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

Touch screen/Draw/Print
Barcode/Verify/Scan

Patient Details Entry/Verify

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

Slope (deming) = 1.008
Correlation Coef = 0.999
Mean Bias = 1.8%

Alternate (Quantitative) Method Comparison

X Method: Manual

Y Method: Automated

Regression Analysis

<table>
<thead>
<tr>
<th></th>
<th>Deming</th>
<th>Regular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope</td>
<td>1.008 (0.976 to 1.040)</td>
<td>1.002 (0.970 to 1.034)</td>
</tr>
<tr>
<td>Intercept</td>
<td>-17.0782 (-44.7156 to 10.5565)</td>
<td>-13.3677 (-49.6899 to 14.2315)</td>
</tr>
<tr>
<td>Std Err Est.</td>
<td>58.9813</td>
<td>56.8992</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

Reverse T3 (SPE) – 2x96-well plates

FTE Time
81 min to 12 minutes
Total time minus 5 min
IS Variance reduced 18% to 11%

ALD is a registered trademark of SPEWare
Islands of Automation

Manual Batch Prep (n=192)

- Prep Batch 10 min
- Pipette Samples 60 min
- Transfer to 96-well plate 10 min
- SLE Extraction 30 min
- Evap/Recon 25 min + 5 min
- Mass Spec Analysis 420 min

125 min Hands on

Automated Batch Prep (n=192)

- Prep Batch 10 min
- Pipette Samples 35 min
- Transfer to 96-well plate 3 min
- SLE Extraction 30 min
- Evap/Recon 25 min
- Mass Spec Analysis 420 min

15 min Hands on

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

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

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

Max. 2.9e6 cps.

API 5000® /5500® Triple Quadrupole

ARIA and Transcend are registered trademarks of Thermo Corporation,
API5000 and 5500 are registered trademarks of AB SCIEX
TFC-LC – Dynamic

TFC-LC Method Folding

Assay Cycle times: 2.1 – 2.8 minutes

MS/MS Acquisition: 24 – 60 seconds
Method Development – TFC-LC Chromatofocusing

Elute Pump Flow Rate
0.5mL/min

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

Benzodiazepine Panel
Nordiazepam, Diazepam, Clonazepam, Norchlordiazepoxide, Chlordiazepoxide

100ng/mL Calibrator
Load Pump Flow = 0.5mL/min
Elute Pump Flow = 0.5mL/min

Tricyclic Antidepressant Panel
Nortriptyline, Desmethyldoxepin, Desipramine, Amitriptyline, Doxepin, Imipramine, Desmethylclomipramine, 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.
Glycerophosphatidyl cholines recovery < 1%
Lysophosphatidyl cholines recovery >10% for ACN and 1:1 ACN:MeOH and >30% for MeOH

TFC-LC-API5000 Selectivity and Reproducibility

Loop composition (A) = 50% elutropic content
Loop composition (B) = 5% elutropic content

Coefficient of Variation = 3.5%

Nortriptyline IS Response

↓ Organic Composition in Loop = ↓ Signal

Sensitive instrumentation allows for signal reduction
Stable labeled internal standards/Calibration Curves

Goal is Reproducible Recovery not Absolute

Methotrexate IS Response
Sample diluted 1:9, then sub-aliquot 50μL + 450μL 50ng/mL (D₃-Dabigatran at 450 x LLOQ)

Total Dabigatran (Including Glucoronide)
pH 11, 2.5 Hr @ 37°C = 182.484 ng/mL

Free Dabigatran (Unconjugated)
pH 3 = 105.470 ng/mL

Sample 50μL + 450μL 50ng/mL D₃-Dabigatran, then dilute 1:9 with Diluent (D₃-Dabigatran at 45 x LLOQ)

Samples (n=6) at 5000ng/mL (5 x ULOQ)
CV = 7.52%
Bias = 12.11%

Samples (n=6) at 5000ng/mL (5 x ULOQ)
CV = 6.57%
Bias = -2.20%
IS selection aids Data Review

