

On The Path To A Random Access LC/MS Workflow:

A Novel Approach To Calibration

Don Cooper, PhD
Clinical Business Operations
Waters Corporation
Manchester UK

- There has been increasing adoption of liquid chromatography coupled with mass spectrometry (LC/MS) by clinical laboratories over the last approx 10 years.
- The adoption has been driven by several factors including:
 - improved specificity and sensitivity over conventional assays for some analytes
 - reduced costs compared to conventional assays for some analytes
 - multiplex capability
 - open architecture allows laboratories to develop LDTs e.g., for research purposes
- There are limitations:
 - high complexity & requires some degree of operator skill
 - relatively high instrument costs
 - limited availability of reagents (kits)
- Successful in niche applications (e.g., for immunosuppressant drug monitoring, steroid analysis, 25OHViD etc)

- Some of the limitations are being addressed to facilitate wider adoption of LC/MS:
 - IVD marked LC & MS instruments and consumables (columns etc)
 - Development of CE/IVD certified and FDA cleared reagent kits
 - Education / training programs (AACCC, MSACL, fellowship programs, degree courses *etc*)
 - Reduced complexity compact bench-top LC/MS instruments
- But the typical batch mode LC/MS operation remains alien to many modern clinical chemistry laboratories that typically rely on automated random access workflows.

