The Scoop on Biological Testing for Detecting or Confirming Drug-Exposed Newborns – What, When and How?

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University of Utah, ARUP Laboratories
Objectives

• Compare and contrast approaches to drug testing.

• Understand the strengths and weaknesses of meconium and umbilical cord tissue for drug testing.

• Discuss “what to do next” when drug testing results are unexpected.
The “perfect” drug test

• Specific – detects the drugs of interest with low or no false positives (~accuracy)
• Sensitive – detects the drugs of interest with low or no false negatives (~cutoff)
• Precise (reproducible)
• Rapid
• Inexpensive
• Not vulnerable to adulteration
• Meets the needs of testing
Detection of drugs depends on

- Quality of specimen
- Drug use patterns
- Patient pharmacokinetics
- Technology used for testing
- Assay design and performance
- Cutoff concentrations
Traditional reason for drug testing: forensic
Detect inappropriate drug use/exposure

- Pre-employment, workplace settings
- Military
- DOT, safety-sensitive settings
- Competitive sports
- Schools
- Insurance companies
- Forensic
  - Traffic violation, accident
  - Crime investigation, death (non-hospital)
  - Court-ordered

- Goal
  - Identify use of prohibited drugs
- Actions
  - Fines
  - Penalties
  - Removal from workplace/activity
  - Incarceration
Traditional Analytical Approach

• Immunoassay-based screen
• Confirm positive results with a mass spectrometric method (GC-MS, LC-MS)

• Prevents false positive results
• False negatives are not addressed unless every relevant analyte that is not detected by the screen is verified by confirmation testing
Immunoassays

Reagent Antibodies

Drugs analytes in Sample

Drug 1

Drug 1 metabolite

Drug 2

Signal

Method schematics provided by Dr Fred Strathmann
What is a false positive immunoassay result?

A positive signal can be generated in an immunoassay by a drug or other compound that the test was not designed to detect, leading to a “falsely” positive result.
What is a false positive immunoassay result?

- Relatively common for **some** (not all) immunoassays
  - Amphetamines
  - Tricyclic antidepressants
- Rates vary with individual assays and drug
- Patients are often familiar with common causes of false positive results, and some causes are not really false positives, but rather, limitations of the testing.
  - Vick’s nasal inhaler
  - Poppy seeds
- The cause of the false positive may not be known.
**Key Points**

- Cutoff is based on a “representative” compound/calibrator.
- Cross-reactivity allows for structurally related compound detection.
- Cross-reactivity is responsible for false positives.

**Table 7 — Concentrations (ng/mL) of Opiate Compounds That Produce a Result Approximately Equivalent to the 300 ng/mL Cutoff**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (ng/mL) at 300 ng/mL Cutoff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Codeine</td>
<td>102-306</td>
</tr>
<tr>
<td>Dihydrocodeine</td>
<td>291</td>
</tr>
<tr>
<td>Hydromorphone</td>
<td>247</td>
</tr>
<tr>
<td>Hydromorphone</td>
<td>498</td>
</tr>
<tr>
<td>Levallophan</td>
<td>&gt;7500*</td>
</tr>
<tr>
<td>Levodropranol</td>
<td>1048</td>
</tr>
<tr>
<td>Meperidine</td>
<td>&gt;500000</td>
</tr>
<tr>
<td>6-Acetylmorphine</td>
<td>435</td>
</tr>
<tr>
<td>Morphine-3-Glucuronide</td>
<td>626</td>
</tr>
<tr>
<td>Nalorphine</td>
<td>9362*</td>
</tr>
<tr>
<td>Naltrexone</td>
<td>828139</td>
</tr>
<tr>
<td>Oxycodone</td>
<td>2550</td>
</tr>
<tr>
<td>Oxymorphone</td>
<td>&gt;20000</td>
</tr>
</tbody>
</table>

Therapeutic doses of ofloxacin (Flagyl) or levofloxacin (Levaquin), non-opiates, may produce positive results with this assay. A positive result from an individual taking ofloxacin or levofloxacin should be interpreted with caution and confirmed by another method.
Monitoring rates of false positive and negative results

• Determined by the laboratory
  • Calculated based on agreement between immunoassay and confirmation testing results; expressed as a percent
  • May vary based on patient population

• Requires alignment of an appropriate confirmation test with each immunoassay
  • Will vary based on the specific tests involved
  • Requires confirmatory testing of all results during a study
  • Data are stronger with higher numbers of samples tested

• Rates of false positives are frequently monitored by laboratories that offer reflex testing.
How bad is a false positive rate of 10%?