**Hydrocodone**
m/z = 300.2 → 257.2

**Codeine**
m/z = 300.2 → 215.2

**Hydromorphone**
m/z = 286.2 → 185.2

**Morphine**
m/z = 286.2 → 201.2

**Oxymorphone**
m/z = 302.2 → 242.2

**Dihydrocodeine**
m/z = 302.2 → 245.2

**Hydrocodone-d3**
m/z = 303.2 → 241.2

**Codeine-d6**
m/z = 306.2 → 115.2

**Hydromorphone-d6**
m/z = 292.2 → 152.2

**Morphine-d3**
m/z = 289.2 → 185.2

**Oxymorphone-d6**
m/z = 308.2 → 202.2

**Dihydrocodeine-d3**
m/z = 305.2 → 230.2

M+2 isotopic contribution
Open Access LC Configuration – Multichannel usage

Tricyclics LLOQ – 3 Independent Channels

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

**Amitriptyline Calibration Curve Overlay from LC Channels 2-4**

- Calibrate on 1 channel
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
Extending Calibration – Validation of Historical Curves

Levetiracetam + 144 hours
Deming Slope = 1.217
Intercept = -1.1966
Corr Coef, r = 0.9892

Oxcarbazepine + 144 hours
Deming Slope = 1.041
Intercept = -0.7982
Corr Coef, r = 0.9927

Lacosamide + 144 hours
Deming Slope = 1.065
Intercept = -0.8589
Corr Coef, r = 0.9920
Clinical Toxicology – Generic Prep/Analytical Platform for Rapid TAT and STAT Testing - Dynamic

>90 Analytes, multiple sub-panels matrix agnostic
50uL sample + 450uL IS in 0.1-1% Formic Acid,
Seal plate/Mix/Centrifuge/Inject
Calibrate Daily or weekly
QC bracket Release
Clinical Toxicology Analytical Cassetting Throughput

A 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).

B 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

Maintain Gradient Pitch (%/sec)*

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)

200µL Serum/Plasma plus 200µL $^{3}\text{C}_{13}$-Testosterone, Precision of Tecan® <2%

SLE, 2-fold concentration, inject 35µL LC-MS/MS

Testosterone 2.5 – 5000ng/dL (25-5000pg/mL): $m/z$ 289 – 97 (QN), 289 – 109 (QL)

$^{3}\text{C}_{13}$-Testosterone (~120ng/dL): 292 – 112 (QN), 292 – 100 (QL)

Female: Red top versus Serum Separator Tube Transition ratio Bias = -3.32% versus 6.57%
Mean SST Bias = -12.75%
Precision (CV, n = 24) = 1.95% versus 3.06%

99 samples in 60 minutes or 2376 samples/system/day – Cycle time = 2.4 min
### Table 3. Percent of specimens that met desirable and minimal performance criteria from currently certified assays in CDC HoSt program.\(^a\)

<table>
<thead>
<tr>
<th>Parameter and performance criteria</th>
<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>Imprecision, (b) (n = 40)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Desirable (5.3%)</td>
<td>93 (100/85)</td>
<td>68 (80/55)</td>
<td>97 (100/93)</td>
<td>55 (75/35)</td>
<td>70 (75/65)</td>
</tr>
<tr>
<td>Minimal (8.0%)</td>
<td>100 (100/100)</td>
<td>85 (100/70)</td>
<td>100 (100/100)</td>
<td>85 (100/70)</td>
<td>80 (80/80)</td>
</tr>
<tr>
<td>Bias, (c) (n = 40)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Desirable (6.4%)</td>
<td>98 (100/95)</td>
<td>48 (65/30)</td>
<td>54 (80/20)</td>
<td>55 (65/45)</td>
<td>88 (90/85)</td>
</tr>
<tr>
<td>Minimal (9.5%)</td>
<td>100 (100/100)</td>
<td>65 (95/35)</td>
<td>66 (95/27)</td>
<td>80 (95/65)</td>
<td>100 (100/100)</td>
</tr>
<tr>
<td>(TE, d) (n = 160)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Desirable (16.7%)</td>
<td>100 (100/100)</td>
<td>79 (99/59)</td>
<td>67 (95/30)</td>
<td>89 (96/83)</td>
<td>98 (99/96)</td>
</tr>
<tr>
<td>Minimal (25.1%)</td>
<td>100 (100/100)</td>
<td>93 (100/86)</td>
<td>76 (100/45)</td>
<td>98 (100/96)</td>
<td>100 (100/100)</td>
</tr>
</tbody>
</table>

