Validation of Cyclosporin on LC-QTOF

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I have reviewed this data and approve this test for clinical use.

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Laboratory Director

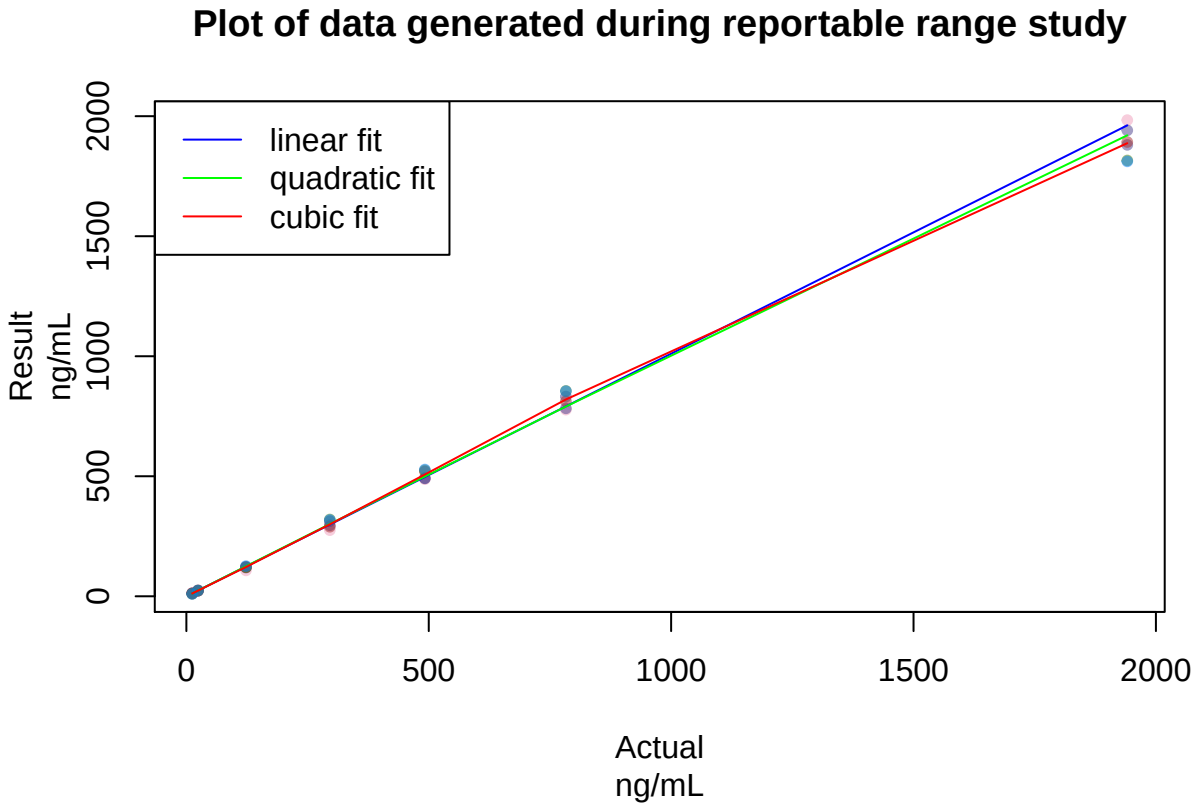
Note: all CV and bias are in percent

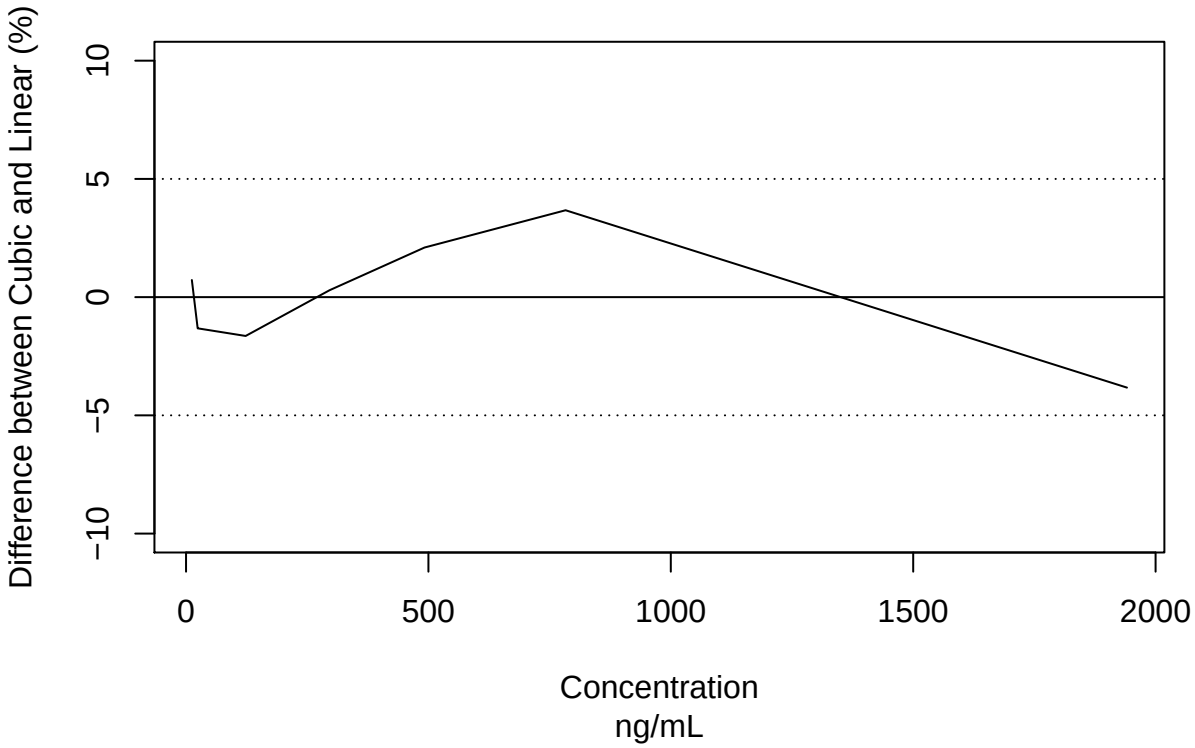
Reportable Range Study

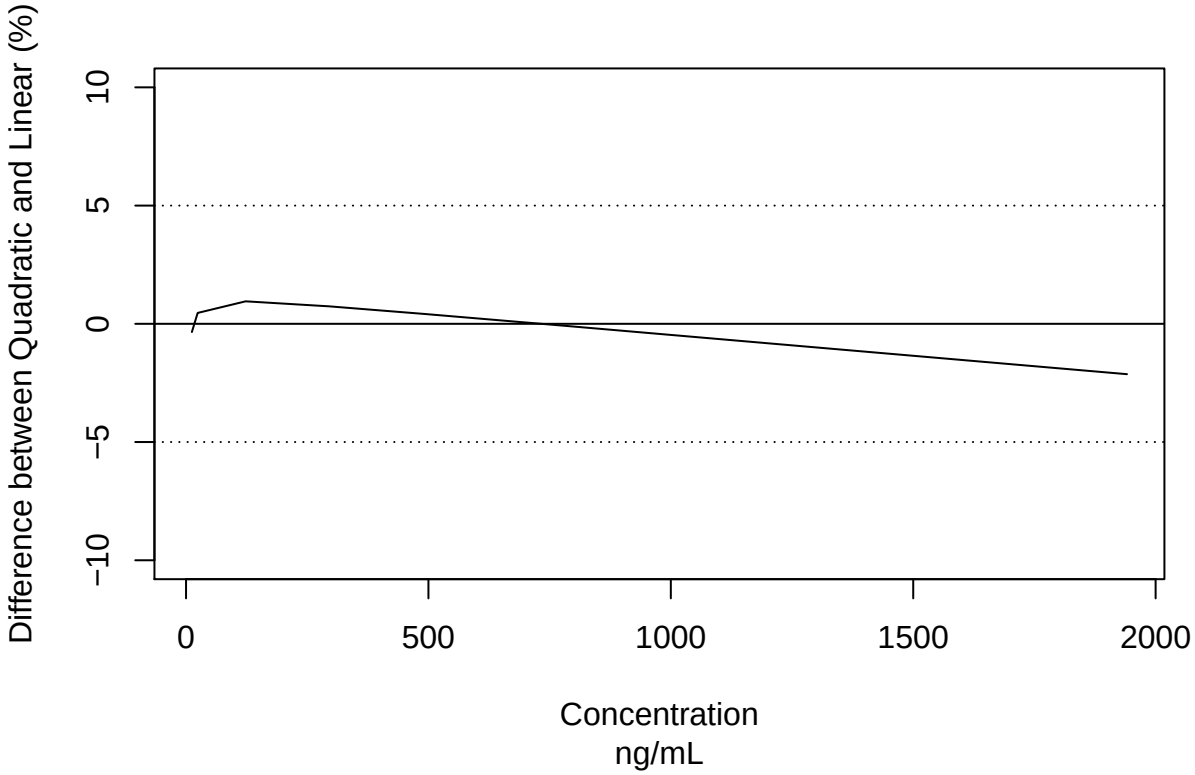
COM.40600

The reportable range study was performed by running standards over 6 days. Analysis was performed by both calculating the %CV at each level and ensuring that it is less than 20% and by using the method of Emancipator and Kroll to evaluate for linearity over the calibration range. A tolerance of 10% for non-linearity was used as the criteria and comparisons were made with both quadratic vs linear and cubic vs quadratic. The assay is validated for the range of 12 - 1941 ng/mL.

Actual.cals	Mean.cals	SD.cals	CV.cals
12	11.6	1.4	11.9
24	23.9	0.9	3.8
123	119.7	6.2	5.2
296	302.0	17.8	5.9
492	507.3	18.9	3.7
783	819.2	34.1	4.2
1941	1887.5	68.0	3.6





