Getting a Handle on Your Biggest Volumes

Dr Russell Grant
Laboratory Corporation of America® Holdings

The Different Types of Volume

- **Common Needs**
  - Redundant systems (Automation and instruments)
  - High-throughput multiplexing LC

- **Many Assays – few samples (Dynamic)**
  - Manual (real time) and automated
  - “Generic” sample preparation
  - Sporadic calibration

- **Same assay – many samples (Batch)**
  - “Common” technology usage (Platform expertise)
  - Process Automation – end to end + electronic

Patient Service Network:
Interaction, Phlebotomy and Triage

- Beacon Touch
- Patient Details Entry/Verify
- Touch screen/Draw/Print
- Barcode/Verify/Scan
- Process/Ship
### Laboratory: Parent tube and IS addition Automation

**TECAN Freedom Evo**

**Benefits to Automated Sample Prep**

- 8 opposable thumbs vs 1
- Automated batch building - ID retained
- Proven accurate and precise
- Error monitoring – PMP
- Platform expertise – Not assay

### Manual v Automated Correlation

<table>
<thead>
<tr>
<th>Variable</th>
<th>Manual</th>
<th>Automated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope</td>
<td>1.008</td>
<td>1.008</td>
</tr>
<tr>
<td>Correlation Coef</td>
<td>0.999</td>
<td>0.999</td>
</tr>
<tr>
<td>Mean Bias</td>
<td>1.8%</td>
<td>1.8%</td>
</tr>
</tbody>
</table>

*Freedom Evo® is a registered trademark of TECAN*

### Intermediate (96-well plate processing (ALD®))

- 2 or 4 x 96-well plates (SPE and SLE)
- 12 solvents
- Positive pressure
- Manifold heating

**FTE Time**

<table>
<thead>
<tr>
<th>Manual</th>
<th>Automated</th>
</tr>
</thead>
<tbody>
<tr>
<td>81 min</td>
<td>12 minutes</td>
</tr>
<tr>
<td>Total time minus 5 min</td>
<td>15 min Hands on</td>
</tr>
<tr>
<td>Variance reduced 18% to 11%</td>
<td></td>
</tr>
</tbody>
</table>

*ALD is a registered trademark of SPEWare*

### Islands of Automation

<table>
<thead>
<tr>
<th>Manual Batch Prep (n=192)</th>
<th>Automated Batch Prep (n=192)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prep Batch 10 min</td>
<td>Prep Batch 10 min</td>
</tr>
<tr>
<td>Pipette Samples 60 min</td>
<td>Pipette Samples 35 min</td>
</tr>
<tr>
<td>Transfer to 96-well plate 10 min</td>
<td></td>
</tr>
<tr>
<td>SLE Extraction 30 min</td>
<td>SLE Extraction 30 min</td>
</tr>
<tr>
<td>Evap/Recon 25 min</td>
<td>Evap/Recon 25 min</td>
</tr>
<tr>
<td>Mass Spec Analysis 420 min</td>
<td>Mass Spec Analysis 420 min</td>
</tr>
<tr>
<td>125 min Hands on</td>
<td>15 min Hands on</td>
</tr>
</tbody>
</table>

*Evo® is a registered trademark of Tecan, ALD® is a registered trademark of SPEWare*
High Throughput Multiplexing Tools

ARIA® Transcend® TLX-4 MS Detects
(2x600bar binary + 2 valves/channel)

Tricyclic antidepressants panel
2.17 Min total cycle time
30 seconds diverted to MS

API 5000® /5500® Triple Quadrupole

Max. 2.9e6 cps.

ARIA and Transcend are registered trademarks of Thermo Corporation,
API 5000 and 5500 are registered trademarks of AB SCIEX

TFC-LC – Dynamic

Assay Cycle times: 2.1 – 2.8 minutes
MS/MS Acquisition: 24 – 60 seconds

Method Development – TFC-LC Chromatofocusing

Elute Pump Flow Rate

1.5mL/min

0.1-100ng/mL Calibrator.
50% Loop Composition
Load Pump Flow = 0.5mL/min

Benzodiazepine Panel
Nordiazepam, Diazepam, Clonazepam,
Norchlordiazepoxide, Chlordiazepoxide

