Title: Multi-center analytical evaluation of the automated electrochemiluminescence immunoassay for Cyclosporine

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SUPPLEMENTAL DIGITAL CONTENT 1

Supplemental Table 1. IPTS samples analyzed per investigational site, with measurement method indicated. The abbreviation “n.m.” indicates not measured at the site.

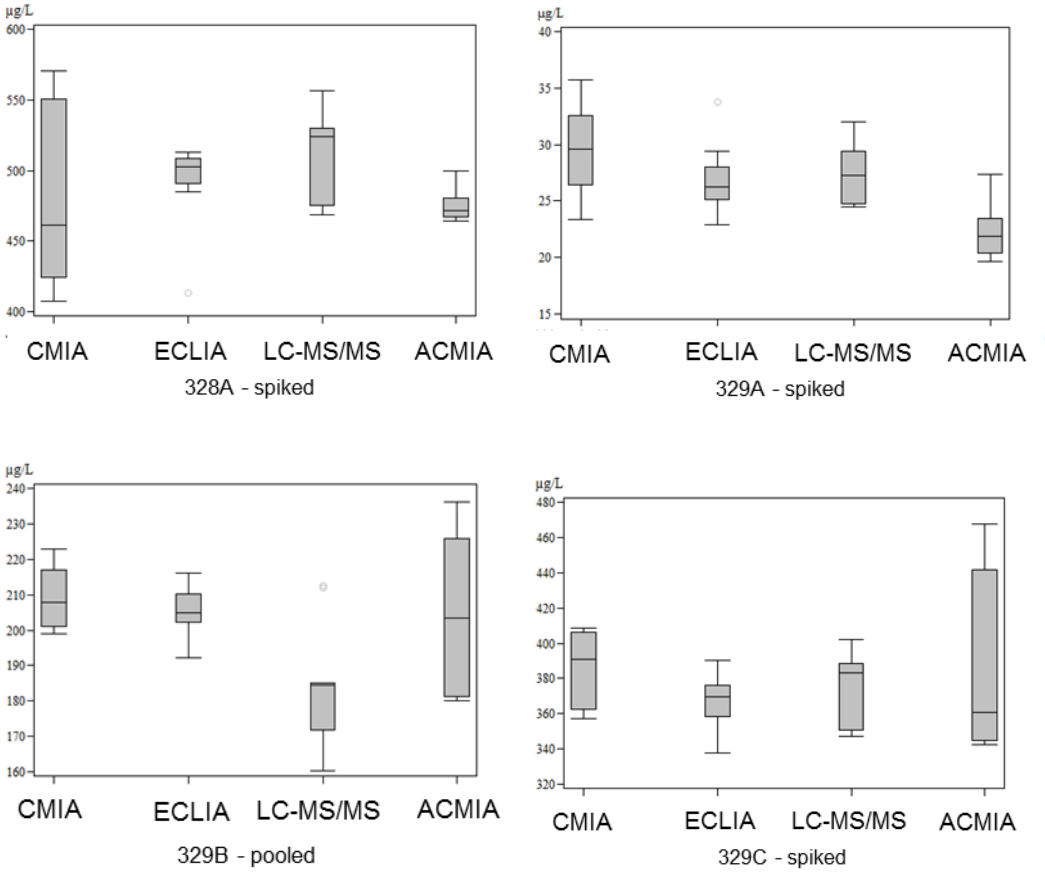
IPTS Samples	Spiked Samples								Pooled Patient Blood Samples		
	328A	329A	329C	335B	337A	337B	340C	342A	329B	338A	339C
Barcelona	ECLIA	ECLIA	ECLIA	ECLIA LC-MS/MS	ECLIA LC-MS/MS	ECLIA LC-MS/MS	ECLIA LC-MS/MS	ECLIA LC-MS/MS	ECLIA	ECLIA LC-MS/MS	ECLIA LC-MS/MS
Brussels	n.m.	n.m.	n.m.	ECLIA LC-MS/MS CMIA	ECLIA LC-MS/MS CMIA	ECLIA LC-MS/MS CMIA	ECLIA LC-MS/MS CMIA	ECLIA LC-MS/MS CMIA	n.m.	ECLIA LC-MS/MS CMIA	ECLIA LC-MS/MS CMIA
Ghent	ECLIA LC-MS/MS ACMIA CMIA	ECLIA LC-MS/MS ACMIA CMIA	ECLIA LC-MS/MS ACMIA CMIA	ECLIA LC-MS/MS ACMIA CMIA	ECLIA LC-MS/MS ACMIA CMIA	ECLIA LC-MS/MS ACMIA CMIA	ECLIA LC-MS/MS ACMIA CMIA	n.m.	ECLIA LC-MS/MS ACMIA CMIA	ECLIA LC-MS/MS ACMIA CMIA	n.m.
Munich	ECLIA LC-MS/MS	ECLIA LC-MS/MS	ECLIA LC-MS/MS	ECLIA LC-MS/MS	ECLIA LC-MS/MS	ECLIA LC-MS/MS	ECLIA LC-MS/MS	n.m.	ECLIA LC-MS/MS	ECLIA LC-MS/MS	n.m.
Stuttgart	ECLIA LC-MS/MS ACMIA CMIA	ECLIA LC-MS/MS ACMIA CMIA	ECLIA LC-MS/MS ACMIA CMIA	ECLIA LC-MS/MS ACMIA CMIA	ECLIA LC-MS/MS ACMIA CMIA	ECLIA LC-MS/MS ACMIA CMIA	ECLIA LC-MS/MS ACMIA CMIA	n.m.	ECLIA LC-MS/MS ACMIA CMIA	ECLIA LC-MS/MS ACMIA CMIA	n.m.