\(^a\) Each assay was challenged with 40 specimens that had been assigned testosterone concentration based on the CDC reference method for quantifying testosterone. The 40 specimens were analyzed by each assay 4 different times over the course of 1 year \((n = 160)\). Data are % all (% male/% female). Assay A, LabCorp, LC/MS/MS; Assay B, Boston University Steroid Hormone Assay Laboratory Section of Endocrinology, Diabetes, and Nutrition, Boston University School of Medicine, and Boston Medical Center, LC/MS/MS; Assay C, Roche Diagnostics, electrochemiluminescence; Assay D, Mayo Clinic, LC/MS/MS; Assay E, Covance Central Laboratories Services, LC/MS/MS.

\(^b\) CV of the 4 individual measurement of each specimen.

\(^c\) Percent difference between the mean of 4 replicate measurements and the assigned value.

\(^d\) Percent difference of an individual measurement \((n = 160)\) so that the specimens’ imprecision is combined with evaluation of bias for each individual measurement.

---

**Isotope-dilution liquid chromatography-tandem mass spectrometry candidate reference method for total testosterone in human serum.**

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

ARIA™ TX4-API 4000:

PPT with 2x Concentration
2D-LC
5 minute cycle-time – 1 min acquisition

ARIA™ Transcend™ 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 25 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
- Inline 48 - 78 secs

Start – 1.25 ml/min, 80% Mobile Phase B

Eluting pump
Loading pump
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>Preanalytical</th>
<th>Matrix</th>
<th>Channels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Androstenedione</td>
<td>SLE+</td>
<td>S/P</td>
<td>1,3,5,7</td>
</tr>
<tr>
<td>Aldosterone</td>
<td>Hydrolysis/SLE</td>
<td>U</td>
<td>1,3,5,7</td>
</tr>
<tr>
<td>Aldosterone SLE+ S/P</td>
<td>U</td>
<td>S/P</td>
<td>1,3,5,7</td>
</tr>
<tr>
<td>DHEA LLE/KMnO4 S/P</td>
<td>S/P</td>
<td>2,6</td>
<td></td>
</tr>
<tr>
<td>17-OHProgestrone U</td>
<td>SLE+</td>
<td>U</td>
<td>4</td>
</tr>
<tr>
<td>17-OHProgestrone SLE+ S/P</td>
<td>U</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Cortisol</td>
<td>SLE+</td>
<td>U</td>
<td>8</td>
</tr>
<tr>
<td>Cortisone</td>
<td>SLE+</td>
<td>U</td>
<td>8</td>
</tr>
</tbody>
</table>

CHANNEL

1 2 3 4 5 6 7 8

VALVING SETUP
Multiplexed LC-MS/MS Data – Acquisition Window

Androstenedione (S/P)  
SLE+, 10ng/dL, 20s

Aldosterone (S/P)  
SLE+, 1ng/dL, 30s

Aldosterone (U)  
SLE+, 20ng/dL, 30s

DHEA (S/P)  
LLE/Ox+, 20ng/dL, 20s

17OHPregesterone (S/P)  
SLE+, 10ng/dL, 30s

17OHPregesterone (U)  
Hydrolysis/LLE, 20ng/dL, 45s

Cortisol (U)  
SLE+, 1ng/mL, 40s

Cortisone (U)  
SLE, 20ng/mL, 40s
MedWatch: 184 Drugs/Metabolites in Urine

Urine + IS/Acid, Seal Plate, Mix, Hydrolysis, Centrifuge, Inject
UPLC-API5500 ESI Scheduled MRM, 354 monitored transitions