Conventional LC/MS Calibration

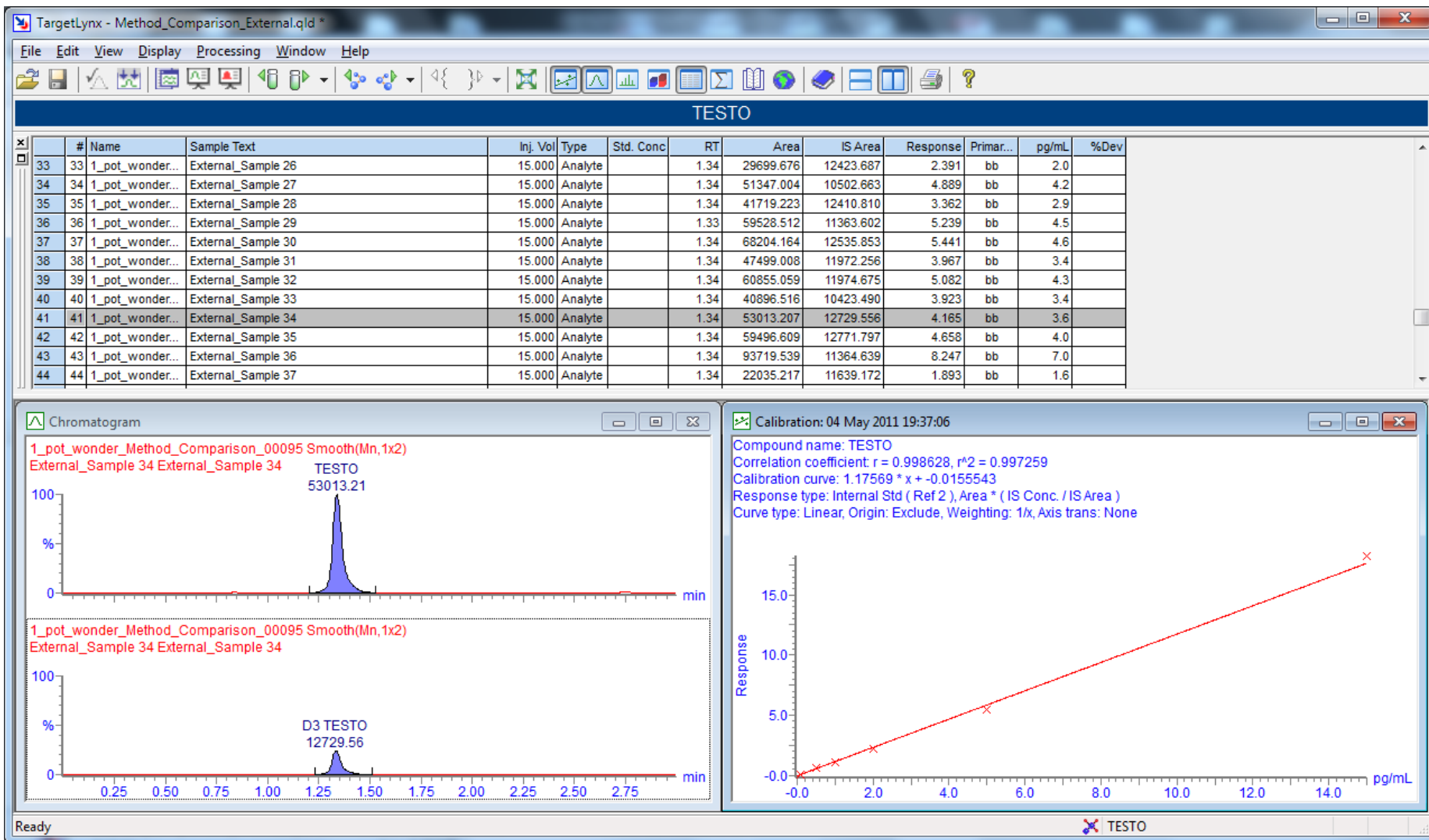
Queue Is Empty

Spectrum Chromatogram Map Edit ▾ Samples ▾

	File Name	Sample ID	Sample Type	MS File	MS Tune File	Inlet File	Bottle	Inject Volume	Conc A
1	Testosterone 001	Blank	Analyte	Testo_d3IS	Testo_29112...	Testosterone	1:1	15	
2	Testosterone 002	CAL 0.1ng/mL	Standard	Testo_d3IS	Testo_29112...	Testosterone	1:2	15	0.1
3	Testosterone 003	CAL 0.5ng/mL	Standard	Testo_d3IS	Testo_29112...	Testosterone	1:3	15	0.5
4	Testosterone 004	CAL 1.0ng/mL	Standard	Testo_d3IS	Testo_29112...	Testosterone	1:4	15	1.0
5	Testosterone 005	CAL 2.0ng/mL	Standard	Testo_d3IS	Testo_29112...	Testosterone	1:5	15	2.0
6	Testosterone 006	CAL 5.0ng/mL	Standard	Testo_d3IS	Testo_29112...	Testosterone	1:6	15	5.0
7	Testosterone 007	CAL 15.0ng/mL	Standard	Testo_d3IS	Testo_29112...	Testosterone	1:7	15	15.0
8	Testosterone 008	Sample 1	Analyte	Testo_d3IS	Testo_29112...	Testosterone	1:8	15	
9	Testosterone 009	Sample 2	Analyte	Testo_d3IS	Testo_29112...	Testosterone	1:9	15	
10	Testosterone 010	Sample 3	Analyte	Testo_d3IS	Testo_29112...	Testosterone	1:10	15	
11	Testosterone 011	Sample 4	Analyte	Testo_d3IS	Testo_29112...	Testosterone	1:11	15	
12	Testosterone 012	Sample 5	Analyte	Testo_d3IS	Testo_29112...	Testosterone	1:12	15	
13	Testosterone 013	Sample 6	Analyte	Testo_d3IS	Testo_29112...	Testosterone	1:13	15	
14	Testosterone 014	Sample 7	Analyte	Testo_d3IS	Testo_29112...	Testosterone	1:14	15	
15	Testosterone 015	Sample 8	Analyte	Testo_d3IS	Testo_29112...	Testosterone	1:15	15	
16	Testosterone 016	Sample 9	Analyte	Testo_d3IS	Testo_29112...	Testosterone	1:16	15	
17	Testosterone 017	Sample 10	Analyte	Testo_d3IS	Testo_29112...	Testosterone	1:17	15	
18	Testosterone 018	QC 1	QC	Testo_d3IS	Testo_29112...	Testosterone	1:18	15	3.0
19	Testosterone 018	Sample 11	Analyte	Testo_d3IS	Testo_29112...	Testosterone	1:18	15	
20	Testosterone 019	Sample 12	Analyte	Testo_d3IS	Testo_29112...	Testosterone	1:19	15	
21	Testosterone 020	Sample 13	Analyte	Testo_d3IS	Testo_29112...	Testosterone	1:20	15	
22	Testosterone 021	Sample 14	Analyte	Testo_d3IS	Testo_29112...	Testosterone	1:21	15	
23	Testosterone 022	Sample 15	Analyte	Testo_d3IS	Testo_29112...	Testosterone	1:22	15	
24	Testosterone 023	Sample 16	Analyte	Testo_d3IS	Testo_29112...	Testosterone	1:23	15	
25	Testosterone 024	Sample 17	Analyte	Testo_d3IS	Testo_29112...	Testosterone	1:24	15	

Example sample list for a typical LC/MS assay. Samples are analysed in batches. Each batch includes a sequence of **calibrators**.

