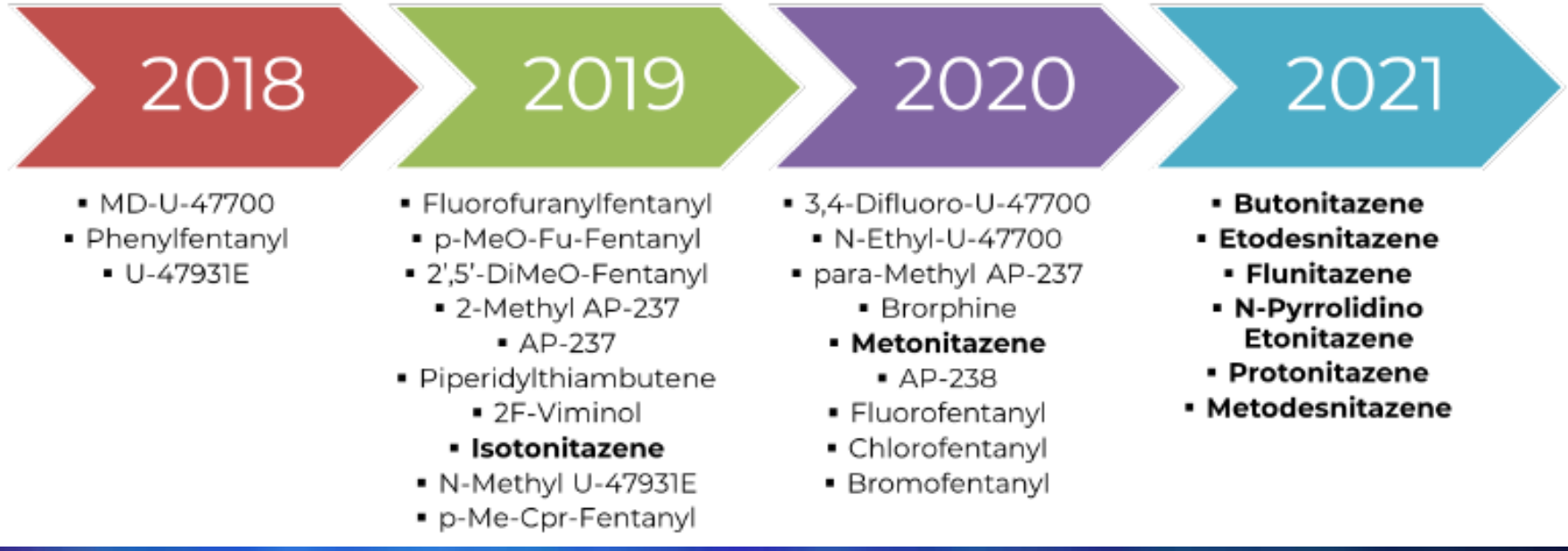
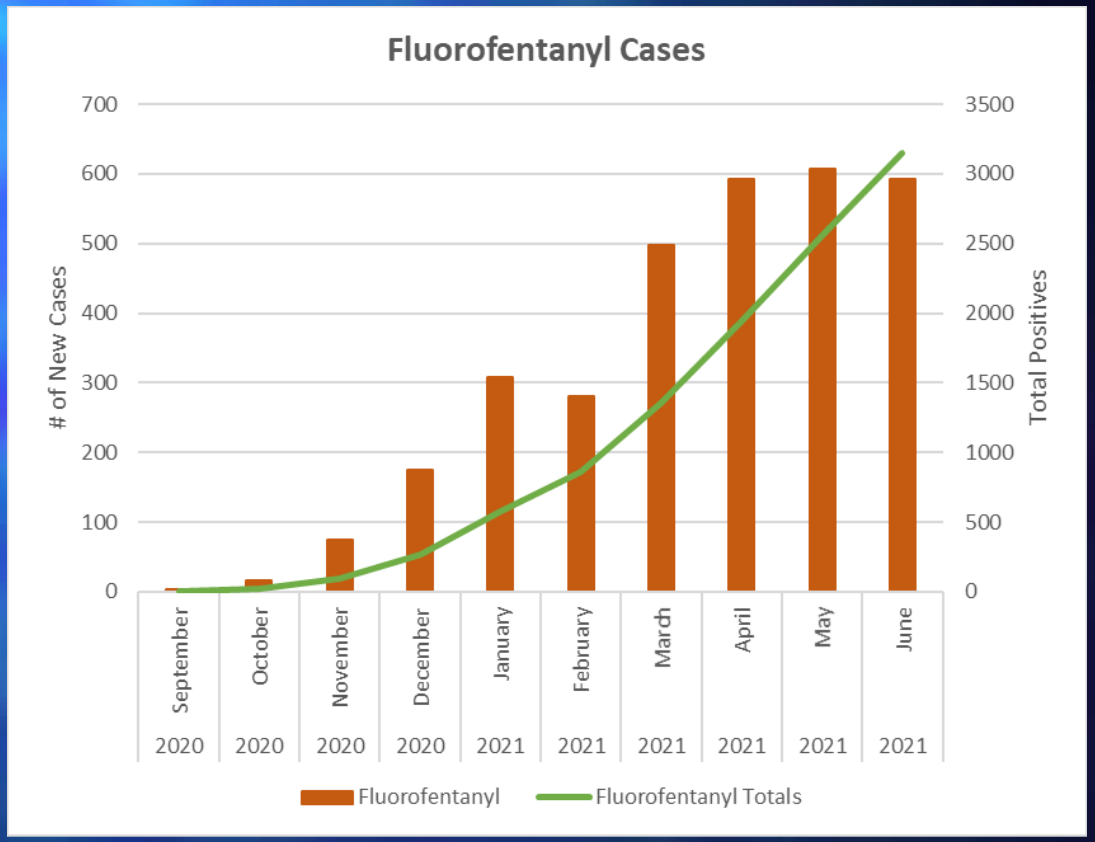
Novel Synthetic Opioids 2018 - 2021