Tricyclic Antidepressant Panel
Nortriptyline, Desmethyldoxepin,
Desipramine, Amitriptyline, Doxepin,
Imipramine, Desmethyliclonipramine,
Clomipramine
**TFC-LC and Phospholipid Depletion [1]**

- **TFC column = Cyclone P, 50 x 0.5mm**
- **Loop Size = 100 µL**
- Loop contents modified from 10-100% for ACN, 1:1 ACN/MeOH or MeOH
- Glycophosphatidyl cholines recovery < 1%
- Lysophosphatidyl cholines recovery >10% for ACN and 1:1 ACN/MeOH and >30% for MeOH

**Protein Precipitated Sample (A)**
- Phospholipid (P) co-elution = additional chromatographic resolution required

**Minimize ESI Matrix effects = LC Flexibility**

---

**TFC-LC-API5000 Selectivity and Reproducibility**

- **Nortriptyline IS Response**
  - Coefficient of Variation = 3.5%

- **Methotrexate IS Response**
  - Coefficient of Variation = 2.4%

**Goal is Reproducible Recovery not Absolute**

---

**Dilution Pre or Post IS addition – Linear 1/x Calibration**

- **Basic Hydrolysis for “Total”**
  - Total Dabigatran (including Glucuronide) pH 11 = 182.448 ng/mL
  - Free Dabigatran (Uncarcograted) pH 3 = 105.470 ng/mL

- **LC-MS/MS versus TFC-LC-MS/MS**
  - Sample diluted 1:9, then sub-aliquot 50 µL + 450 µL, 50ng/mL, D3-Dabigatran at 45 x LLOQ
  - CV = 7.52%, Bias = 12.11%

- **Sample 50 µL + 400 µL, 50mg/mL, D3-Dabigatran, then dilute 1:9 with Diluent (D), Dabigatran at 45 x LLOQ**
  - CV = 6.57%, Bias = -2.20%
Open Access LC Configuration – Multichannel usage

Tricyclics LLOQ – 3 Independent Channels

Channel 2  Channel 4

Analyte Reproducibility across 3 channels in Samples and QC’s

Amitriptyline 0.0 – 3.8% CV
Nortriptyline 0.4 – 5.3% CV
Desipramine 1.8 – 5.3% CV
Imipramine 0.7 – 4.1% CV
Doxepin 0.0 – 7.5% CV
Desmethyldoxepin 0.7 – 8.6% CV
Clomipramine 1.0 – 6.5% CV
Desmethylclomipramine 0.0 – 2.3% CV

Extending Calibration – Validation of Historical Curves

Levetiracetam + 24 hours
Deming Slope = 1.016
Intercept = -0.7749
Corr Coef, r = 0.9955

Oxcarbazepine + 24 hours
Deming Slope = 1.039
Intercept = -0.8327
Corr Coef, r = 0.9956

Lacosamide + 24 hours
Deming Slope = 1.039
Intercept = -0.9056
Corr Coef, r = 0.9927
Clinical Toxicology Analytical Cassetting Throughput

Analysis of 6 assays on 4 channels is executed in 6 minutes (A).
Analytical throughput of 1440 samples/system/day.
Clinical Tox profile (C), Opiates Profile 2 (O2), Cocaine Profile (Co), Benzodiazepine Profile (B), Tricyclics Profile (T) and Opiates Profile 1 (O1).

Analysis of a subset of assays 4 samples in 2.5 minutes (B).
Analytical throughput of 2304 samples/system/day.
Tricyclics Profile (T), Clinical Tox profile (C), Cocaine Profile (Co), Benzodiazepine Profile (B)
LC optimization for Batch Mode

Screening Gradient:
- 45 Sec Load
- 90 Sec Ramp
- 45 Sec Wash
- 60 Sec Equilibration

CHANGE
- Test reduced load times, truncate ramp, reduce wash and equilibration times

Final Gradient:
- 10 Sec Load
- 40 Sec Ramp
- 30 Sec Wash
- 40 Sec Equilibration
- 0.5 minute data window

Testosterone UHT - 4 channel LC-MS/MS (25-5000pg/mL)

- 20µL Serum/Plasma plus 20µL of 3C13-Testosterone. Precision of Tecan<2%.
- SLE, 2-fold concentration, inject 35µL buffer.