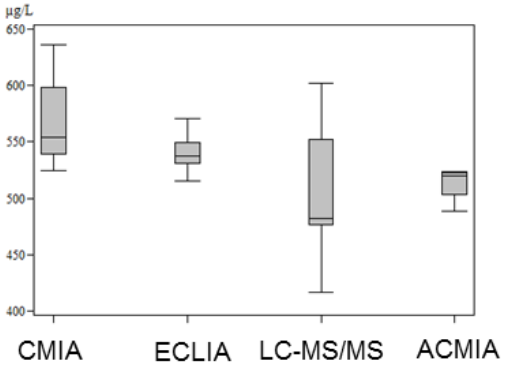
Supplemental Table 2. Cross-reactivity with metabolites commonly found in whole blood samples for the ECLIA Cyclosporine assay. Cross-reactivity was evaluated based on guidance from CLSI document EP7-A2.

Metabolite	Maximum concentration of metabolite added µg/L	Cross-reactivity %
AM1	2000	2
AM19	2000	n. d.[a)]
AM1c	2000	n. d.
AM1c9	2000	n. d.
AM4n	2000	2
AM9	2000	6

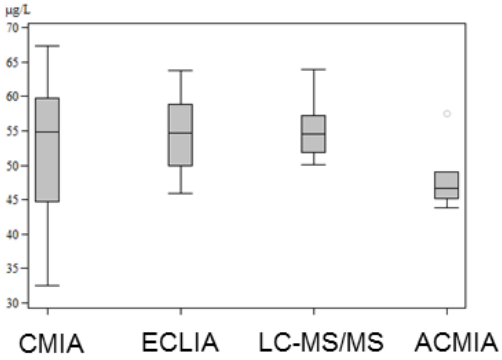
[a)] n.d. = not detectable. Cross-reactivity was designated as “not detectable” if the obtained value was less than the sensitivity of the assay.

Supplemental Figure 1. Box and whisker plots for IPTS samples evaluated (pooled patient blood and spiked). Respective commercial assays are referred to in the x-axis.