Conventional External Calibration Curve



- Several barriers to random access quantitative mass spectrometry:
 - Manual review of results
 - Mobile phase switching & equilibration
 - Column switching & equilibration
- Can be addressed by:
 - Software / informatics
 - Instrument design / engineering
 - Assay design (e.g., common fit-for-purpose mobile phases)
- **The need to run calibrators and batches of samples remains the major barrier**

Internal Calibration

The Internal Calibration Concept*

- The internal calibrators are added directly to the individual sample which is then processed in the normal way (e.g., SPE, LLE etc ...)
- Each point on the calibration curve is derived from a unique internal calibrator that can be differentiated from the analyte of interest and from the other internal calibrators using mass spectrometry.
- The ideal situation is to use a different stable isotope labelled form of the analyte of interest for each calibration point so that each internal calibrator has a unique mass.
- The sample is then analysed by LC/MS(/MS), simultaneously monitoring the analyte and all the added internal calibrators.
- From that single analysis, the integrated peak areas for the internal calibrators can be used to construct a calibration line from which the analyte concentration can be calculated.

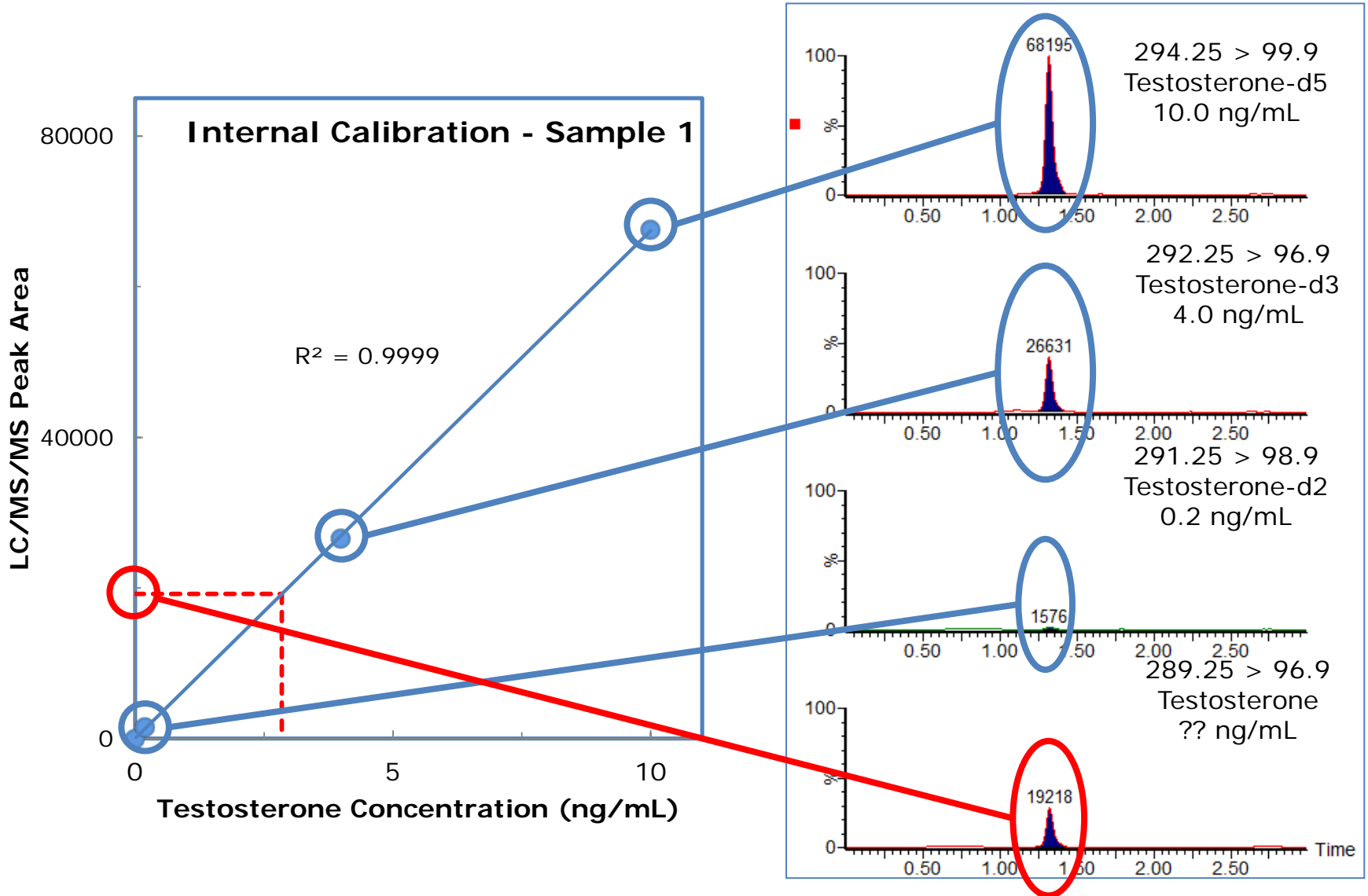
* Patent pending

Example Internal Calibration for Testosterone

Analyte * / Calibrator	MS/MS	Final Concentration (ng/mL)
Testosterone*	289.25 > 96.9	
Testosterone-D ₂	291.25 > 98.9	0.2
Testosterone-D ₃	292.25 > 96.9	4.0
Testosterone-D ₅	294.25 > 99.9	10.0

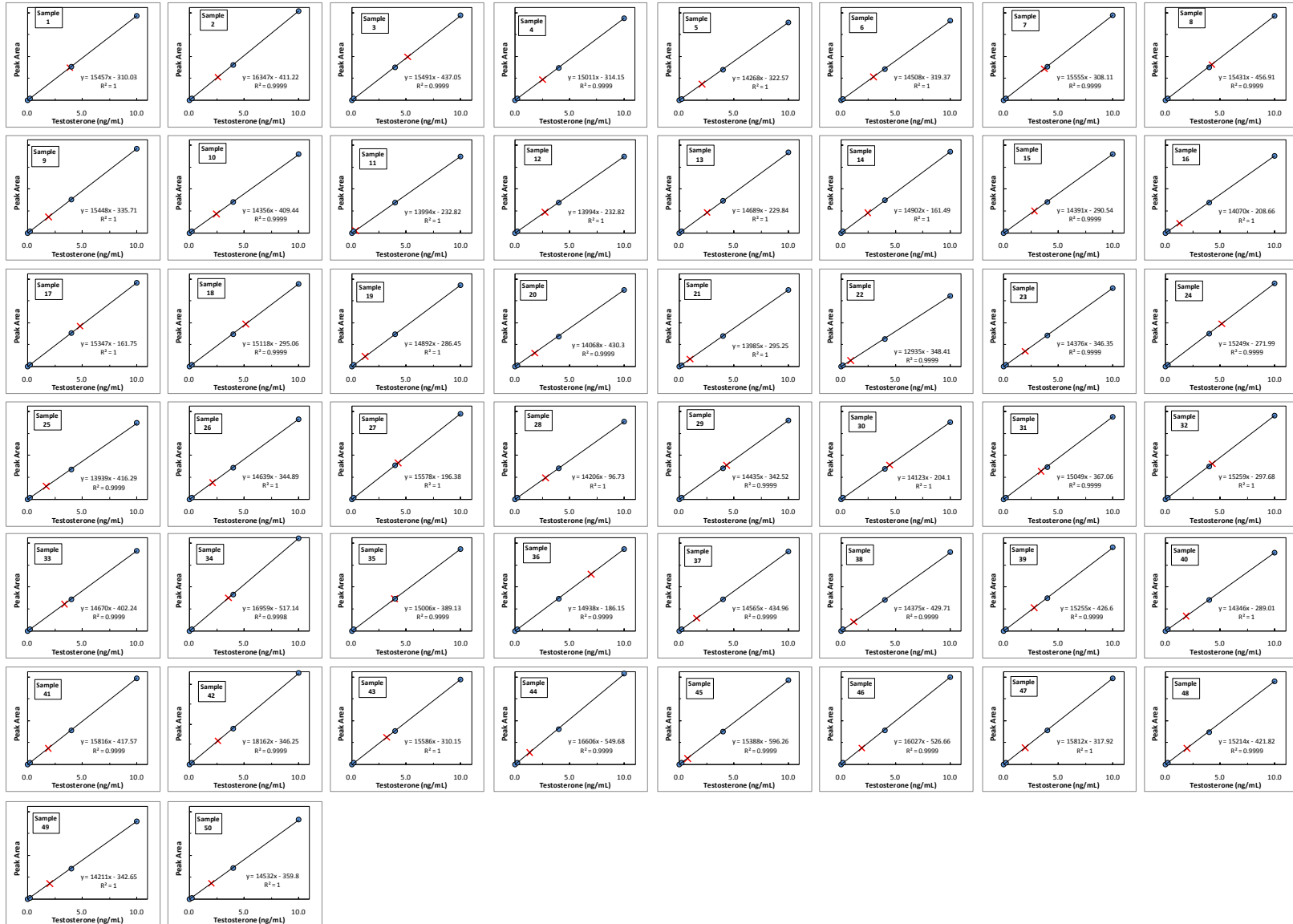
- A 20x concentrated mixture of internal calibrators was prepared.
- The internal calibrator mixture was added to each sample and the samples prepared as usual (liquid-liquid extraction).
- The samples were analysed by LC/MS/MS simultaneously monitoring the 4 MRMs above.