CDC Certification

Table 3. Percent of specimens that met desirable and minimal performance criteria from currently certified assays in CDC test program. a,b

<table>
<thead>
<tr>
<th>Assay A</th>
<th>Assay B</th>
<th>Assay C</th>
<th>Assay D</th>
<th>Assay E</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intra-assay precision</strong> (n = 20)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Samples (% CV)</td>
<td>63.9 (6.2%)</td>
<td>60.0 (6.0%)</td>
<td>65.9 (5.7%)</td>
<td>60.8 (5.6%)</td>
</tr>
<tr>
<td><strong>Inter-assay precision</strong> (n = 20)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Samples (% CV)</td>
<td>72.9 (7.0%)</td>
<td>72.9 (6.9%)</td>
<td>72.9 (7.0%)</td>
<td>72.9 (6.9%)</td>
</tr>
<tr>
<td><strong>Intra-assay accuracy</strong> (n = 10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Samples (pg/mL)</td>
<td>50.0 (5.0%)</td>
<td>50.0 (5.0%)</td>
<td>50.0 (5.0%)</td>
<td>50.0 (5.0%)</td>
</tr>
<tr>
<td><strong>Inter-assay accuracy</strong> (n = 10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Samples (pg/mL)</td>
<td>50.0 (5.0%)</td>
<td>50.0 (5.0%)</td>
<td>50.0 (5.0%)</td>
<td>50.0 (5.0%)</td>
</tr>
</tbody>
</table>

Notes:
- a) All results are reported in pg/mL.
- b) The percent of all samples that met the performance criteria are shown.
- c) The results are calculated using the 95th percentile of the distribution.
- d) The results are calculated using the 99th percentile of the distribution.
- e) The results are calculated using the 99.5th percentile of the distribution.
- f) The results are calculated using the 99.9th percentile of the distribution.
- g) The results are calculated using the 99.99th percentile of the distribution.
- h) The results are calculated using the 99.999th percentile of the distribution.
- i) The results are calculated using the 99.9999th percentile of the distribution.
- j) The results are calculated using the 99.99999th percentile of the distribution.
- k) The results are calculated using the 99.999999th percentile of the distribution.
- l) The results are calculated using the 99.9999999th percentile of the distribution.
- m) The results are calculated using the 99.99999999th percentile of the distribution.
- n) The results are calculated using the 99.999999999th percentile of the distribution.
- o) The results are calculated using the 99.9999999999th percentile of the distribution.
- p) The results are calculated using the 99.99999999999th percentile of the distribution.
- q) The results are calculated using the 99.999999999999th percentile of the distribution.
- r) The results are calculated using the 99.9999999999999th percentile of the distribution.
- s) The results are calculated using the 99.99999999999999th percentile of the distribution.
- t) The results are calculated using the 99.999999999999999th percentile of the distribution.
- u) The results are calculated using the 99.9999999999999999th percentile of the distribution.
- v) The results are calculated using the 99.99999999999999999th percentile of the distribution.
- w) The results are calculated using the 99.999999999999999999th percentile of the distribution.
- x) The results are calculated using the 99.9999999999999999999th percentile of the distribution.
- y) The results are calculated using the 99.99999999999999999999th percentile of the distribution.
- z) The results are calculated using the 99.999999999999999999999th percentile of the distribution.

References:
2D-LC to 1D LC – 25-Hydroxyvitamin D2/D3

**ARIATM TX4-API 4000:**
- PPT with 2x Concentration
- 2D-LC
- 5 minute cycle-time – 1 min acquisition

**ARIATM TranscendTM TX4-API 5000:**
- PPT with 3x Dilution – Supernatant injection
- 1D-LC
- 2.5 minute cycle-time – 30 sec acquisition
- CDC Certified 2014

Dead volume/recycling and Divert Valve – 1D

- Ramp to 95% B over 75 seconds at 1.25 mL/min
- Mobile phase B forward-washes the column at 2 mL/min for 20 seconds
- Loading pump back-washes the column at 2.5 mL/min for 20 seconds
- Step to 100% B for 23 seconds at 1.25 mL/min, then 2 mL/min for 5 sec
- Column is reconditioned with 80% Mobile Phase B at 1.25 mL/min