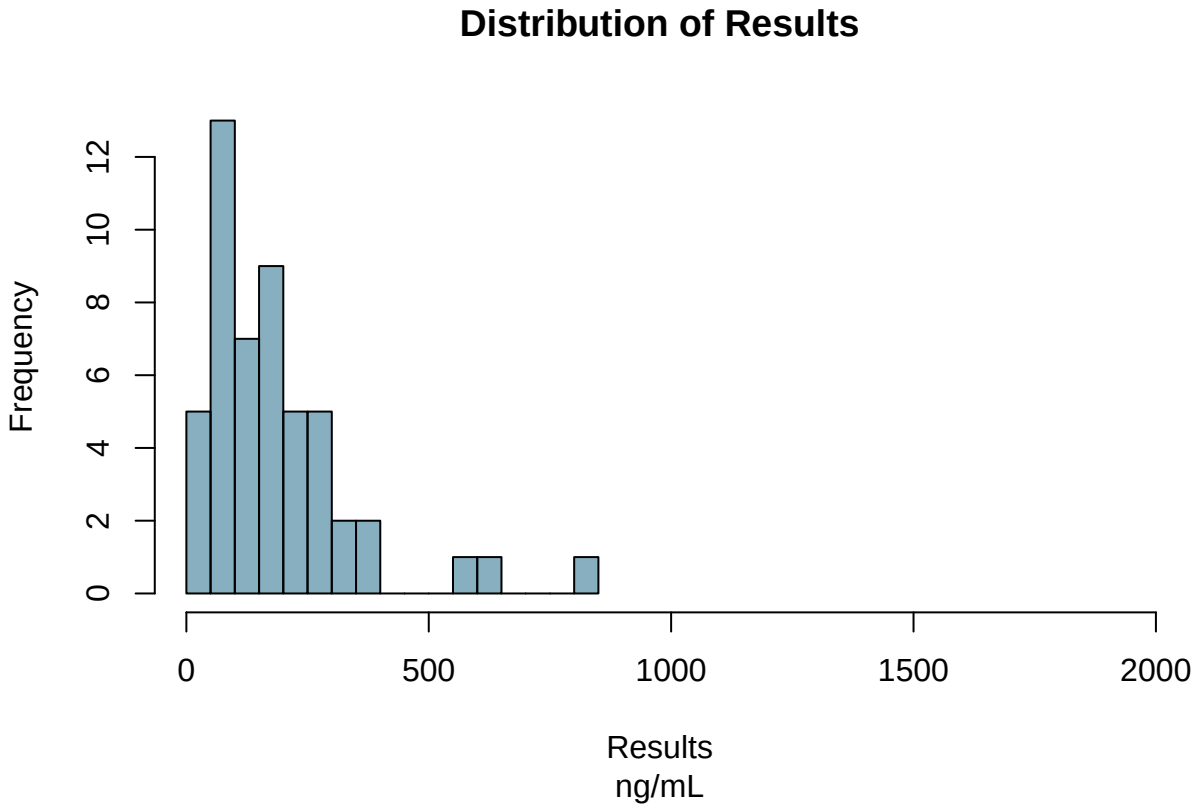


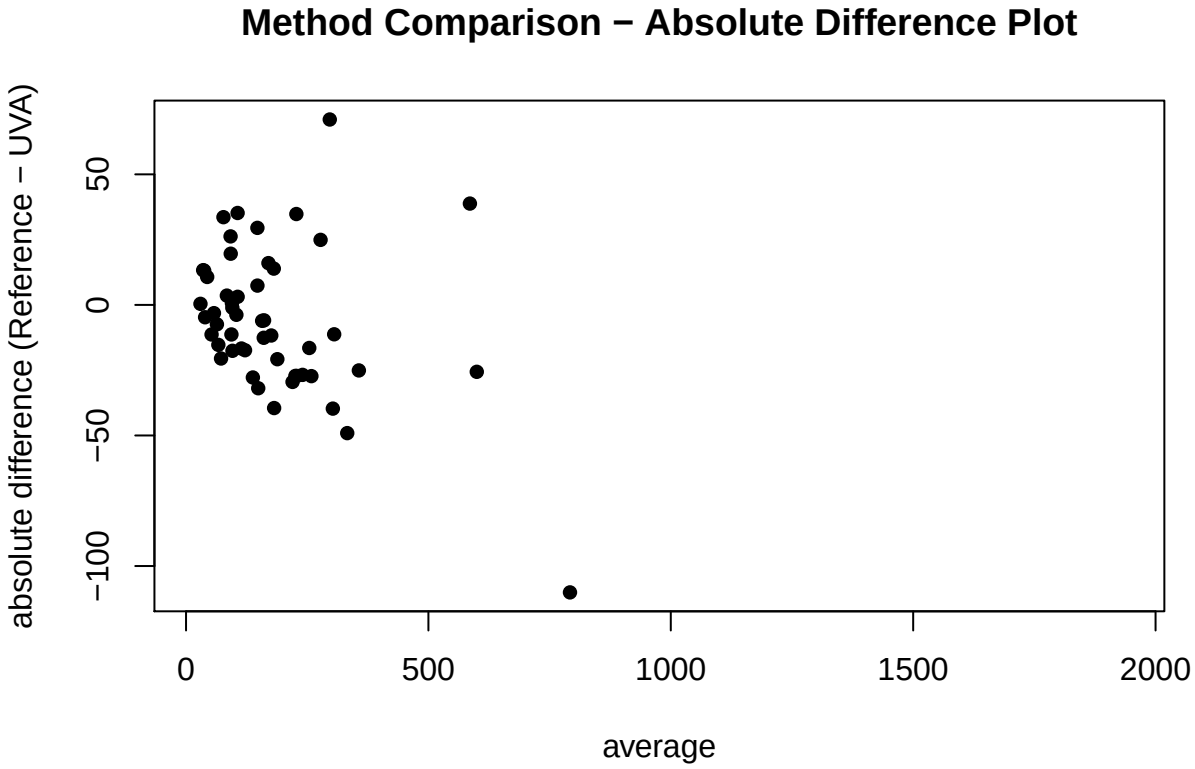
Accuracy & Method comparison Study

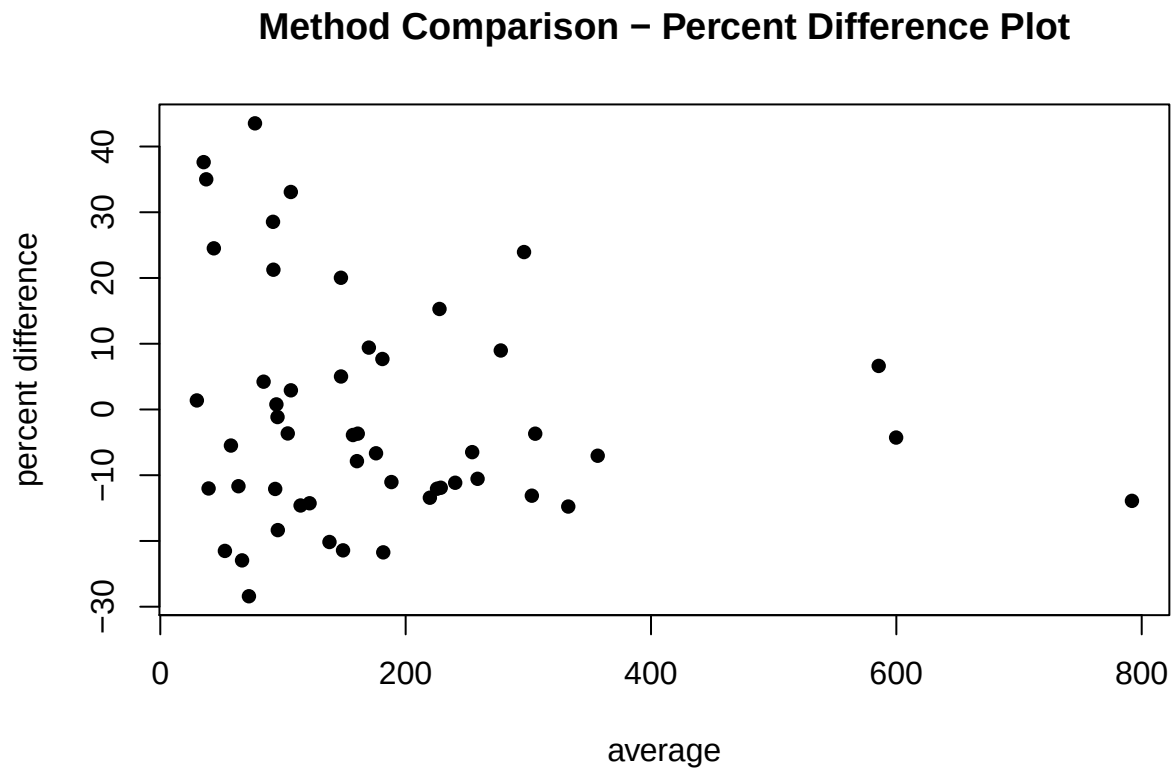