Simultaneous Calibration and Quantification for Testosterone.

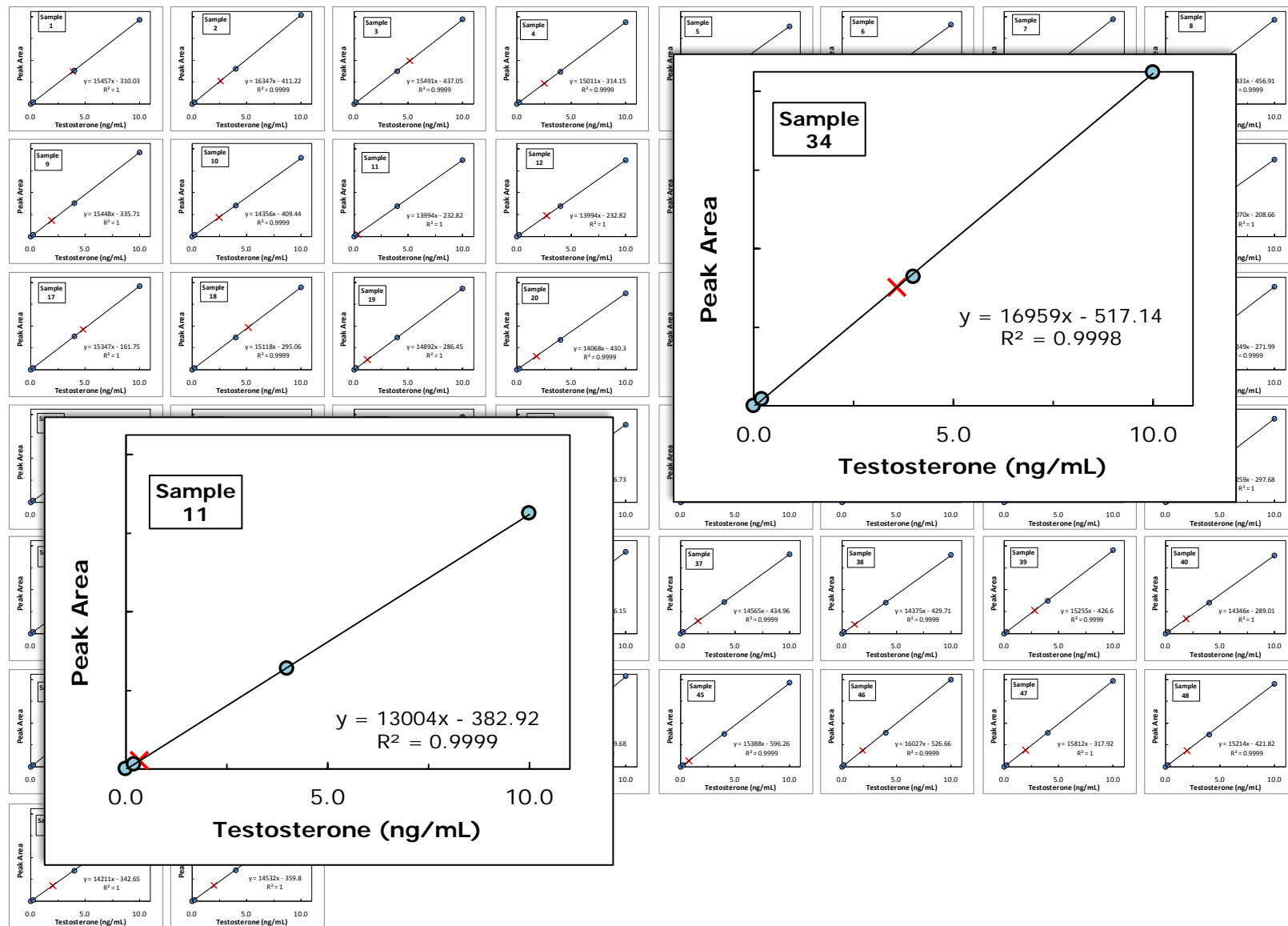


There is a unique calibration line for each sample.