2 Pump/2 Valve Assay Switching – Speed and Open Access

(A) Common Solvents Alternate LC column Functionality
- Second (Load) Pump primed ready for redundancy

(B) Unique Solvents and Alternate LC column Functionality
- 4-Plex combination of solvent systems and LC columns per channel
8-Channel/8-Assay Open Access at >2000 samples/24Hr

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Method</th>
<th>Matrix</th>
<th>Samp. Volume</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Androstenedione</td>
<td>SLE+ S/P 1,3,7,11</td>
<td>B</td>
<td>U</td>
<td>20s</td>
</tr>
<tr>
<td>Aldosterone</td>
<td>SLE+ S/P 1,3,7,7</td>
<td>B</td>
<td>U</td>
<td>30s</td>
</tr>
<tr>
<td>DHEA</td>
<td>LLE/KMnO4 S/P 2,6</td>
<td>B</td>
<td>U</td>
<td>45s</td>
</tr>
<tr>
<td>17-OHProgesterone</td>
<td>SLE+ S/P 4</td>
<td>B</td>
<td>U</td>
<td>8</td>
</tr>
<tr>
<td>Cortisol</td>
<td>SLE+ U 8</td>
<td>B</td>
<td>U</td>
<td>8</td>
</tr>
</tbody>
</table>

Multiplexed LC-MS/MS Data – Acquisition Window

- Androstenedione (S/P) SLE+, 10ng/dL, 20s
- 17-OH-Progesterone (S/P) SLE+, 10ng/dL, 30s
- DHEA (S/P) SLE+, 20ng/mL, 20s
- 17-OH-Progesterone (S/P) SLE+, 20ng/mL, 45s
- Cortisol (U) SLE, 1ng/mL, 40s
- Cortisol (U) SLE+ U 8

MedWatch: 184 Drugs/Metabolites in Urine

- Opiates
- Opioids
- Benzodiazepines
- Drugs of abuse
- Antiepileptics
- Antipsychotics
- Antidepressants
- Analgesics
**Immunoassay and LC-MS/MS Concordance**

Pre ASCENT™ Implementation Analyst/Reporter

Post ASCENT™ Implementation Ascent
**Automated Data review**

If [examined value] exceeds [a threshold], then flag with [text string].

<table>
<thead>
<tr>
<th>Calibration Concentration Solvent</th>
<th>Implied from reconstituted</th>
<th>See Radio-Detection</th>
<th>Qual/Quant detection from expected by R.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibration Nitrile</td>
<td>Implied from reconstituted</td>
<td>See Radio-Detection</td>
<td>Qual/Quant detection from expected by R.</td>
</tr>
<tr>
<td>Calibration Regression slope</td>
<td>Calibration curve analyzable</td>
<td>Predikt Quality</td>
<td>Alignment of peaks with data +10</td>
</tr>
<tr>
<td>Calibration Standards Included</td>
<td>More than the product capacity, set plate for calibration</td>
<td>Mass High Signal No Peak</td>
<td>As peak and lowest intensity +10</td>
</tr>
<tr>
<td>Automated Data review Impact</td>
<td>40 QA rules (18 custom)</td>
<td>20 “Batch rules”</td>
<td>20 “Chromatogram rules”</td>
</tr>
</tbody>
</table>

- Requires a LOT of well designed and validated QA rules in Ascent®
- Identical to Autoanalyzer Autoverification

Ascent is a registered product of Indigo Biosystems.

**Isotope Dilution versus Reference Method**

Towards “Internal Calibration + Random Access”

Isotope Dilution: Range Analyte concentrations (Ac), one IS concentration (Ic),

- Analyte peak area (Ar)
- IS peak area (Ir)

Analyte concentration (Ac) / Internal standard concentration (Ic)

Reference method: Spike IS at concentration close to Analyte Concentration from Isotope Dilution

\[
Ac = \frac{(Ar \times Ic)}{Ir}
\]

Ac = 93474 * 1000 ng/dL / 108564 = 861.034 ng/dL

Internal standard and analyte MUST be analytically equivalent.
Use of IS near Medical Decision Points