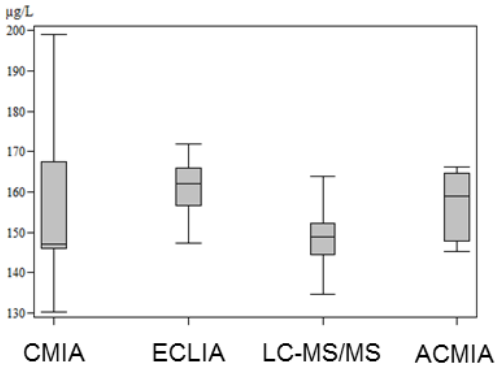




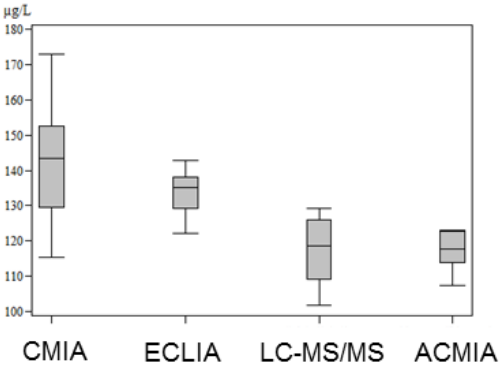
335B - spiked



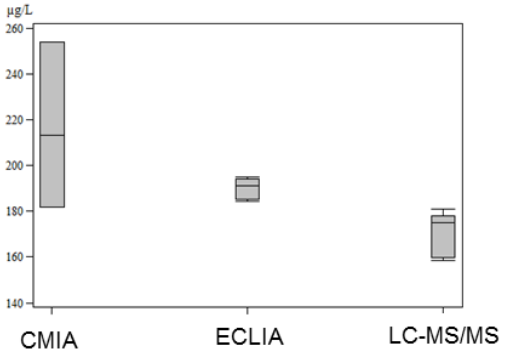
337A - spiked



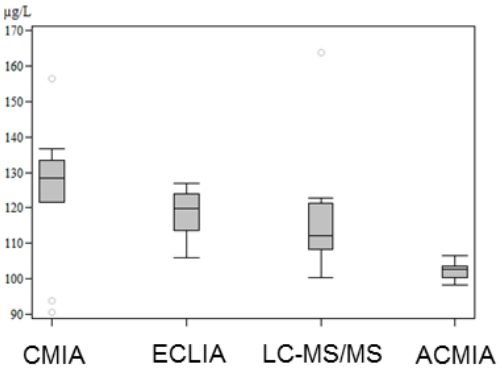
337B - spiked



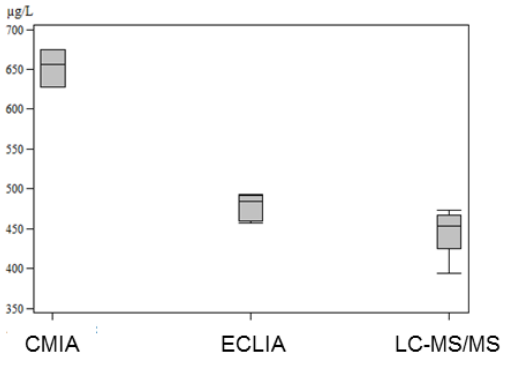
338A - pooled



339C - pooled

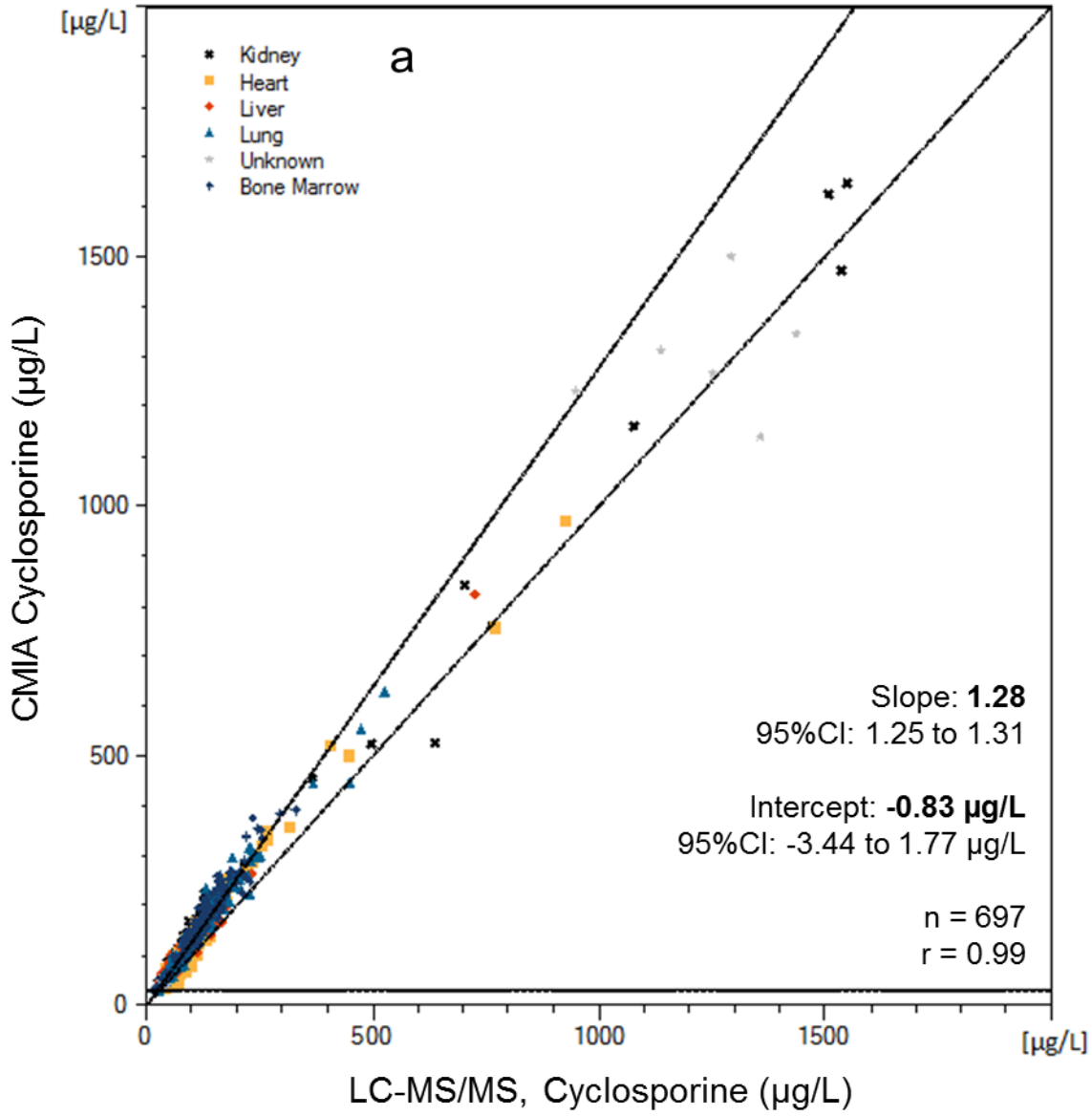