- New NPS Opioids reported by NPS Discoverry – datat adapted by B. Logan CFSRE -2021

Para-Fluorofentanyl

- Analysis of residual precursor, by-products and synthetic pathways suggests this trend is driven by change in precursors in fentanyl synthesis.



- New NPS Opioids reported by NPS Discoverry – datat adapted by B. Logan CFSRE -2021

Case #1

- 17 YOA Female is randomly selected by HS for UDT per school policy
  - 10/15/21 Monday
- Sample Test positive for THC + FEN/NorFEN
  - Per Director's policy, schools are notified of all Opiate positives and other high risk drug results to include: Hallucinogens (MDMA) and Stimulants (MET/COC).
  - Amended report is issued to include drug quantitative results
  - Parents are notified and counseled on future compliance testing and school regulations

Case 1 cont'd

- Parents discussion with the daughter report that she had gone to a concert on either Sat (10/23) or (Sun night_10/24) and a male friend gave her a pill and MJ.
  - Daughter claims she did not know what the pill contained.

Case #1

Days (Batch)	DOB	Collection Date	THC/Ratio	THC	CRE	CRE/100	T2/T1	ETG	ETS	COT	FEN	Norfen
			0.0									
(AG1:B223)	12/27/2021	10/25/2021_1400	503.2	473	94	0.94	#DIV/0!	0.0	0.0	Not rpt'd 1647	100	264
9 dys		11/2/2021	170.8	123	72	0.72	0.3	Not tested	Not tested	1474	1.31	39.4
15dys		11/8/21_602	571.4	360	63	0.63	3.34	Not tested	Not tested	1343	0.4	2.11
15dys		11/8/21_8:01	172.1	191	111	1.11	0.301	Not tested	Not tested	2450	0.00	1.24
17dys		11/10/2021_635	326.05	388.00	119.00	1.19	1.89	Not tested	Not tested	2181	0.00	1.29
18dys		11/11/21_826	157.2	228	145	1.45	0.48	Not tested	Not tested	3194	0.00	0.00 (0.88)
18dys (AG1:B240)		11/12/21_0939	362.9	127	35	0.35	2.3	4239.0	2078.0	Not rpt'd (953)	0.00	0.00 (0.27)
21dys (AG1:B241)		11/15/2021_627	102.0	102	100	1.00	0.6	Not tested	Not tested	2027	0.00	0.00 (0.27)
22dys (AG1:B241)		11/16/21_0733	264.7	225	85	0.85	2.6	Not tested	Not tested	1827	0.00	0.00 (0.58)
1123-11M (AG1:B246)		11/21/21_1633	0.0	0	32	0.32	0.0	Neg	Neg	4.1 <500	Neg	Neg
(AG1:B246)		11/23/21_1400	269.5	512	190	1.9	#DIV/0!	Neg	Neg	Not rpt'd 2750	39.11	317.78
(AG1:B246)		11/23/21_1400	386.7	58	15	0.15	1.4	Neg	Neg	Not rpt'd 308	7.35	29.38
AG1:B250		11/29/21_1812	0.0	0	38	0.38	0.0	Not tested	Not tested	240	0.00	0.00
AG1:B250		11/30/21_0821	0.0	0	107	1.07	#DIV/0!	Not tested	Not tested	1694	0.00	0.00
AG1:B254		12/06/21_1852	0.0	0	12	0.12	#DIV/0!			NEG	0.00	0.00