• Means that 10% of positive results are false (not that 10% of all results are false)

• Depends on the positivity rate for the drug (population dependent)
  • Cocaine positivity rate of 5% means that 50 out of 1000 patients will be positive
  • False positive rate of 10% means that 5 of those 50 (5 of 1000) will be false positives

• In a forensic setting, all positive results are confirmed before being reported.
Key Points

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<td>Morphine-3-Glucuronide</td>
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<td>Nalorphine</td>
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<td>Naloxone</td>
<td>693130</td>
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<td>Oxycodone</td>
<td>2550</td>
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</table>

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Are results consistent with pre-test expectations?

Will the unexpected result impact patient management decisions?
Chromatography (LC or GC)

1. Everything starts at the same time
2. Mobile phase moves in one direction
3. Drug analytes have a unique “retention time” as they repeatedly “choose” mobile phase or stationary phase
4. Requires calibration with known drug standards
5. Must be paired to a detection technology
Tandem Mass Spectrometry (MS/MS)

Key Concepts
1. Precursor and Product Ion filtered by mass to charge (m/z) ratios
2. Instrument parameters are designed such that only subsets of ions reach the detector
3. Signal intensity is correlated with concentration of drug analytes
Clinical drug testing

• Therapeutic monitoring
• Qualify for surgery
• Organ transplantation
• Emergency room visit
• Mental health
• Addiction management
• Labor & Delivery

• Goals
  • Improve patient care
  • Identify use of legal and illegal drugs that may cause adverse outcomes
  • Verify adherence to prescribed medications
  • Detect undisclosed drug use/exposure
  • Protect clinic/providers

• Actions
  • Medical decision making
  • Social decision making
  • Promote safety to individuals, families and communities
Evolving approach

- Understand needs
- Understand testing options and limitations
- Select best test(s)
- Evaluate results
- Consider targeted/secondary testing for unexpected or inadequate results
Pregnancy is a unique opportunity to identify and manage drug use/misuse

May represent the only time a woman seeks medical care and is forthcoming about drug use/misuse
Women are an important demographic in the opioid epidemic

• Women receive more opioid prescriptions than men  
  US Opioid Prescription Claims, 2015

• Heroin use increased 100% among women between 2002 and 2013, 
  compared with a 50% increase among men  
  National Survey on Drug Use and Health

• Every 3 minutes a woman seeks emergency care related to prescription 
  opioid misuse  
  SAMSHA 2013

• Deaths from prescription opioids among women increased more than 
  400% from 1999-2010, compared with a 237% increase among men  
  CDC, 2013
## Methadone vs Buprenorphine in pregnancy

<table>
<thead>
<tr>
<th></th>
<th>Methadone</th>
<th>Buprenorphine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Administration</strong></td>
<td>Observed</td>
<td>Out-patient</td>
</tr>
<tr>
<td><strong>Half-life</strong></td>
<td>8-20 hrs</td>
<td>30 hrs</td>
</tr>
<tr>
<td><strong>Retention rates</strong></td>
<td>78.1%</td>
<td>57.7%</td>
</tr>
<tr>
<td><strong>Overdose mortality risk</strong></td>
<td>4.18/1000</td>
<td>0.98/1000</td>
</tr>
<tr>
<td><strong>Success with polysubstance</strong></td>
<td>Yes</td>
<td>Limited</td>
</tr>
<tr>
<td><strong>NAS incidence</strong></td>
<td>57%</td>
<td>47%</td>
</tr>
<tr>
<td><strong>NAS onset</strong></td>
<td>3-5 days</td>
<td>within 48 hrs</td>
</tr>
</tbody>
</table>

Illicit drug usage (US, all populations)

**ILlicit DRUG USE IMPACTS MILLIONS: MARIJUANA MOST WIDELY USED DRUG**

- **Marijuana**: 13.9% (37.6 million)
- **Misuse of Psychotherapeutic Rx Drugs**: 6.9% (18.7 million)
- **Cocaine**: 1.9% (5.1 million)
- **Hallucinogens**: 1.8% (4.9 million)
- **Inhalants**: 0.6% (1.7 million)
- **Methamphetamines**: 0.5% (1.4 million)
- **Heroin**: 0.4% (948,000)

*2016 NSDUH REPORT AMERICA’S BEHAVIORAL HEALTH CHANGES & CHALLENGES. WWW.SAMHSA.GOV/DATA*
Marijuana use during pregnancy, by age (self-report)