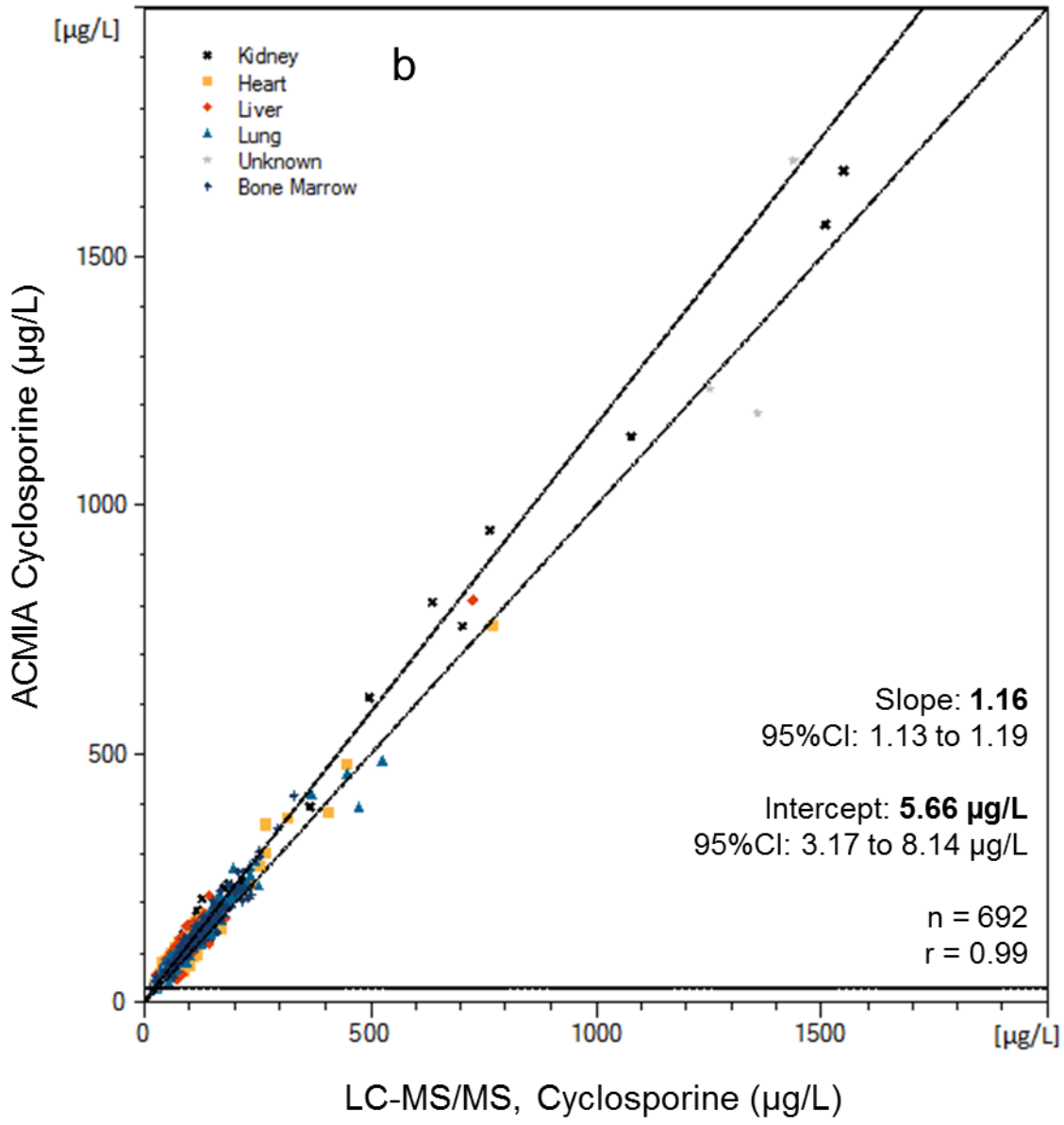
340C - spiked



342A - spiked

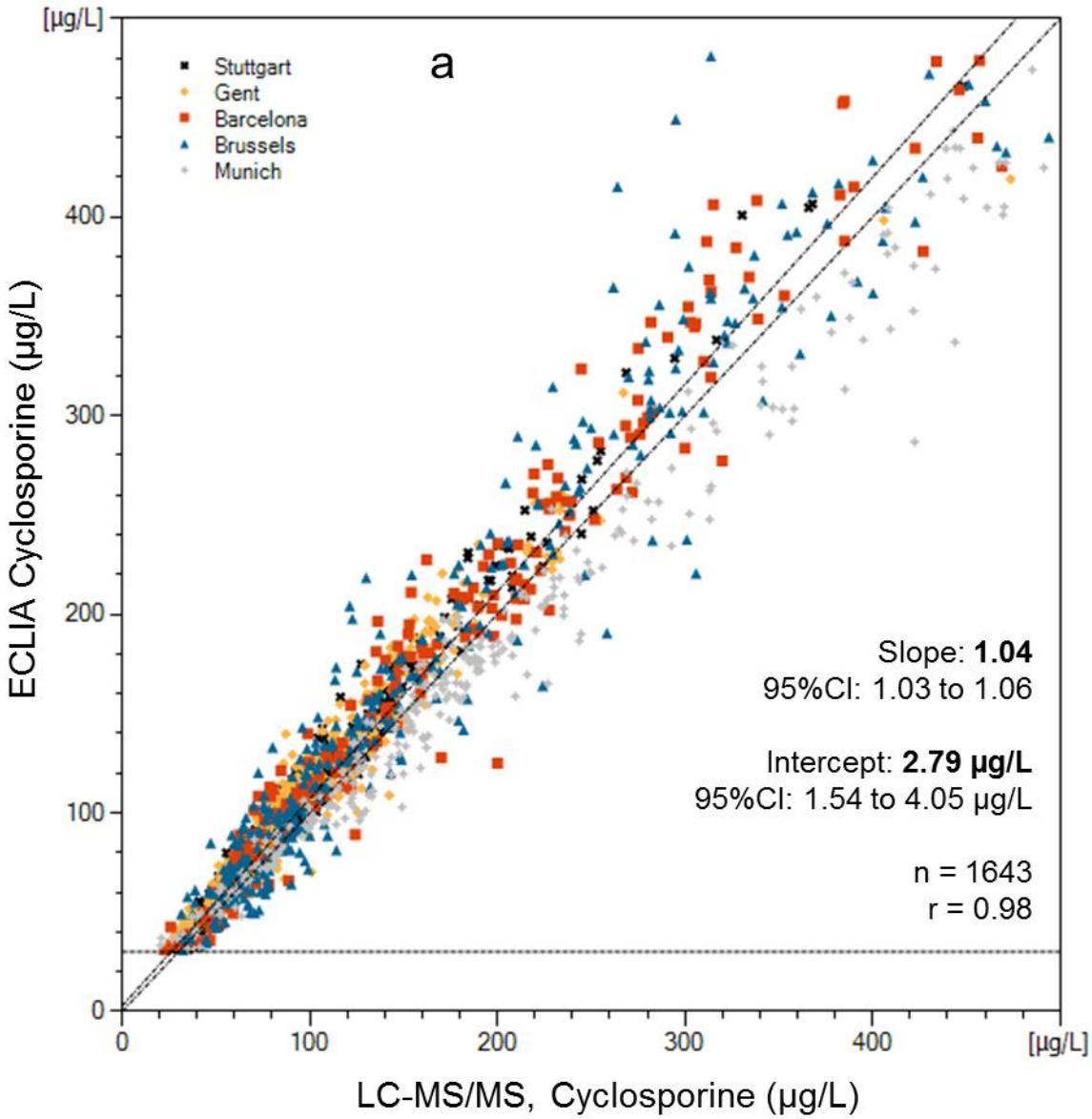
Supplemental Figure 2a-b. Method Comparison of LC-MS/MS methods versus CMIA and ACMIA. Method comparison of LC-MS/MS methods against CMIA (a) and ACMIA (b) was performed using weighted Deming regression. Regression parameters calculated using the entire measuring range are indicated within the plot. Five transplant cohorts and commercially obtained samples of unknown transplant type are included. The dashed line in the regression plot parallel to the x-axis represents the lower limit of the ECLIA assay measuring range. The unity line and the fitted line are also indicated with dashed lines.