ETOH = 176 mg/dL

Case #2

- 31 YOA Male is required to submit regular UDS for compliance monitoring
- Continues to deny drug use including METH and FEN
- Monitored for 111 days

Case #2

Collection	Delata days					3/22/21	19 (incld 22nd)			3/29/21	8(incld 29th)			5/24/21	62(incld 24th)			6/22/21	111 (3/4/21)		
3/4/21	n/a	Drug	Concentration (ng/mL)	Conc-ratio	CRE 177	Drug	Concentration (ng/mL)	Conc-ratio	CRE 14	Drug	Concentration (ng/mL)	Conc-ratio	CRE 22	Drug	Concentration (ng/mL)	Conc-ratio	CRE 157	Drug	Concentration (ng/mL)	Conc-ratio	CRE 93
		MET >	27500	15536.7		MET	2393	17,092		MET	2176	9891		MET	23	15	NEG	MET	0	0	
		AMP	3800	2146.9		AMP	577	4121		AMP	247	1123		AMP	26	17	NEG	AMP	0	0	
		FEN	500	282.5		FEN	427	3050		FEN	79.9	363		FEN	44.7	28		FEN	1.53	2	
		N-FEN >	14000	7909.6		N-FEN	727	5192		N-FEN	802	3645		N-FEN	239	152		N-FEN	7.45	8	
		MTD >	10200	5762.7		MTD	6424	45855		MTD	3935	17886		MTD >	5000	3185	**	MTD	>5000	#VALUE!	
		EDDP >	35000	19774.0		EDDP	2305	16464		EDDP	4276	19436		EDDP >	5000	3185	**	EDDP	>5000	#VALUE!	
		THCA-R*	dil			THCA	26	185		THCA	38	173		THCA	118	75		THCA	221	238	
						THCA-R*	185			THCA-R*	172			THCA-R*	75			THCA-R*	238		
						COT	866			COT	1016.2	4619		COT	3093	1970		COT	2351	2528	
						Diphen	440	3142		Diphen	122.7	558		Diphen	7.8	5		Diphen	0	0	