Marijuana use during pregnancy, by trimester (self-report)

Cannabis and the developing brain

Grant et al. *Pharmacol Ther* 182:133-51, 2018

**GRP55:**
G protein-coupled receptor

**2-AG:**
2-Arachidonoylglycerol

**AEA:**
Anandamide

**CB1:**
Cannabinoid receptor 1

**CB2:**
Cannabinoid receptor 2
Colorado study (Metz et al, presented at SMFM, 2018)

• Cross-sectional study of all deliveries at two urban medical centers in Colorado over a two-week period; 116 women completed the study

• Data collected
  • Self-report of cannabis use to the healthcare provider at the time of admission was collected.
  • Anonymous survey detailing cannabis use during pregnancy.
  • Biological drug testing for cannabis exposure.
Self-report is NOT as accurate as biological testing

• Self-report
  • 2.6% reported marijuana use to healthcare providers at admission.
  • 6.0% reported use in the last 30 days on anonymous survey.
  • All births associated with self-report of cannabis use were associated with positive drug tests in newborns.

• Drug testing
  • 28% of samples were positive for cannabis analytes.
  • Positivity varied based on specific patterns of analytes.
Smoking and drinking while pregnant

Cigarettes in pregnancy:
• 15.9% 15-44 yrs. of age
• 65.4% also use alcohol
• 23.0% also use illicit drugs

Alcohol in pregnancy:
• 8.5% of 15-44 yrs. of age
• 2.7% binge drinkers
• 0.3% heavy drinkers

2012 SAMHSA National Survey on Drug Use and Health, NSDUH Series H–46, HHS Publication 13–4795, Rockville, MD, USA

Universal screening during pregnancy?

- Unbiased, by definition.

- Test performance characteristics and content must be understood to select the most appropriate test(s).

- When to test?
  - First pre-natal visit
  - At delivery
  - Sometime in between?

- To be used in cooperation with psychosocial evaluations, physical exam, history, etc. with the overall goal to promote safety – should not be punitive.
Newborn testing
Is testing mandated in the US? ...No


• The 2015 Protecting Our Infants Act requires the Department of Health and Human Services (HHS) to review its activities related to prenatal opioid use, including NAS, and develop a strategy to address gaps in research and gaps and overlap in programs.

• Testing criteria need to be unbiased because testing for illicit substances in the absence of medical indications may violate a patient’s civil rights.
Highlights of State Policies: addressing substance abuse during pregnancy

• 24 states and the District of Columbia consider substance use during pregnancy to be child abuse under civil child-welfare statutes, and 3 consider it grounds for civil commitment.

• 23 states and the District of Columbia require health care professionals to report suspected prenatal drug use, and 7 states require them to test for prenatal drug exposure if they suspect drug use.

• 19 states have either created or funded drug treatment programs specifically targeted to pregnant women, and 17 states and the District of Columbia provide pregnant women with priority access to state-funded drug treatment programs.

• 10 states prohibit publicly funded drug treatment programs from discriminating against pregnant women.
California status

• SUBSTANCE USE DURING PREGNANCY CONSIDERED: no comments

• WHEN DRUG USE SUSPECTED, STATE REQUIRES: reporting required; no comments on testing

• TARGETED DRUG TREATMENT FOR PREGNANT WOMEN: yes, has been created

https://www.guttmacher.org/state-policy/explore/substance-use-during-pregnancy
Which births to test? Risk factors...

**Mothers who**
- History of drug use/abuse
- Known at-risk home environment
- <18 yrs
- Had no or little pre-natal care (<5 visits)
- History of hepatitis, AIDS, prostitution
- Present with placental abruption
- Present with unexplained premature labor

**Newborns with**
- Unexplained neurological complications
- Unexplained intrauterine growth retardation
- S/S of neonatal abstinence syndrome (NAS)

Most common risk factors for positive results

- 68.6% Maternal history of non-medical drugs
- 51.1% Maternal history of tobacco
- 90.8% Maternal history of tobacco and/or non-medical drugs
- 96.9% Maternal history of tobacco, non-medical drugs, poor prenatal care, and/or social risk factors (e.g., domestic violence, incarceration, history of prostitution, HIV, etc.)