**Reference Method**

- IS: \( m/z \) 120 – 76 (Y-axis ratio max 15)
- IS: \( m/z \) 120 – 76* 300nM (Y-axis ratio max 58)
- IS: \( m/z \) 120 – 76 (Y-axis ratio max 100)

**IS Transition ratio Low MDP/High MDP**

- MMA Concentration (nM)
- Reference method IS at 300 nM
  - Slope [Deming] = 0.961
  - Correlation Coefficient = 0.9946
  - Mean Bias = -1.55%
  - Patient mean = 271nM
- Reference method IS at 80 nM
  - Slope [Deming] = 1.077
  - Correlation Coefficient = 0.9921
  - Mean Bias = 4.45%
  - Patient mean = 308nM
- Reference method Mean of 300 and 80nM
  - Slope [Deming] = 1.010
  - Correlation Coefficient = 0.9976
  - Mean Bias = 0.97%
  - Patient mean = 298nM

**Medical Decision Points and IS position**

<table>
<thead>
<tr>
<th>Testosterone Medical Decision Points (MDP, ng/dL)</th>
<th>Age (y)</th>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &amp; Sx (F)</td>
<td>11-18</td>
<td>&lt;3</td>
<td>10</td>
</tr>
<tr>
<td>Age &amp; Sx (M)</td>
<td>11-18</td>
<td>&lt;3</td>
<td>970</td>
</tr>
<tr>
<td>20 – 50 (F Pre)</td>
<td>10</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>20 – 50 (M)</td>
<td>350</td>
<td>1030</td>
<td></td>
</tr>
<tr>
<td>60 – 80 (F Post)</td>
<td>7</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>60 – 80 (M)</td>
<td>7</td>
<td>40</td>
<td></td>
</tr>
</tbody>
</table>

**Testosterone**

- Testosterone: \( m/z \) 292 – 112 (4000ng/dL - 2.6e6)
- Testosterone: \( m/z \) 292 – 112 (1000ng/dL - 8.6e5)
- Testosterone: \( m/z \) 292 – 112 (50ng/dL - 2.1e4)
- Testosterone: \( m/z \) 292 – 112 (10ng/dL - 2.1e3)
- Testosterone: \( m/z \) 292 – 112 (5ng/dL - 1.5e3)
- Testosterone: \( m/z \) 292 – 112 (2.5ng/dL - 7.6e2)
### Determining IS Concentration - Gravimetry

#### Internal Standard Concentration (Ic) = \( \frac{(Ac \times Ir)}{Ar} \)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Analyte Peak Area (Ar)</th>
<th>IS Peak Area (Ir)</th>
<th>IS #1 (ng/mL)</th>
<th>IS #2 (ng/mL)</th>
<th>IS #3 (ng/mL)</th>
<th>IS #4 (ng/mL)</th>
<th>IS #5 (ng/mL)</th>
<th>IS #6 (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>10522.26</td>
<td>3.312</td>
<td>5.488</td>
<td>10.548</td>
<td>12.864</td>
<td>18.448</td>
<td>32.848</td>
<td>56.364</td>
</tr>
<tr>
<td>5</td>
<td>23821.96</td>
<td>7.550</td>
<td>55.229</td>
<td>96.847</td>
<td>1547.950</td>
<td>4161.766</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>95306.78</td>
<td>97.164</td>
<td>55.389</td>
<td>98.328</td>
<td>1583.146</td>
<td>4181.146</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>406503.06</td>
<td>6.062</td>
<td>54.440</td>
<td>97.318</td>
<td>1541.248</td>
<td>3981.718</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>1073077.63</td>
<td>9.322</td>
<td>96.437</td>
<td>96.437</td>
<td>1583.272</td>
<td>4475.856</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5000</td>
<td>528781.50</td>
<td>10603.594</td>
<td>95.907</td>
<td>55.655</td>
<td>100.661</td>
<td>1985.970</td>
<td>4049.095</td>
<td></td>
</tr>
<tr>
<td>10000</td>
<td>1018077.57</td>
<td>9845.678</td>
<td>94.787</td>
<td>58.074</td>
<td>104.044</td>
<td>1657.150</td>
<td>4269.227</td>
<td></td>
</tr>
</tbody>
</table>