Article – Issues Protracted Renal Clearance Fentanyl OUD patients

Drug and Alcohol Dependence 214 (2020) 108147

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Drug and Alcohol Dependence

journal homepage: www.elsevier.com/locate/drugalcdep

Short communication

Protracted renal clearance of fentanyl in persons with opioid use disorder

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Article continued

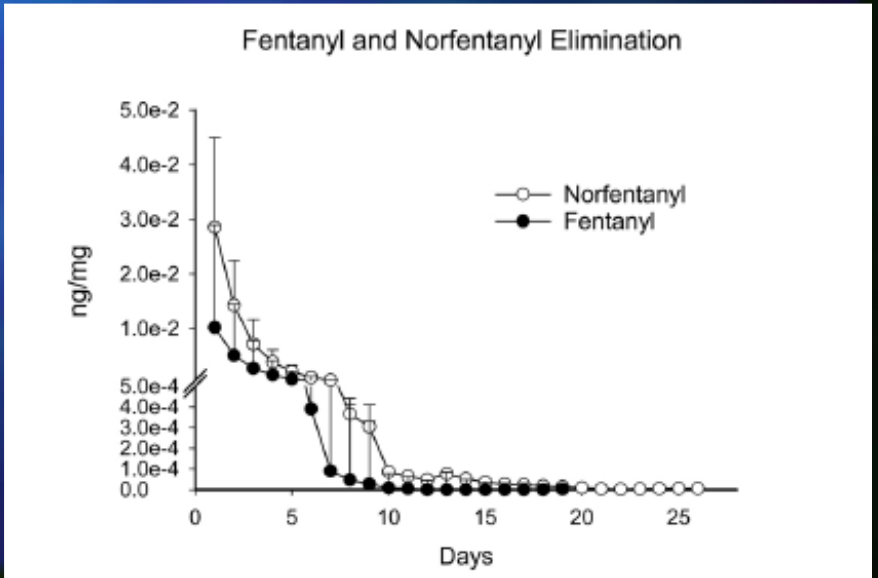
ABSTRACT

Introduction: The illicit opioid supply in the U.S. is increasingly adulterated with fentanyl. As such, persons with opioid use disorder (OUD) may be regularly exposed to fentanyl, however, the pharmacokinetics of repeated fentanyl exposure are not well understood. The current study aimed to quantify renal clearance of fentanyl in OUD patients presenting to residential treatment.

Methods: Participants (N = 12) who presented to a 28-day residential treatment program were enrolled if they tested positive for fentanyl at intake. Urine samples were collected every 2–3 days and were quantitatively tested for fentanyl, norfentanyl, and creatinine via liquid chromatography mass spectrometry (LC–MS). Fentanyl clearance was defined as the time since last illicit opioid use and the median time between last positive and first negative fentanyl urine screen.

Results: Participants had a mean and standard deviation (SD) age of 28.9 (11.0), were 67 % male, and 83 % white. The mean (SD) time for fentanyl and norfentanyl clearance was 7.3 (4.9) and 13.3 (6.9) days, respectively. One participant continued to test positive for fentanyl for 19 days and norfentanyl for 26 days following their last use, and left treatment without testing negative for norfentanyl.

Conclusion: Fentanyl clearance in persons with OUD is considerably longer than the typical 2–4 day clearance of other short-acting opioids. The findings of this study might explain recent reports of difficulty in buprenorphine inductions for persons who use fentanyl, and point to a need to better understand the pharmacokinetics of fentanyl in the context of opioid withdrawal in persons who regularly use fentanyl.



Case #3
Fentanyl Abuse or Overdose?

- Case History
  - Gender: M Race: Caucasian YOA: 28 Ht: 5'10 Wt: 165
  - Scene: July, 10:43 AM Officer dispatch reference a medical call
    Male found on the floor unconscious;
  - Father (caller) attempts CPR – chest compressions.
  - Subject lying on the floor (police arrival) unconscious and unresponsive Officer attempts AED – negative result. Detectives and coroner dispatched to the scene.
  - Scene details: Father explained that at apprx 0330 AM hr noticed son was awake listening to music. 0530 AM hr Father notices son is asleep in the bed.
  - Father 10:20 AM finds son laying face up on the bed. Move him to the floor.

