Drug Screening in Biological Samples using High Resolution Mass Spectrometry

William Clarke, PhD, MBA, DABCC
Johns Hopkins University School of Medicine
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RAPID ANTIRETROVIRAL (ARV) SCREENING
Rapid Analysis with Q Exactive and LC-MS

• Preparation: protein precipitation plates on a Tecan Evo robotic station

• Two minute chromatographic method for 20 compounds

• Full scan MS-data dependent MS2 (ddMS2): fragmentation is triggered if a compound of interest is detected above a threshold; exact mass for analysis of fragments
  • Positive mode electrospray ionization; resolution = 17.5K at m/z 200

• Detection utilizes 1-3 product ions per compound; verification possible through data query for precursor exact mass
Nevirapine (NVP) 20 ng/mL in serum

- NVP precursor XIC
- NVP product ion 1 XIC
- NVP product ion 2 XIC
- NVP product ion 3 XIC
High Throughput Screening Assay

• Automated sample preparation
  • 30 min/96-well plate (active run time); 0.3 min/sample

• 4 min to first result by LC-MS
  • 2 min sample to sample

• Approx. 3h/plate (172 min for subjects + QC)

• Overnight runs (18h) = 6 plates per instrument

• 2 instruments = 972 specimens/day

• LOD = 2-20 ng/mL for all ARV drugs
Applications of the multi-drug assay in HPTN studies

Discrepant HIV diagnostic test results

**HPTN 043:** Most HIV-infected adults with discordant rapid tests were virally suppressed without ARV drugs

Fogel et al. J AIDS. 2015; 69:446

Cross-sectional HIV incidence (as part of a multi-assay algorithm)

**HPTN 043:** 6.7% of MAA-positive individuals had ARV drugs and were excluded from incidence assessments

Applications of the multi-drug assay in HPTN studies

Transmitted HIV drug resistance

**HPTN 061**: Analysis of ARV drug resistance in seroconverters; estimation of transmitted drug resistance was reduced (23% → 12%) after accounting for ARV drug use

Chen et al. J AIDS 2015; 69:446
Applications of the multi-drug assay in HPTN studies

Undisclosed ART use among HIV-infected participants

**HPTN 052**: 45 (46.9%) of 96 “ARV naïve” index participants who had a VL<400 at enrollment were on ART; many continued off-study ART after enrollment


Undisclosed knowledge of HIV status

**HPTN 061**: >40% of 155 men initially characterized as “newly diagnosed, ARV naïve” were on ART at enrollment; many had unusual patterns of ARV drugs detected

Applications of the multi-drug assay in HPTN studies

Use of ARV drugs in HIV-uninfected cohorts

**HPTN 064:** 2% of 1,806 HIV-uninfected women had ARV drugs detected at enrollment (15% in Baltimore; 5% in Bronx; NNRTIs and PIs; 1-4 drugs/sample)


**HPTN 068:** None of >2,000 HIV-uninfected young women had ARV drugs detected at enrollment

Zhang, Sivay et al. Manuscript in preparation

**HPTN 073:** Two of 208 HIV-uninfected Black MSM were taking off-study TDF/FTC at enrollment

Zhang, Manuscript submitted
Applications of the multi-drug assay in HPTN studies

Population-level ARV drug use

**HPTN 043:** ARV drug use was analyzed in a large community-randomized clinical trial; ARV drug use was associated with sex (women>men), pregnancy, older age, and study site; increased ARV drug use was associated with reduced HIV incidence at one study site

Fogel et al. J AIDS. 2017; 74:158
UNTARGETED TOXICOLOGY SCREENING
Untargeted LC-HRMS Screening

• Samples prepared by simple protein precipitation and dilution; 30-minute mixed mode chromatography

• Mass peaks are selected based on minimum intensity threshold (ion current in quadrupole)

• Selected peaks are fragmented and analyzed by high-resolution orbitrap
  • Data-dependent fragmentation and analysis

• Resulting pattern matched to stored mass spectra patterns
  • Curated spectra: MZ cloud
  • Theoretical spectra: ChemSpider
Analysis of known cocaine positive specimen
Analysis of known cocaine positive specimen
Analysis of known cocaine positive specimen
Analysis of known cocaine positive specimen
Analysis of urine toxicology negative control
Analysis of urine toxicology negative control
Analysis of urine toxicology negative control
Analysis of urine toxicology negative control
Next Steps

• Post-analysis data processing is necessary to exclude endogenous metabolites

• Analyze “drug-free” urines to set exception list

• Analyze known pain management samples to optimize algorithm

• Analyze blinded samples from external reference lab
Next Steps

• Repeat workflow optimization with serum samples

• Analyze known serum/plasma TDM samples

• Analyze serum toxicology samples (collaboration with Medical Examiner?)

• Validation of both urine and serum workflows and sample preparation
Specimen preparation and LC-HRMS

Generate compound list from library match

Remove excluded compounds

Preliminary Report

Obtain compounds from prelim report

Analyze & build local library

Final Report (confirmed)
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If there are no questions, we'll move on to the next chapter.

I have a question.

Certainly, Calvin. What is it?

What's the point of human existence?

I meant any questions about the subject at hand.

Oh.

Frankly, I'd like to have the issue resolved before I expend any more energy on this.

Questions??

wclarke@jhmi.edu