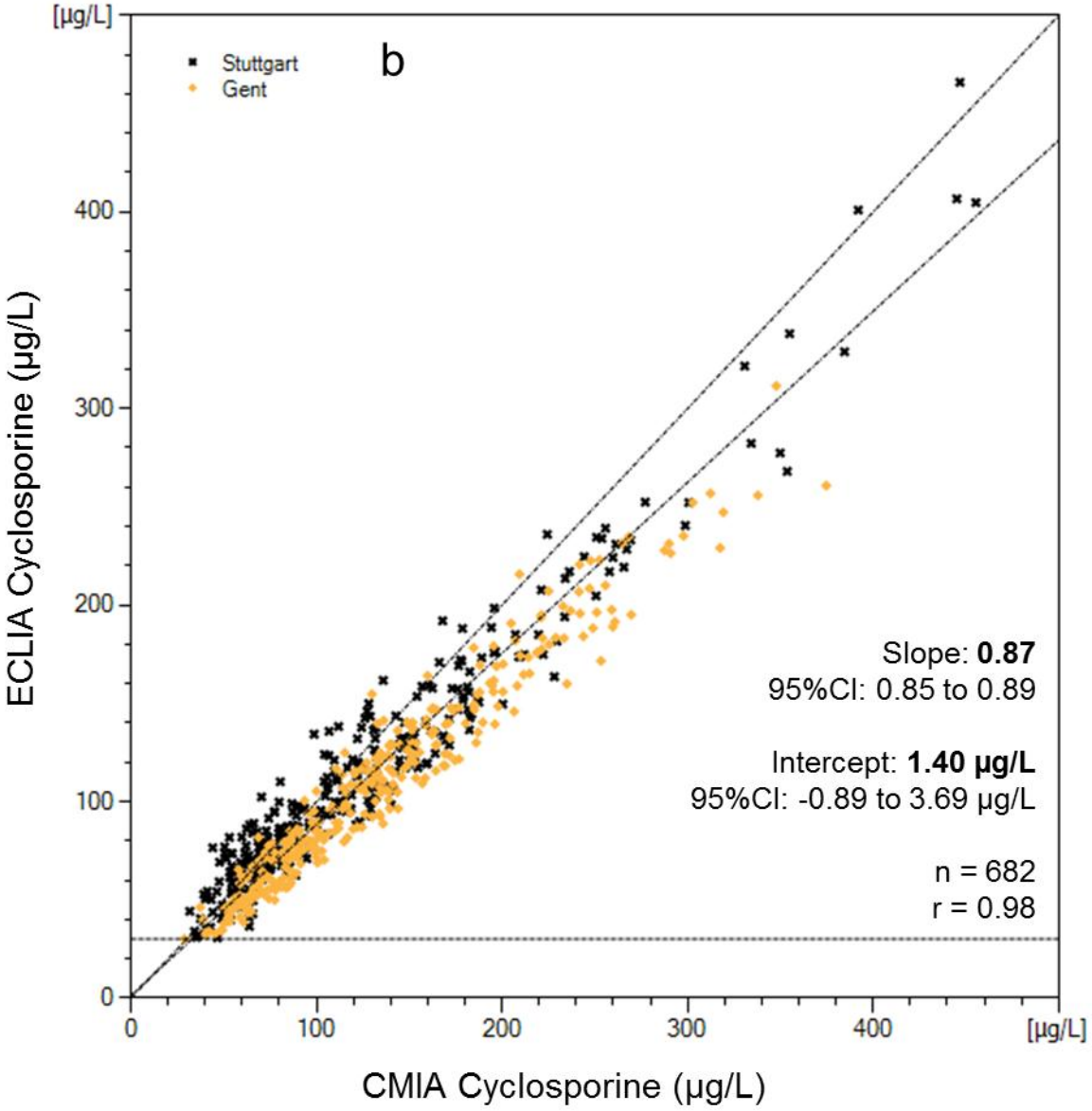

[µg/L]
b
Kidney
Heart
Liver
Lung
Unknown
Bone Marrow
ACMIA Cyclosporine (µg/L)
1500
1000
500
0
0
500
1000
1500
[µg/L]
Slope: 1.16
95%CI: 1.13 to 1.19
Intercept: 5.66 µg/L
95%CI: 3.17 to 8.14 µg/L
n = 692
r = 0.99
LC-MS/MS, Cyclosporine (µg/L)

Supplemental Figure 3a-c. Method Comparison of the ECLIA assay versus LC-MS/MS, CMIA and ACMIA (sites indicated). Method comparison of ECLIA against LC-MS/MS (a), CMIA (b) and ACMIA (c) was performed using weighted Deming regression. Data points from 0 to 500 µg/L are displayed due to the highest concentration of data points being in this region. Five transplant cohorts and commercially obtained samples of unknown transplant type are included, and the investigational site that is the source of each data point is indicated with different symbols in the graph. The dashed line in the regression plot parallel to the x-axis represents the lower limit of the ECLIA assay measuring range. The unity line and the fitted line are also indicated with dashed lines.