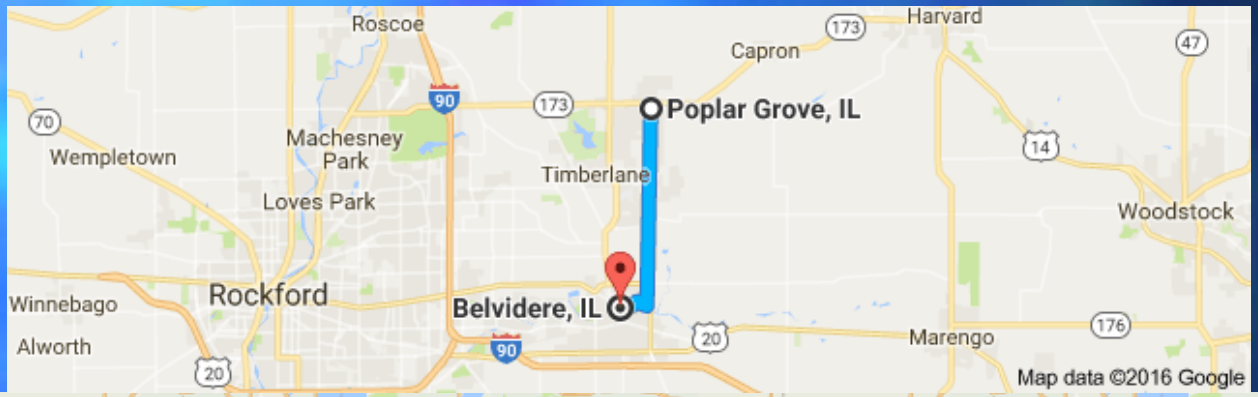
Case Hx, Cont'd

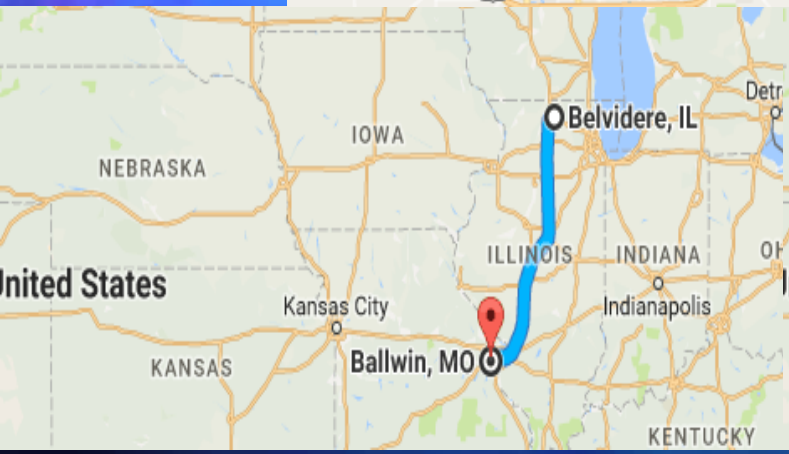
- Medical Hx
  - Treated for chronic headaches
  - Prior Hx of heroin use
    - Father not aware of reuse.
  - Evidence observed
  - Prx Hx:
    - Propranolol
    - Clonazepam
    - Fiorinal
    - Acetaminophen + Diphenhydramine

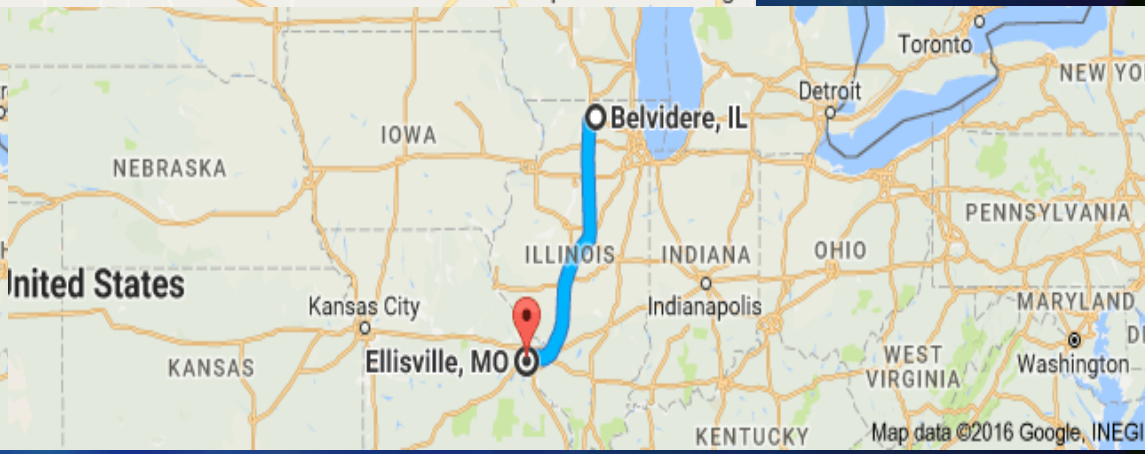
Scene Evidence and Prx. Hx.