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

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

| Calibration | Concentration Deviation | Calibration No Intercept | response undefined on a non-linear calibration curve | Ion Ratio Deviation | Ion Ratio Peak Missing | Peak Fit Quality | Peak High Signal No Peak | Subsequent to Carryover | QC Concentration Deviation | QC Exceeds Standard Deviation | QC Negative Control | Present but Below LLOQ | QC Flag Positive Unknowns | Contamination of Blank | blank and concentration > N | RT Quant Qual Difference | RT Relative Deviation | Internal Standard Area Deviation | deviates from expected by N | RT Shift From Standards | Quant RT deviates from expected by N |
|-------------|--------------------------|--------------------------|-----------------------------------------------------|---------------------|------------------------|-------------------|---------------------------|--------------------------|--------------------------|-----------------------------|-------------------------|----------------------|----------------------------|--------------------------|--------------------------|-----------------------------|--------------------------|-----------------------------|-----------------------------|--------------------------|
| Calibration Standards Excluded | more than N standard samples not used for calibration curve | Calibration Regression Failed | calibration curve uncalculatable | Calibration | 40 QA rules (18 custom) | 20 “Batch rules” | 20 “Chromatogram rules” |

31
Automated Data review Impact

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),

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

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

\[ \text{Ac} = \frac{93474 \times 1000 \text{ ng/dL}}{108564} = 861.034 \text{ ng/dL} \]

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

MMA = 373 nM

IS: (m/z) 120 – 76 1000nM (Y-axis ratio max 15)

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

IS: (m/z) 120 – 58 90nM (Y-axis ratio max 190)

IS Transition ratio Low MDP/High MDP

MMA Concentration (nM)
No “Calibration” – IS at MDP Pseudo Reference Method

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

Testosterone Medical Decision Points (MDP, ng/dL)

<table>
<thead>
<tr>
<th>Age (y)</th>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-10</td>
<td>&lt;3</td>
<td>10</td>
</tr>
<tr>
<td>11-18 (F)</td>
<td>&lt;3</td>
<td>38</td>
</tr>
<tr>
<td>11-18 (M)</td>
<td>&lt;3</td>
<td>970</td>
</tr>
<tr>
<td>20 - 50 (F Pre)</td>
<td>10</td>
<td>55</td>
</tr>
<tr>
<td>20 - 50 (M)</td>
<td>350</td>
<td>1030</td>
</tr>
<tr>
<td>60 - 80 (F Post)</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>60 - 80 (M)</td>
<td>7</td>
<td>40</td>
</tr>
</tbody>
</table>

IS: \((m/z)\) 292 – 112 (4000ng/dL - 2.6e6)
IS: \((m/z)\) 292 – 112 (1500ng/dL - 9.6e5)
Testosterone \((m/z)\) 289 – 97 = 517ng/dL (3.4e5)
IS: \((m/z)\) 292 – 112 100ng/dL - 6.8e4
IS: \((m/z)\) 292 – 112 (50ng/dL - 3.7e4)
IS: \((m/z)\) 292 – 112 (10ng/dL - 5187)
IS: \((m/z)\) 292 – 112 (5ng/dL - 2950)
## Determining IS Concentration - Gravimetry

### Internal Standard Concentration (Ic) = \((A_c \times I_r) / A_r\)

<table>
<thead>
<tr>
<th>Analyte Concentration (Ac)</th>
<th>Analyte Peak Area (Ar)</th>
<th>IS Peak Area (Ir)</th>
<th>IS #1</th>
<th>IS #2</th>
<th>IS #3</th>
<th>IS #4</th>
<th>IS #5</th>
<th>IS #6</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>7054.517</td>
<td>10532.26</td>
<td>3.732</td>
<td>5.747</td>
<td>45.956</td>
<td>80.630</td>
<td>1266.153</td>
<td>3224.713</td>
</tr>
<tr>
<td>5</td>
<td>11290.82</td>
<td>8825.85</td>
<td>3.908</td>
<td>6.495</td>
<td>49.458</td>
<td>88.675</td>
<td>1403.244</td>
<td>3622.708</td>
</tr>
<tr>
<td>10</td>
<td>23821.803</td>
<td>11314.437</td>
<td>4.750</td>
<td>7.138</td>
<td>55.229</td>
<td>96.047</td>
<td>1547.910</td>
<td>4016.756</td>
</tr>
<tr>
<td>50</td>
<td>95506.79</td>
<td>9710.595</td>
<td>5.084</td>
<td>7.067</td>
<td>55.869</td>
<td>98.328</td>
<td>1587.144</td>
<td>4103.246</td>
</tr>
<tr>
<td>200</td>
<td>456837.937</td>
<td>10603.594</td>
<td>4.642</td>
<td>7.484</td>
<td>54.440</td>
<td>97.518</td>
<td>1543.240</td>
<td>3901.718</td>
</tr>
<tr>
<td>1000</td>
<td>1970307.633</td>
<td>9122.446</td>
<td>4.630</td>
<td>6.954</td>
<td>56.312</td>
<td>99.417</td>
<td>1583.272</td>
<td>4171.816</td>
</tr>
<tr>
<td>2500</td>
<td>5253780.503</td>
<td>9596.876</td>
<td>4.567</td>
<td>6.997</td>
<td>55.655</td>
<td>100.661</td>
<td>1595.970</td>
<td>4049.695</td>
</tr>
<tr>
<td>5000</td>
<td>10390778.7</td>
<td>10347.679</td>
<td>4.979</td>
<td>7.042</td>
<td>58.074</td>
<td>104.044</td>
<td>1607.158</td>
<td>4205.207</td>
</tr>
</tbody>
</table>