COM.40300, COM.40350, CHM.14200 & CHM.18800

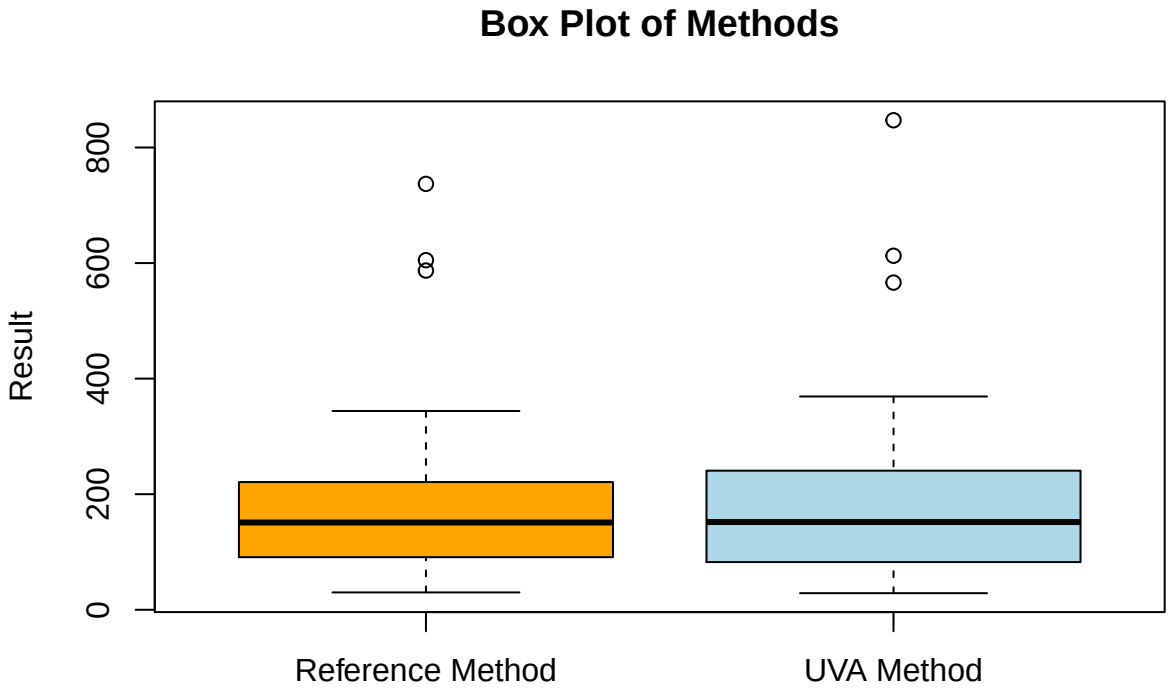
This study consisted of analyzing 51 quantitatively positive patient samples. The UVA method utilizes