Drug	Subj Name	Pharmacy	Prx no	Dose	Qty prscribed	Number Refills	Qty found	Prx Directions	Date Prx issued	DOD or found
Propranolol	TMB	X	X	X	180	0	8	X	X	7/31/2013
Acetamin/Diphen	"	"	X	X	X	X	6	X	X	"
Fiorinal (Butal/Asp/Caf)	"	"	1865840-047xx	X	60	X	40	Q.D	6/26/2013	"
Clonazepam	*"*	*Sam's club Chesterfield MO*	*441829x-xxxxx*	*1 mg*	*30*	*X*	*0*	*Q.D*	*6/24/2013*	
Clonazepam	*"*	*Walmart Ellisville MO*	*186537-047xx*	*1 mg*	*30*	*3*	*0*	*Q.D*	*6/26/2013*	*"*
Indomethacin	MGB (father)	Wallgreens Belvidere IL	two 30 dy supplies	50 mg	15	0	5	TID for 5 days	-	"

Distance traveled







Autopsy Finding - External

- Ht: 69” Wt: 181 lbs
- Healed cutaneous scars (hands, legs, knees)
- Eyes brown; 4mm – normal
  - Cornea clear; conjunctivae w/o petechial
- Tongue, buccal mucosa + pharynx normal
- Neck symmetrical w/o abnormalities
- Chest/Back – unremarkable
- Skin – psoriasis-like elbow

- Evidence of Injury - None
- Rigor complete/fixed equal distribution all extremities

Autopsy Internal

- Cardio - Unremarkable
  - 350g LV 15mm RV 4mm Interventricular Septum 19mm
  - No Atherosclerosis, Thrombosis, Calcification
  - Chambers/valves – normal
  - Pulmonary artery – major branches - normal
  - Vena cava – major tributaries/pulmonary veins – free of thrombi
- Liver/Biliary
  - Liver – **2425g** smooth/glistening/intact
  - Moderately congestion
  - No focal lesions
  - Gallbladder - unremarkable
  - Extrahepatic biliary - patent
- Spleen – 310 g
  - Regional lymph nodes – unremarkable; Parenchyma – moderately firm

Autopsy Internal cont'd

- Respiratory
  - R **800g** / L **625g** fully inflated
  - Intraparenchymal pulmonary arteries – normal, patent, w/o thrombus or embolus
  - Moderate vascular congestion/edema
    - No emphysema, consolidation, granulomata or focal lesions
  - Mild anthracosis
- CNS
  - Brain 1475g –
  - External surface/configuration – normal
  - Dura mater/falx cerebri intact w/o hemorrhage
  - Base including cranial nerves/blood vessels w/o atherosclerosis
  - Coronal section through cerebral hemisphere cortex, white matter + basal nuclei – normal
  - Brain stem + cerebellum – transverse sectional – normal
  - Cervical spinal cord - unremarkable

Reference Ranges - 95% Inclusion

- Brain, 1179-1621 g
- Heart, 233 – 383 g
- Liver, 968-1860 g
- Spleen, 28-226 g
- Right lung, 155-720 g
- Left lung, 112-675 g
- Right kidney, 81-160 g
- Left kidney, 83-176 g

Am J Forensic Med Pathol. 2012 Dec;33(4):368-72. doi: 10.1097/PAF.0b013e31823d29ad. – Molina, DiMajo
Am J Forensic Med Pathol. 2012 Dec;33(4):362-7. doi: 10.1097/PAF.0b013e31823d298b. – Molina, DiMajo

Toxicology

- Comprehensive Testing
- Peripheral Blood

		~~Therapeutic Range~~
– 7-Aminoclonazepam	35.3 ng/mL	
– Alprazolam	88.6 ng/mL	10 – 40 ng/mL
– Fentanyl	10.0 ng/mL	1 – 3 ng/mL

- Urine

– Alprazolam	957 ng/mL
– Alpha-OH-Alpraz	1805 ng/mL
– 7-Aminoclonazepam	1348 ng/mL
– Fentanyl	12.6 ng/mL
– Norfentanyl	> 100 ng/mL
– Hydrocodone	217 ng/mL

Additional samples: vitreous

Pathologist Findings

- Few generalized cutaneous scars present
- Chronic dermatitis, elbow, consistent w psoriasis
- Pulmonary vascular congestion and edema, combined weight of lungs 1425 g
- Pulmonary anthracosis, moderate, w/o emphysema
- No evidence of significant natural disease, injury, active infection or congenital anomaly