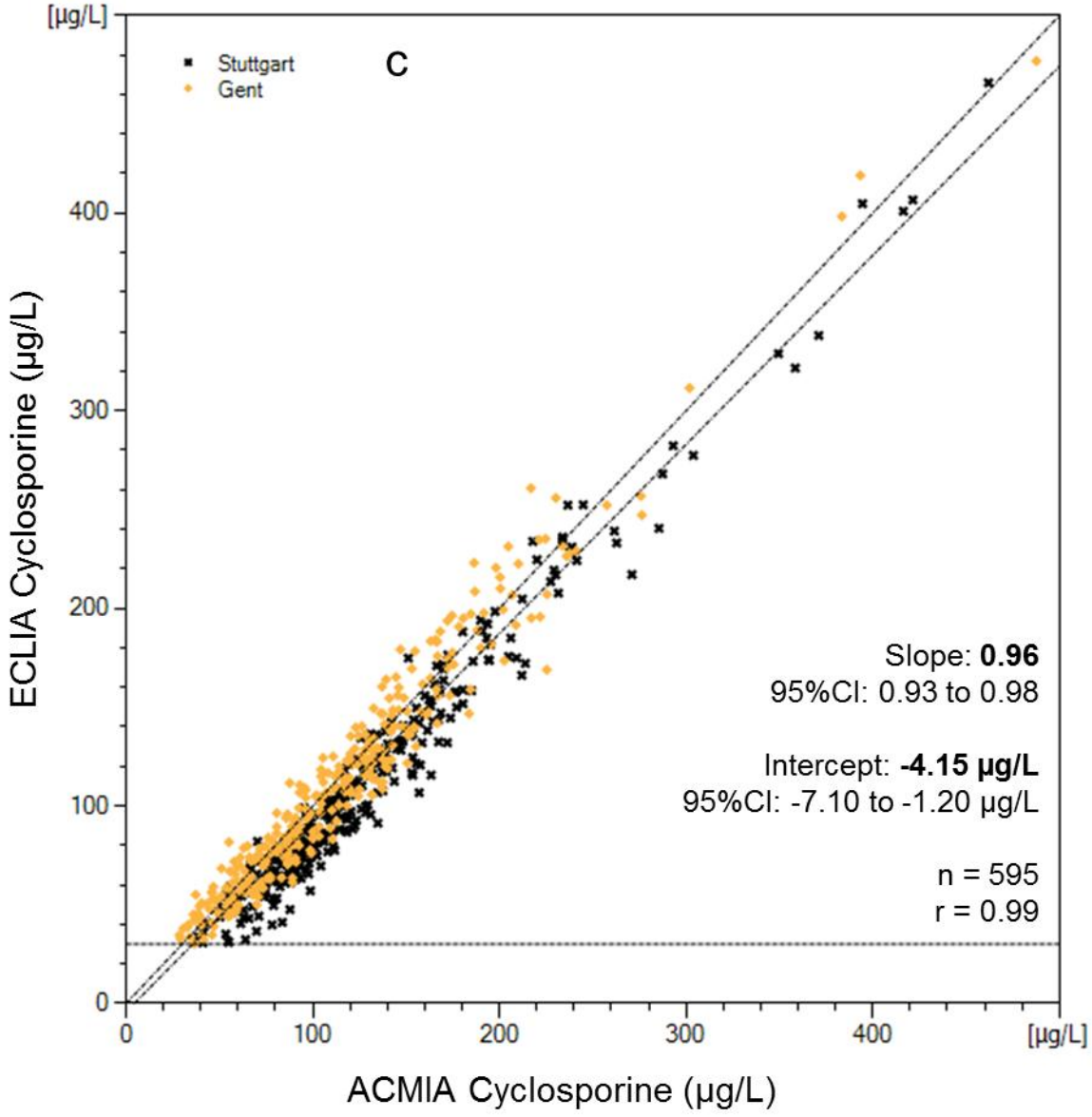
a) ECLIA vs LC-MS/MS, Deming regression