Wood et al., *BMC Pregnancy and Childbirth* 14:250, 2014
Typical approach to testing

Consider local and/or state policies
Evaluate risk of drug exposure during pregnancy
Collect specimen(s)
Submit specimen(s) for testing or store specimens
Monitor for NAS or other concerns
Specimens

- Amniotic fluid
- Vernix
- Placenta
- Umbilical cord

- Urine
- Oral fluid
- Sweat
- Blood
- Meconium
- Hair
- Nails
### Estimated Detection Window for Various Biological Specimens

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Hours</th>
<th>Days</th>
<th>Weeks</th>
<th>Months</th>
<th>Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral Fluid</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Breast Milk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placenta</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweat*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vernix</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amniotic Fluid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nails*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hair, Neonatal</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Umbilical Cord</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meconium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hair, Maternal*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Actual detection window is drug-dependent and also reflects patterns of use, dose, and performance of laboratory testing.*

*Characterization of chronology and duration of drug use depends on time reflected by the collection.*

Wabuyele, et al.. *TDM* 40(2):166-85, 2018
Short-term detection specimens

• Oral fluid
• Blood

• Limited specimen availability for newborns.
• Detection limit of hours for most drugs; parent drugs are predominate analytes.
• Not routinely evaluated for newborns.
Medium-term detection specimens

- Urine
- Sweat
- Amniotic fluid
- Vernix
- Placenta

- Missing the first void of urine is likely to compromise detection.
- Diagnostic yield for chronic exposure has been poor for newborns.
- Reports of soap contamination affects detection.
- Typical cannabis analytes targeted for adults often screen positive but do not confirm with infant urine.

Long-term detection specimens

- Hair
- Nails
- Meconium
- Umbilical cord

- May verify expected drug use and detect unexpected drug use during approximately the last trimester of a full term birth.
- Risk of external contamination
- Target analytes?
- Appropriate cutoffs for detection?
Meconium

• First stool of the newborn.
• Used for drug testing for ~25 yrs.
• Begins to form at ~12-16 wks gestation.
• Accumulates over remainder of pregnancy (a non-linear process).
• Usually passes within 48 hours of birth.
• Collection requires coordinated efforts and may not be available.
Umbilical cord tissue

• Forms ~5th week of gestation.
• Grows with fetus throughout pregnancy (non-linear process).
• Drugs appear to deposit consistently across the length of cord.
• Easy to collect at time of birth. *** MAJOR ADVANTAGE ***
• Particularly useful for high-risk scenarios wherein time to result is critical.
• Concentrations of drug analytes are lower in cord than in meconium, but can be detected with appropriate methods.
• Detection depends on many factors including quality of collection and drug use-patterns.
Major advantages of these specimens:

**Meconium**
- Concentrations of drug analytes in meconium are much higher for most drugs, than in cord.
- No debate about specimen reflecting exposure to the infant versus the mother.
- Substantial research to support interpretation.

**Cord**
- All infants are born with a cord for which ample specimen is available at birth; time to result may be faster than meconium.
- Avoids detection of drugs that are administered directly to the infant after birth.
- Less likely to be discrepant in multiple births.
Major disadvantages of these specimens:

**Meconium**
- Collection process
- Specimen availability

**Cord**
- Not always as sensitive as meconium for many drugs

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Specimen heterogeneity

- Risk of contamination from other specimens
- Negative results do not exclude possibility of exposure
- No standardized methods or cutoff concentrations
Common questions about newborn drug testing

• Can we detect first trimester exposures?
Considerations

- Size matters

- Samples are mixed/homogenized in lab
- Stability/metabolism of drugs in meconium
Observations of the heroin metabolite 6-monoacetylmorphine (6-AM)

- Rarely observed in most specimens
- Generally in low concentration
- Stability concerns

Wu et al., JAT, 41(3):196-204, 2017
Patterns of opiates

McMillin et al, Ther Drug Monit 37(5):568-80, 2015
Common questions about newborn drug testing

• Can we detect first trimester exposures?
• Can we detect how frequently the mother used drugs, or how much?
ARUP/IHC nicotine study

• Consented mothers at delivery.

• Surveyed smoking history:
  • Smoked consistently (3-20 cigarettes/day) during pregnancy, n = 14.
  • Stopped smoking during pregnancy, n = 2.
  • Exposed to second-hand smoke only during pregnancy, n = 3.

• Determined nicotine and metabolites in paired cord and meconium samples.