- Opinion: Death attributed to the adverse effects of Fentanyl

Fentanyl Drug Concentration Changing Landscape

- Baselt, Randall C.
  - Disposition of Toxic Drugs and Chemical in Man 8th Ed.
  - Fatal Fentanyl Ranges (ug/L = ng/mL or ug/kg)

	Blood	Brain	Liver	Kidney	Urine
Avg	8.3	20	37	18	28
	(3.0-28)	(9.2-30)	(5.9-78)	(6.1-42)	(5.0-93)

Fentanyl and Driving Impairment

Abstract

The incidence of fentanyl in forensic toxicology analyses in the USA has dramatically increased over the past several years. The increase in death cases has been well studied; however, little has been reported on the impact to drug impaired driving. Fentanyl driving while under the influence of drugs (DUID) case data from 2014 to 2019 is presented. The data were obtained from three toxicology laboratories in the Northeast, Southeast, and Midwest regions of the USA. Fentanyl whole blood concentrations ranged from 0.1 to 157 ng/mL in living drivers with a 466% to 524% increase in fentanyl-positive DUID cases from 2014 to 2019, depending on the US region. The vast majority of fentanyl cases involved poly-drug use. Twenty case histories are presented where fentanyl was the only drug identified. The mean (standard deviation) fentanyl concentration for these cases was 5.2 ± 3.8 ng/mL with a median of 3.7 ng/mL, and the concentrations ranged from 2.0 to 16 ng/mL. Naloxone administration was documented in exactly half of these cases similar to another study involving carfentanil-impaired driving. The case histories also demonstrate that some recreational opioid users may display limited signs of impairment either due to tolerance or naloxone administration. The top three observations in common among the cases were the driver was found unresponsive behind the wheel, the vehicle left the travel lane or roadway, and the driver was involved in a crash. The increase in fentanyl use not only poses a risk for overdose and death, but is also a significant concern for traffic safety. This study supports the movement of fentanyl from a Tier II drug to Tier I due to its significant potential for impairment and increase in prevalence in impaired driving cases.

- JAT 2021; 45 389-396 Rohrig, T; Scarneo, C. Tiscione N.B. et. al.

Police

- Conclude Fentanyl OD
  - Concentration > 3x's the therapeutic range
- Medication Diversion
  - Phone records

Final Thoughts + Recommendations

- 1. Develop a comprehensive drug testing program and use it…..
  - Assess the patient baseline with regular testing over the first 2-3 months.
  - Evaluate results relative to drug use and prescription history.
  - Maintain a regular (random) testing routine based on patient risk assessment
    - Consider alternate matrices for difficult patients (OF, Hair, Blood vs Urine)
- 2. Become familiar with the test panels, platforms and your laboratory
  - Identify the toxicologist/specialist for interpretations and clarification (PD/PK, stability, windows of detection (they aren't what they use to be!).
    - We like to talk to people…. Call us ☺
  - Know the strength and weaknesses of the tests
    - Sample collection, storage, time of collection, patient risk for alteration, storage conditions
    - Lab test cutoffs, drugs identified in the panel, the labs limitations vs your needs
- 3. Adulterated samples or evidence of diversion
  - Pill shaving, sample substitution etc.

Sample Testing Drug Menu

DRUG SCREENS AND CONFIRMATIONS PERFORMED AT WITHAM TOXICOLOGY LAB

DRUG CLASS (Screening cutoff)	SPECIFIC DRUG	ANALYTE IDENTIFIED
Amphetamines	Amphetamine	Amphetamine
Cutoff 500 ng/ml	Methamphetamine	Methamphetamine & amphetamine Includes D&L stereoisomer analysis
	Ecstacy (MDMA)	Methylenedioxymethamphetamine
Barbiturates	Amobarbital	Amobarbital
Cutoff 200 ng/ml	Butalbital	Butalbital
	Pentobarbital	Pentobarbital
	Phenobarbital	Phenobarbital
	Secobarbital	Secobarbital
Benzodiazepines	Alprazolam metab. (xanax)	Alpha-hydroxyalprazolam
Cutoff 200 ng/ml	Chlordiazepoxide	Nordiazepam & Oxazepam
	Clonazepam	7-aminoclonazepam
	Diazepam (Valium)	Nordiazepam, Oxazepam, & Temazepam
	Flunitrazepam (Rohypnol)	7-aminoflunitrazepam
	Flurazepam (Dalmane)	2-hydroxy-ethyl-flurazepam
	Lorazepam (Ativan)	Lorazepam
	Triazolam (Halcion)	Alpha-hydroxytriazolam
	Temazepam	Temazepam & oxazepam