Mean IS Concentration equivalent (ng/dL) | 4.537 | 6.866 | 53.874 | 95.665 | 1516.761 | 3911.982 |

---

**Normalized Ic**

**Calibrator level**
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 = 1.962 (0.332 – 3.597)
Correlation Coefficient = 0.9996
Mean Bias = -4.899%

“Approximate Matching” IS versus Isotope Dilution

Slope [Deming] = 1.002 (0.998 – 1.006)
Intercept = 0.080 (-1.546 – 1.707)
Correlation Coefficient = 0.9997
Mean Bias = 0.251%
### Population Pooling Testosterone Men (N=400)

<table>
<thead>
<tr>
<th>Pool</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>376</td>
<td>550</td>
<td>393</td>
<td>465</td>
<td>433</td>
<td>562</td>
<td>526</td>
<td>494</td>
<td>427</td>
<td>409</td>
<td>402</td>
<td>499</td>
<td>499</td>
<td>479</td>
<td>417</td>
<td>465</td>
</tr>
<tr>
<td></td>
<td>356</td>
<td>520</td>
<td>362</td>
<td>439</td>
<td>414</td>
<td>518</td>
<td>495</td>
<td>475</td>
<td>408</td>
<td>382</td>
<td>388</td>
<td>456</td>
<td>475</td>
<td>472</td>
<td>375</td>
<td>435</td>
</tr>
<tr>
<td></td>
<td>365</td>
<td>555</td>
<td>384</td>
<td>449</td>
<td>441</td>
<td>527</td>
<td>534</td>
<td>507</td>
<td>436</td>
<td>407</td>
<td>416</td>
<td>502</td>
<td>501</td>
<td>472</td>
<td>404</td>
<td>451</td>
</tr>
<tr>
<td>Mean</td>
<td>367</td>
<td>539</td>
<td>375</td>
<td>447</td>
<td>431</td>
<td>533</td>
<td>516</td>
<td>485</td>
<td>420</td>
<td>399</td>
<td>400</td>
<td>485</td>
<td>491</td>
<td>467</td>
<td>397</td>
<td>448</td>
</tr>
<tr>
<td>CV (%)</td>
<td>2.39</td>
<td>3.07</td>
<td>4.19</td>
<td>3.13</td>
<td>2.69</td>
<td>3.76</td>
<td>3.44</td>
<td>3.88</td>
<td>3.42</td>
<td>3.08</td>
<td>2.99</td>
<td>4.34</td>
<td>2.45</td>
<td>3.22</td>
<td>4.50</td>
<td>2.97</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Target</th>
<th>453</th>
<th>411</th>
<th>482</th>
<th>500</th>
<th>409</th>
<th>443</th>
<th>479</th>
<th>422</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>440</td>
<td>403</td>
<td>409</td>
<td>514</td>
<td>394</td>
<td>444</td>
<td>479</td>
<td>412</td>
</tr>
<tr>
<td></td>
<td>465</td>
<td>426</td>
<td>424</td>
<td>540</td>
<td>409</td>
<td>472</td>
<td>498</td>
<td>449</td>
</tr>
<tr>
<td></td>
<td>435</td>
<td>396</td>
<td>397</td>
<td>513</td>
<td>389</td>
<td>455</td>
<td>490</td>
<td>433</td>
</tr>
<tr>
<td></td>
<td>451</td>
<td>416</td>
<td>416</td>
<td>534</td>
<td>416</td>
<td>458</td>
<td>497</td>
<td>441</td>
</tr>
<tr>
<td>Mean</td>
<td>448</td>
<td>410</td>
<td>412</td>
<td>525</td>
<td>402</td>
<td>457</td>
<td>491</td>
<td>434</td>
</tr>
<tr>
<td>CV (%)</td>
<td>2.97</td>