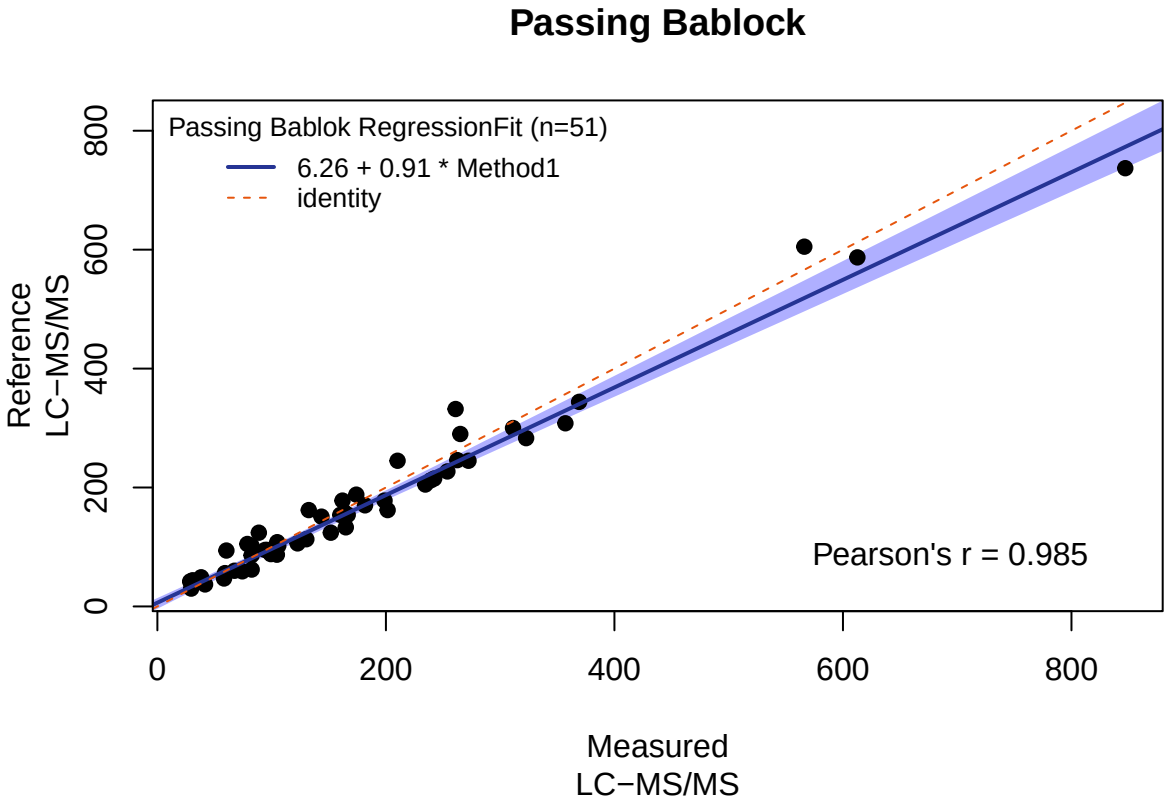
LC-MS/MS while the comparator method is LC-MS/MS performed by previous UVA method. The sections that fall within this section include qualitative comparison and accuracy spike.






Method Comparison − Percent Difference Plot
percent difference
40
30
20
10
0
−10
−30
0
200
400
600
800
average


Box Plot of Methods
Result
800
600
400
200
0
Reference Method
UVA Method



Qualitative Comparison

This data compares values where one of the methods reports a value outside of the AMR, since quantitative comparison is not possible. The goal is to evaluate if the interpretation of the samples are in agreement. At least 82 samples were analyzed and were in qualitative agreement by both the reference and UVA method. The chart below shows any discrepancies.

method.comp.dis.Reference	method.comp.dis.Measured

Accuracy Spike

This experiment evaluates the accuracy of the assay by spiking a known amount of analyte into at least 10 different patient samples.