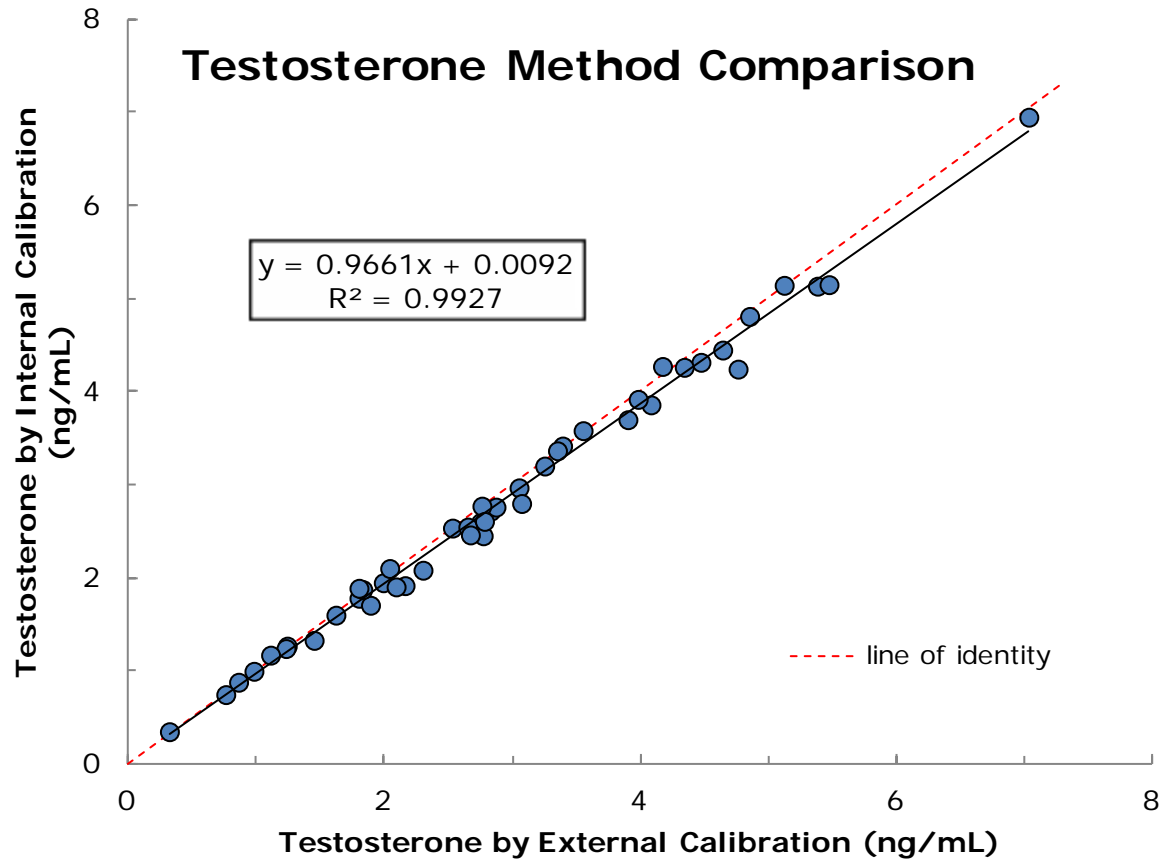
- 50 samples in this example.



There is a unique calibration line for each sample.



Testosterone Method Comparison



Preliminary imprecision estimate = 2.5%CV
(N=5, mean = 1.97ng/mL, SD = 0.0463)

- We have developed an approach to quantitative LC/MS compatible with the demands of the routine clinical chemistry customer.
- Acceptable preliminary validation performed for several different analytes.
- No requirement to analyse separate, external calibrators
- Simplified workflow
- Calibration (internal) is perfectly matrix matched
- Time to first result is reduced (4min vs. 32min for testosterone example)
- Potential to develop random access LC/MS-based clinical analysers
- Such an instrument linked to a LIS and with on-board reagents could be used to run personalised, multiplexed analyte panels.