**Mean IS Concentration equivalent (ng/dL)**

|          | 6.866 | 53.874 | 95.665 | 1516.180 | 3911.982 |

**Standard Deviation (% CV)**

|          | 10.548 | 7.671 | 7.526 | 7.851 | 8.512 |

#### Mean of 6 IS results (Cals) vs CDC Phase 1

**Slope [Deming] = 0.980 (0.972 – 0.988)**

**Intercept = 0.880 (-1.899 – 3.658)**

**Correlation Coefficient = 0.9997**

**Mean Bias = -1.662%**

#### Average IS versus Isotope Dilution

**Slope [Deming] = 0.945 (0.941 – 0.949)**

**Intercept = 0.080 (-1.546 – 1.707)**

**Correlation Coefficient = 0.9996**

**Mean Bias = 0.251%**

#### “Approximate Matching” IS versus Isotope Dilution

**Slope [Deming] = 1.002 (0.998 – 1.006)**

**Intercept = 0.000 (-1.506 – 1.507)**

**Correlation Coefficient = 0.9997**

**Mean Bias = 0.251%**
### Population Pools as Calibrators

<table>
<thead>
<tr>
<th>Age (y)</th>
<th>Low (ng/dL)</th>
<th>High (ng/dL)</th>
<th>N</th>
<th>Mean</th>
<th>Median</th>
<th>Measured (ID)</th>
<th>Measured (CDC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-10</td>
<td>&lt;3</td>
<td>10</td>
<td>10782</td>
<td>5.43</td>
<td>4.70</td>
<td>2.75</td>
<td>3.42</td>
</tr>
<tr>
<td>11-18 (F)</td>
<td>&lt;3</td>
<td>38</td>
<td>19453</td>
<td>30.68</td>
<td>28.70</td>
<td>27.56</td>
<td>28.35</td>
</tr>
<tr>
<td>11-18 (M)</td>
<td>&lt;3</td>
<td>970</td>
<td>25434</td>
<td>303.00</td>
<td>285.45</td>
<td>261.81</td>
<td>261.17</td>
</tr>
<tr>
<td>20-50 (F Pre)</td>
<td>10</td>
<td>55</td>
<td>6551</td>
<td>24.78</td>
<td>23.00</td>
<td>30.07</td>
<td>29.99</td>
</tr>
<tr>
<td>20-50 (M)</td>
<td>350</td>
<td>1030</td>
<td>28000</td>
<td>382.69</td>
<td>365.83</td>
<td>450.38</td>
<td>450.25</td>
</tr>
<tr>
<td>60-80 (F Post)</td>
<td>7</td>
<td>10</td>
<td>5830</td>
<td>18.62</td>
<td>17.05</td>
<td>16.98</td>
<td>17.48</td>
</tr>
<tr>
<td>60-80 (M)</td>
<td>7</td>
<td>40</td>
<td>21452</td>
<td>371.63</td>
<td>354.63</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

#### Boxcox transformation

- 60-80 F
- 60-80 M
- 11-18 M
- 20-50 F
- 20-50 M

#### Population Pools as Calibrators vs CDC Phase 1

Population Median
- Slope (Deming) = 0.901
- Intercept = 1.037
- Correlation Coefficient = 0.9992
- Mean Bias = -0.992%

Pool Targets from ID
- Slope (Deming) = 0.994
- Intercept = -0.526
- Correlation Coefficient = 0.9992
- Mean Bias = -0.799%

Pool Targets CDC Assigned
- Slope (Deming) = 0.991
- Intercept = -0.161
- Correlation Coefficient = 0.9992
- Mean Bias = -0.823%
Conclusions

Dynamic volume:
- Bracketing QC’s
- Generic sample preparation (good QQQ)
- Generic Chromatographic platform
- Longitudinal Calibration
- Automated data release

Batch volume:
- Contiguous Automation
- Very high throughput multiplexing
- Advanced LC configuration
- Robust LC methods
- Automated data release

Acknowledgements

LabCorp: Dr Christopher Shafor, Patricia Holland, Matt Crawford, Stacy Dee, Yvonne Wright, Martin Green, Dr Marcia Eisenberg, Gregory Jans, Dr Karla Walker

Essential Testing: Brian Rappold