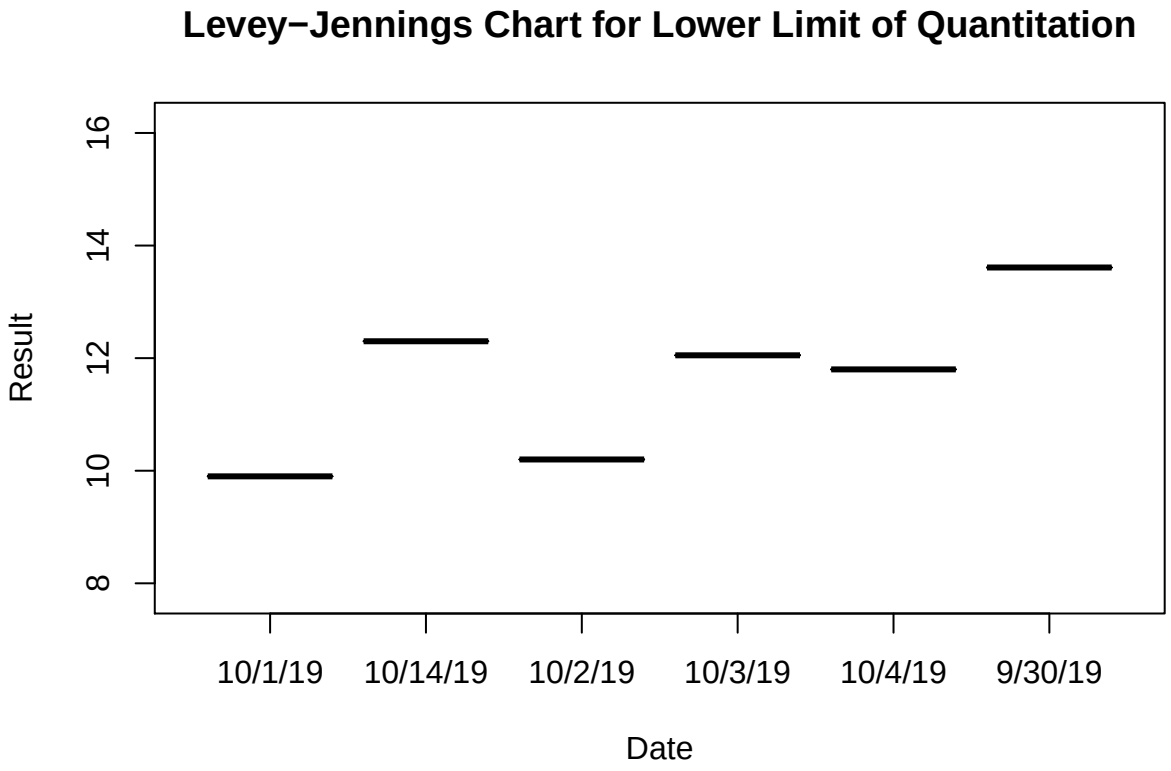
Specimen	Bias	Calculated	Measured
Spike Patient 1	-12.8	40	45.1
Spike Patient 2	-5.5	40	42.2
Spike Patient 3	-8.0	200	216.1
Spike Patient 4	-4.5	200	209
Spike Patient 5	1.8	500	490.8
Spike Patient 6	-2.0	500	510.2
Spike Patient 7	-10.2	1000	1102.5
Spike Patient 8	-3.8	1000	1038.5
Spike Patient 9	-9.4	1600	1750
Spike Patient 10	-6.2	1600	1698.4
Average	-6.7		

Imprecision Study

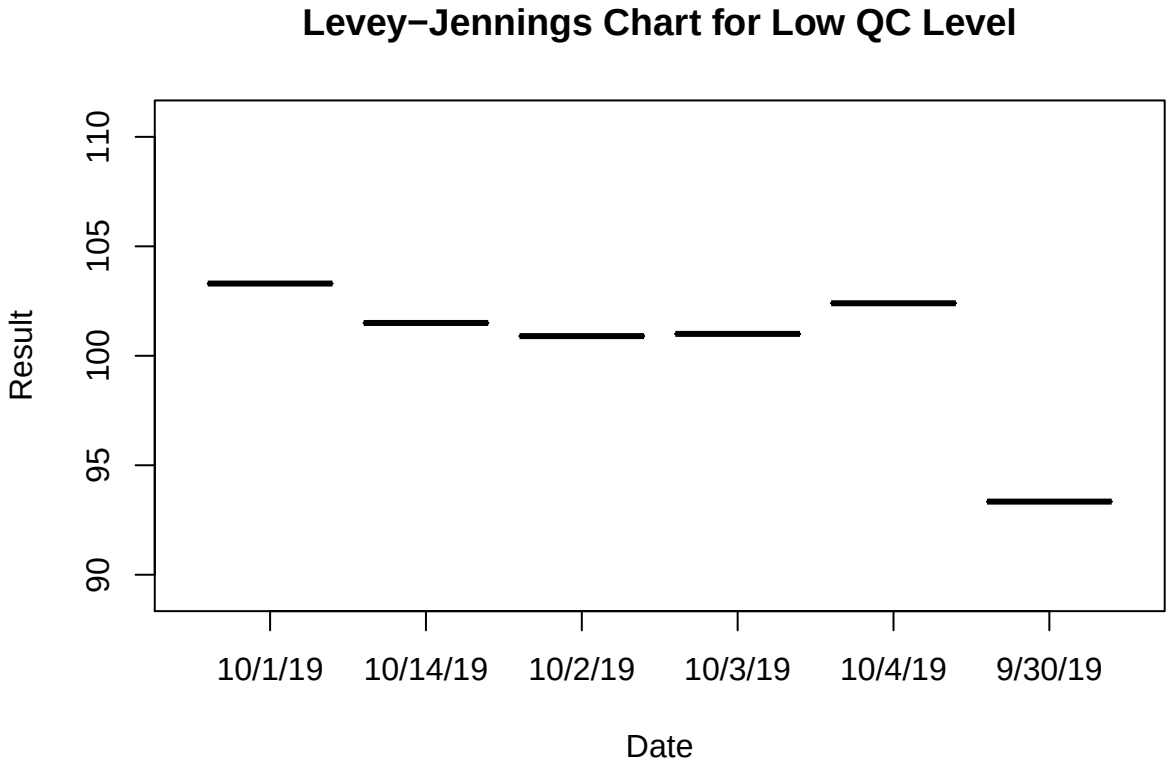
COM.40300

Intra-day and inter-day precision was determined at multiple concentrations, and is expressed as the coefficient of variation. The acceptability criteria is an imprecision $< 15\%$.