Testing cont'd

Cannabinoids Cutoff 20 ng/ml	Marijuana	11-nor-9-carboxy-delta-9-tetrahydrocannabinol
Cocaine Metabolites Cutoff 150 ng/ml	Cocaine	Benzoylecgonine
Ethanol Cutoff 20 mg/dl	Alcohol	Ethanol
Methadone Cutoff 300 ng/ml	Methadone	Methadone EDDP
Opiates Cutoff 300ng/ml	Codeine Heroin Hydrocodone Hydromorphone Morphine Oxycodone Oxymorphone	Codeine,morphine, & hydrocodone 6-monoacetyl morphine & morphine Hydrocodone & hydromorphone Hydromorphone Morphine & hydromorphone Oxycodone & oxymorphone Oxymorphone
Phencyclidine Cutoff 25 ng/ml Cutoff 200 ng/ml	Phencyclidine (PCP)	Phencyclidine
Tramadol	Tramadol	Tramadol

Testing cont'd

Meperidine (Demerol) Cutoff 200 ng/ml	Meperidine	Meperidine
Propoxyphene (Darvon) Cutoff 300 ng/ml	Propoxyphene	Propoxyphene Norpropoxyphene
Buprenorphine (Suboxone) Cutoff 5 ng/ml	Buprenorphine	Buuprenorphine Norbuprenorphine
Fentanyl Cutoff 1 ng/ml	Fentanyl	Fentanyl norfentanyl
Carisoprodol (Soma) Cutoff 100 ng/ml	Carisoprodol	Carisoprodol Meprobamate
Ethylglucuronide (alc metabolite) Cutoff 500 ng/ml	Ethyl glucuronide	Ethyl glucuronide Ethyl Sulfate

DRUG PANELS Meconium

13 PANEL 12 PANEL 9 PANEL 7 PANEL 5 PANEL

Amphetamines
Cannabinoids
Cocaine
Opiates
Phencyclidine
Methadone
Barbiturates
Benzodiazepines
Propoxyphene
Oxycodone
Meperidine
Tramadol
Buprenorphine
Fentanyl *
* recently added

Alcohol Biomarkers

- ETG/ETS – Most commonly used
- FAEE – Fatty Acid Ethyl Ester
  - Nonoxidative metabolites of ethanol formed by an enzymatic esterification of ethyl alcohol with free fatty acids and other lipids by FAEE synthase and acetyl-CoA/ethanol O-acyl-transferase.
    - Meconium
- Phosphatidylethanol (PEth)
  - Provides a detection of alcohol abuse with 99% sensitivity (far higher than traditional blood testing methods) A blood spot/micro sample can be collected by finger prick making it less invasive than a full venipuncture (needle and vial collection) PEth testing is not affected by medications, illnesses, previous drinking habits, age or the health of the donor The result provides a detection period of up to 3-4 weeks Ideally combined with Hair or Nail testing.
  - Phosphatidylethanol testing, known as PEth testing, is a highly reliable blood test allowing the detection of chronic excessive alcohol abuse over the previous 3-4 weeks. With a sensitivity and accuracy rate of over 99% it is being widely adopted as a replacement to CDT, LFT & MCV testing which offers up to a 77% sensitivity rate

Overall Approach

- The approach should consist therefore of the identification of the probable drug class(es), selection of assays which will affect management, and advice/support to the clinician in sample collection and the interpretations of results.
- The diagnosis depends not only on the results of lab tests, but also on the wealth of historical and clinical information that may be missing, overlooked, or mis-interpreted.
- Laboratory testing has many challenges: administrative, analytical and financial but the service is an important tool in your resources for better patient care.