<table>
<thead>
<tr>
<th>Nicotine (ng/g)</th>
<th>Cot (mec)</th>
<th>3-OH Cot (mec)</th>
<th>Smoked/day/trimester</th>
</tr>
</thead>
<tbody>
<tr>
<td>322</td>
<td>150</td>
<td>354</td>
<td>20</td>
</tr>
<tr>
<td>224</td>
<td>103</td>
<td>231</td>
<td>5-7</td>
</tr>
<tr>
<td>322</td>
<td>188</td>
<td>223</td>
<td>10</td>
</tr>
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<td>172</td>
<td>70</td>
<td>222</td>
<td>8-10</td>
</tr>
<tr>
<td>590</td>
<td>317</td>
<td>187</td>
<td>3-4</td>
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<tr>
<td>183</td>
<td>53</td>
<td>110</td>
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</tr>
<tr>
<td>84</td>
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<td>76</td>
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<td>5-6</td>
</tr>
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<td>43</td>
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<td>18</td>
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<td>0</td>
</tr>
<tr>
<td>&lt;4</td>
<td>&lt;2</td>
<td>&lt;4</td>
<td>0</td>
</tr>
</tbody>
</table>

Common questions about newborn drug testing

• Can we detect first trimester exposures?
• Can we detect how frequently the mother used drugs, or how much?
• Can we detect drugs administered in the hospital?
## History of morphine in hospital

<table>
<thead>
<tr>
<th></th>
<th>Morphine (ng/g)</th>
<th>Hydrocodone (ng/g)</th>
<th>Hydromorphone (ng/g)</th>
<th>Known Maternal Prescription</th>
<th>Known Infant Prescription</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1014</td>
<td>Neg</td>
<td>34</td>
<td>Morphine</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>510</td>
<td>Neg</td>
<td>11</td>
<td>Morphine</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>390</td>
<td>34</td>
<td>Neg</td>
<td>Hydrocodone</td>
<td>Morphine</td>
</tr>
<tr>
<td>6</td>
<td>226</td>
<td>3</td>
<td>32</td>
<td>Morphine</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>42</td>
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<td>Neg</td>
<td>Morphine</td>
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<tr>
<td>8</td>
<td>3159</td>
<td>Neg</td>
<td>1066</td>
<td>Hydromorphone</td>
<td>Morphine</td>
</tr>
<tr>
<td>3</td>
<td>41</td>
<td>26</td>
<td>Neg</td>
<td>Morphine</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>23</td>
<td>Neg</td>
<td>11</td>
<td>Morphine</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>41</td>
<td>2</td>
<td>Neg</td>
<td>Morphine</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>31</td>
<td>Neg</td>
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<td>Morphine</td>
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• Can we detect how frequently the mother used drugs, or how much?
• Can we detect drugs administered in the hospital?
• What if results from multiple births (e.g. twins) don’t agree?
Discrepant results in twins
(n= 1,084 pairs)

Why would results be discrepant?

• Differential treatment of the infants after birth
• Timing of meconium passage/collection fell before drug administration for one infant, and after drug administration for the other
  • Barbiturates (33% mismatch rate)
  • Opiates (30% mismatch rate)
  • Benzodiazepines (28% mismatch rate)
Why would results be discrepant?

• Results are close to the reporting/detection limit for the test
  • Amphetamine (13% mismatch rate)
  • THC (9% mismatch rate)
  • Methadone (8% mismatch rate)
  • Methamphetamine (5% mismatch rate)
  • Cocaine (4% mismatch rate)
9-carboxy-THC results for twins

Postpartum monitoring

• Pediatric monitoring/care as needed.

• Maternal urine drug testing is recommended. Postpartum compliance with opioid substitution is generally poor.
  • 56% discontinuation of methadone within 6 months of birth (Wilder et al, *Drug and Alcohol Dependence* 149:225-31, 2015)

• Counsel women not to breastfeed if
  • Concomitant use of multiple prescription medicines
  • Evidence of illicit or non-prescribed drug use
  • Behavioral indicators of aberrant drug use
  • Chronic alcohol use
  • Lack of family and community support systems
Breast or bottle?

The Academy of Breastfeeding Medicine Protocol #21, 2015 provides guidelines for breastfeeding when drug use is known.

- Concentrations of methadone and buprenorphine in breast milk are low and breastfeeding is encouraged.

- Concentrations of cannabis analytes are up to 8 times higher than in maternal plasma. Breastfeeding is discouraged unless mother discontinues use of cannabis products.

Summary and conclusions

• Biological testing can detect drug use/exposure that can improve patient care decisions.

• Meconium and umbilical cord testing are useful tools for identification of *in utero* drug exposure.

• Interpretation of results depends on a good understanding of
  • Maternal history
  • Infant history
  • Specimen collection and handling
  • Limitations of testing (laboratory contacts)
Thank-you for your attention!!!