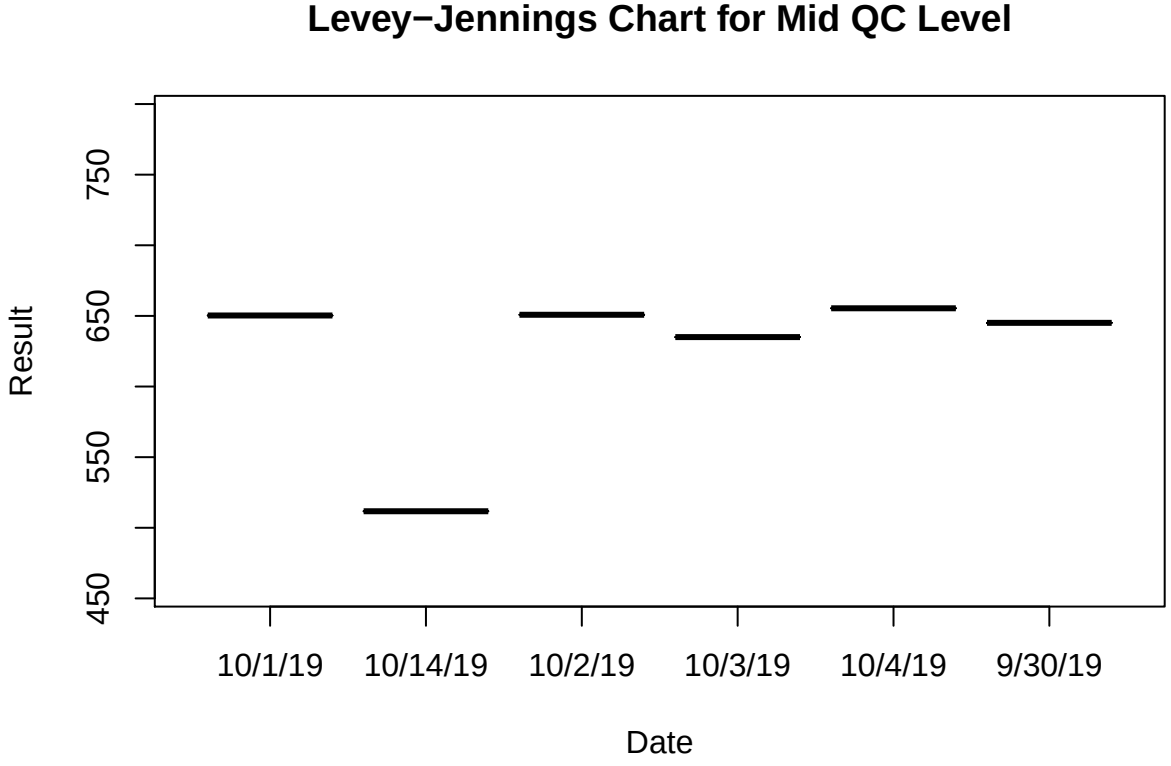
QC Data over multiple days:



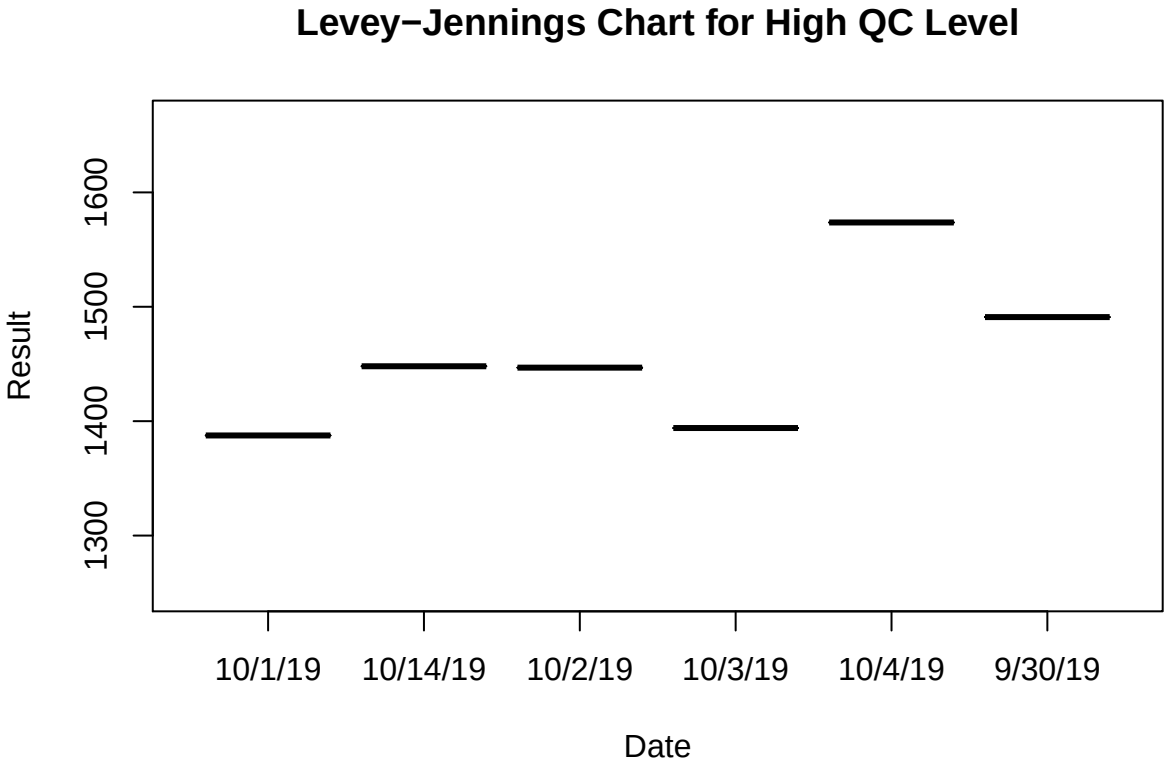
target mean = 12 ng/mL
calculated mean = 12 ng/mL
SD = 1.4 ng/mL
CV = 11.9%
Bias = 0 %