<td>3.26</td>
<td>2.78</td>
<td>2.63</td>
<td>3.14</td>
<td>2.52</td>
<td>1.78</td>
<td>3.67</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Target</th>
<th>429</th>
<th>468</th>
<th>430</th>
<th>462</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>420</td>
<td>469</td>
<td>425</td>
<td>497</td>
</tr>
<tr>
<td></td>
<td>420</td>
<td>484</td>
<td>444</td>
<td>495</td>
</tr>
<tr>
<td></td>
<td>421</td>
<td>468</td>
<td>413</td>
<td>488</td>
</tr>
<tr>
<td></td>
<td>405</td>
<td>478</td>
<td>435</td>
<td>480</td>
</tr>
<tr>
<td>Mean</td>
<td>417</td>
<td>475</td>
<td>429</td>
<td>490</td>
</tr>
<tr>
<td>CV (%)</td>
<td>1.84</td>
<td>1.61</td>
<td>3.10</td>
<td>1.57</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Target</th>
<th>446</th>
<th>460</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>443</td>
<td>445</td>
</tr>
<tr>
<td></td>
<td>470</td>
<td>473</td>
</tr>
<tr>
<td></td>
<td>437</td>
<td>469</td>
</tr>
<tr>
<td></td>
<td>457</td>
<td>484</td>
</tr>
<tr>
<td>Mean</td>
<td>452</td>
<td>468</td>
</tr>
<tr>
<td>CV (%)</td>
<td>3.27</td>
<td>3.51</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Target</th>
<th>460</th>
</tr>
</thead>
<tbody>
<tr>
<td>400</td>
<td>449</td>
</tr>
<tr>
<td></td>
<td>470</td>
</tr>
<tr>
<td></td>
<td>437</td>
</tr>
<tr>
<td></td>
<td>448</td>
</tr>
<tr>
<td>Mean</td>
<td>451</td>
</tr>
<tr>
<td>CV (%)</td>
<td>3.27</td>
</tr>
</tbody>
</table>
## Population Pools as Calibrators

<table>
<thead>
<tr>
<th>Age (y)</th>
<th>Low</th>
<th>High</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>

### Graphs
- **28000 Males 20 – 50 All**
- **Minus Outliers**
- **Boxcox transformation**
- **Cumulative Frequency and Regression Line 20 – 50 M**
Population Pools as Calibrators vs CDC Phase 1

Population Median
Slope [Deming] = 0.901
Intercept = 1.057
Correlation Coefficient = 0.9992
Mean Bias = -9.902%

Pool Targets from ID
Slope [Deming] = 0.994
Intercept = -0.528
Correlation Coefficient = 0.9992
Mean Bias = -0.799%

Pool Targets CDC Assigned
Slope [Deming] = 0.991
Intercept = 0.101
Correlation Coefficient = 0.9992
Mean Bias = -0.821%
The Litmus Test

A: Standard Isotope Dilution
B: IS Mean (Cals)
C: IS Approximately Matched (Cals)
D: IS Mean (CDC)
E: IS Approximately Matched (CDC)
F: Population Pool (Median)
G: Population Pool (Assigned by ID)
H: Population Pool (Assigned by CDC)
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 Shuford, Patricia Holland, Matt Crawford, Stacy Dee, Yvonne Wright, Martin Green, Dr Marcia Eisenberg, Gregory Janis, Dr Karla Walker

Essential Testing: Brian Rappold