target mean = 105 ng/mL
calculated mean = 100 ng/mL
SD = 3.6 ng/mL
CV = 3.6%
Bias = 4.8 %



target mean = 550 ng/mL
mean = 625 ng/mL
SD = 55.8 ng/mL
CV = 8.9%
Bias = -13.6 %



target mean = 1400 ng/mL
calculatedmean = 1457 ng/mL
SD = 68.9 ng/mL
CV = 4.7%
Bias = -13.6 %

QC Data within-run imprecision:

Actual	Mean	CV	Bias
12	10.0	7.00	16.70
105	103.0	1.19	1.90
550	640.0	1.35	-16.36
1400	1368.6	2.03	2.24

Matrix Effect Assessment

CHM.18825

This was performed by spiking the same concentration of drug into 10 separate patient samples. We compare either the peak area or the relative response (RR) for each sample back to a sample where the concentration of

drug was spiked into solvent and generate a ratio. Acceptability includes matrix suppression or enhancement of <25% OR a CV of the effect <15% across all samples.

Specimen	Response	ratio
Solvent spike	1876698	0.95
Patient #1	1858085	0.94
Patient #2	1900218	0.96
Patient #3	1828758	0.93
Patient #4	1881936	0.95
Patient #5	1939030	0.98
Patient #6	1927748	0.98
Patient #7	2000672	1.01
Patient #8	1863361	0.94
Patient #9	2056677	1.04
Patient #10	1976174	
Average	1913318	0.97
% Matrix Effect		3.2
%CV	3.66	

Autosampler Stability

Acceptability was determined by preparing and running samples, allowing them to sit on the autosampler for 72 hours, and then repeating the injections. Data is acceptable.

Specimen.Type	First.run	Repeat.run	Bias
std 1	13.6	14.3	4.8
std 2	25.5	24.5	-3.9
std 3	107.9	110.4	2.3
std 4	275.5	278.3	1.0
std 5	487.7	476.2	-2.4
std 6	777.4	738.8	-5.2
std 7	1983.6	2028.6	2.2
QC 1	93.3	93.6	0.3
QC 2	645.1	599.0	-7.7
QC 3	1490.6	1496.0	0.4
A	78.8	75.3	-4.6
B	272.3	267.5	-1.8
C	143.6	139.8	-2.7
D	242.2	244.1	0.8
E	58.3	55.4	-5.3
F	612.6	618.1	0.9
G	82.5	76.2	-8.3
H	104.6	100.6	-4.0
I	181.7	174.0	-4.4
J	30.9	27.7	-11.5
K	96.1	84.6	-13.6
K	311.3	310.6	-0.2
M	163.9	159.7	-2.6
N	99.3	94.0	-5.7
O	41.7	40.3	-3.5
P	130.4	127.4	-2.3
Q	164.9	166.3	0.8
R	82.4	86.8	5.0
S	322.7	310.6	-3.9
T	105.8	98.3	-7.6
U	67.4	64.9	-3.9
V	94.3	94.6	0.3
W	29.6	29.3	-1.0
X	59.2	56.7	-4.3

Dilutional Linearity

QC3 was diluted 2, 5 and 10-fold to evaluate quantitation once diluted. Data is acceptable.

Specimen.Type	Dilution.Factor	Measured.Results	Bias
blood	2	1454.6	-0.5
blood	2	1443.2	0.3
blood	2	1453.8	-0.5
blood	5	1433.0	1.0
blood	5	1470.0	-1.6
blood	5	1494.5	-3.3
blood	10	1607.0	-11.1
blood	10	1642.0	-13.5
blood	10	1634.0	-12.9

Analytical Specificity & Analytical Interferences

COM.40450, COM.40500, GEN.42030

We determined analytical specificity through by comparing our assay with an assay that is clinically validated, as well as comparing patient samples with spiked standard material. We ran specimens by both methods and showed that when they identified the analyte, we also identified and accurately quantitated the same analyte by our method. To further minimize the potential of interferences, we monitor multiple ions for each drug and use ion ratios in our criteria for identifying the presence of a drug.

Carryover

A urine blank is included after the calibration curve to assess for any carryover up until the highest point of the AMR and is repeated over multiple days. The acceptability criteria is a relative response <50% of the relative response of the LLOQ. No carryover was detected up to 1941 ng/mL.