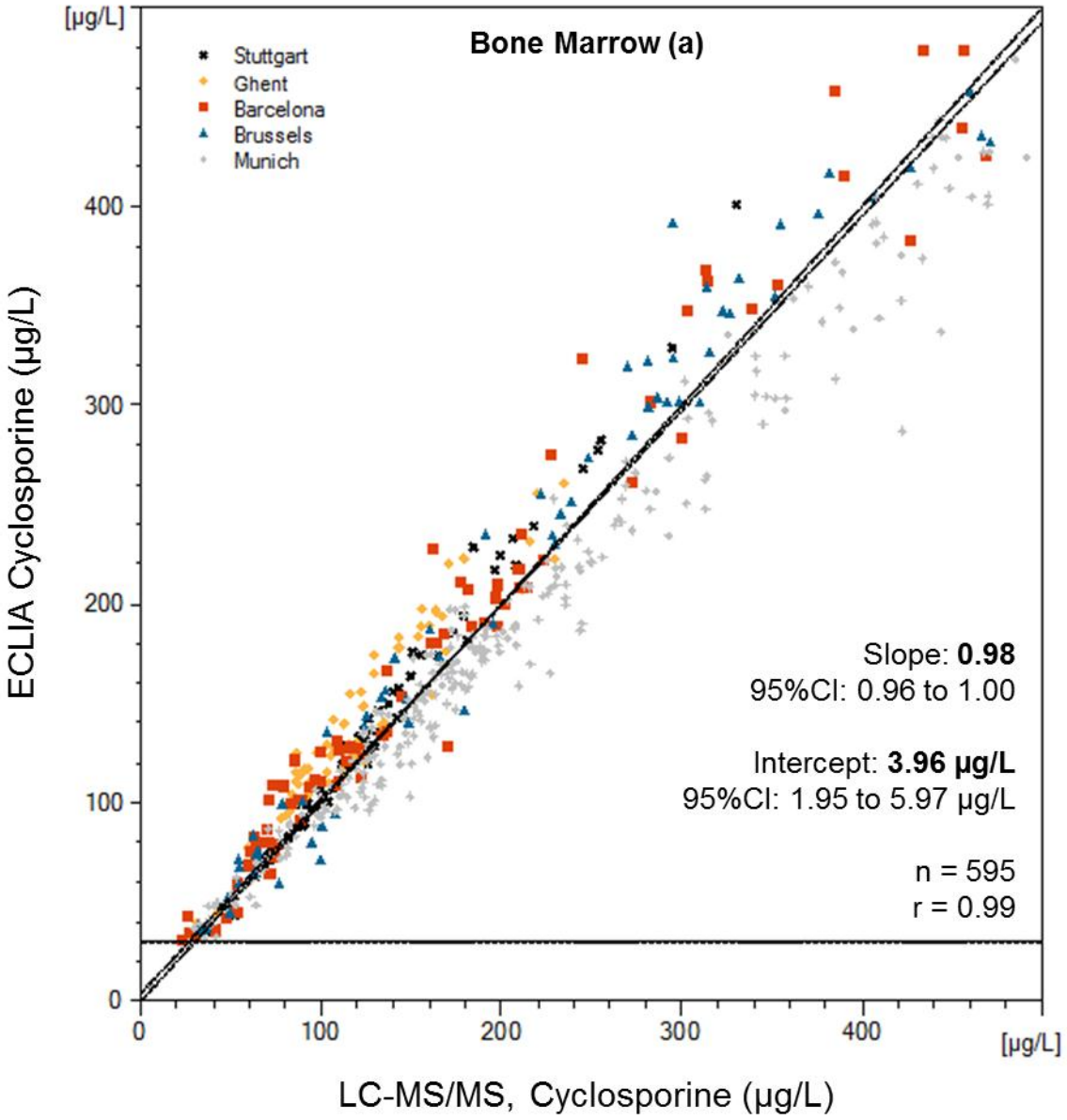
b) ECLIA vs CMIA, Deming regression

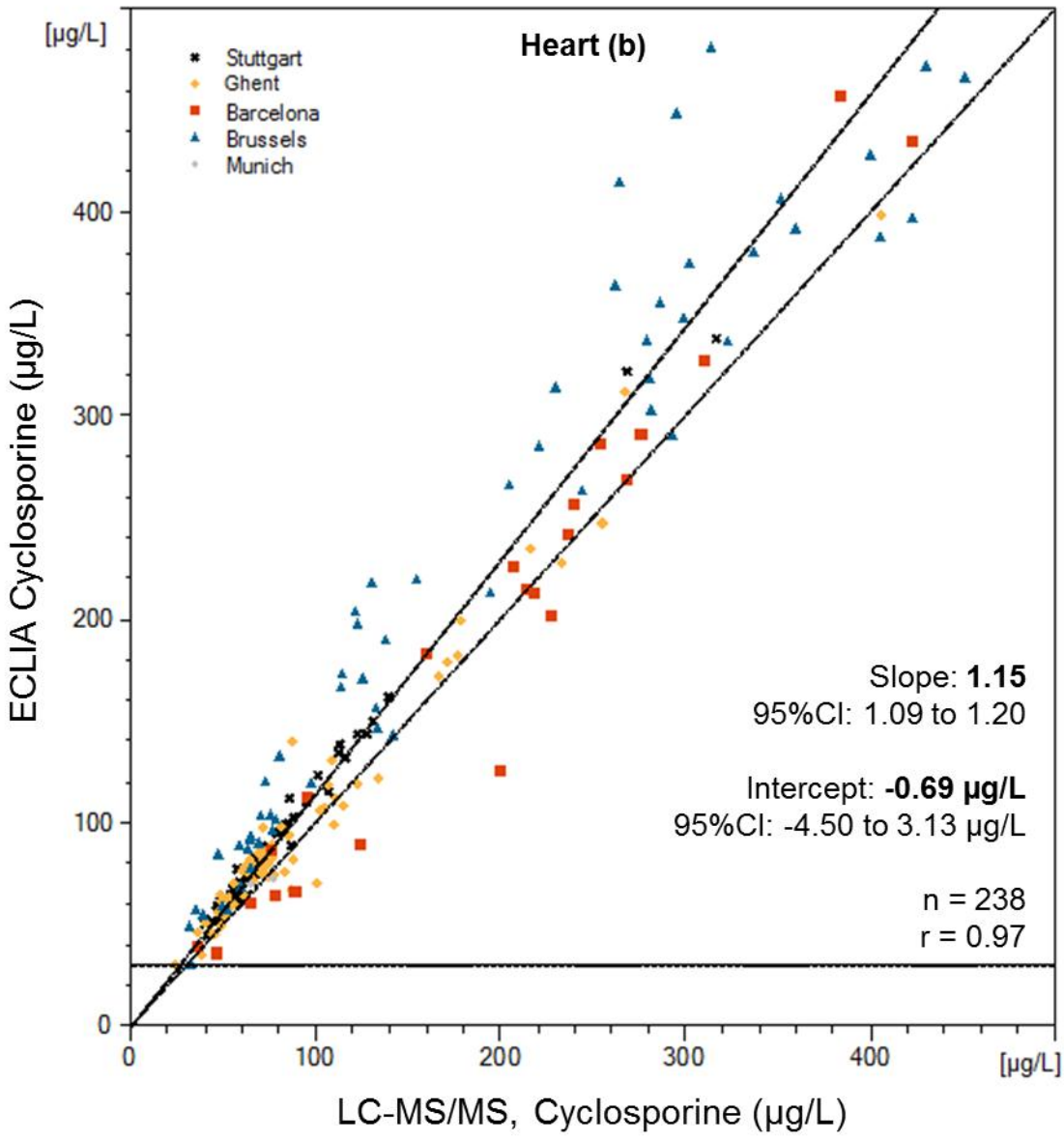


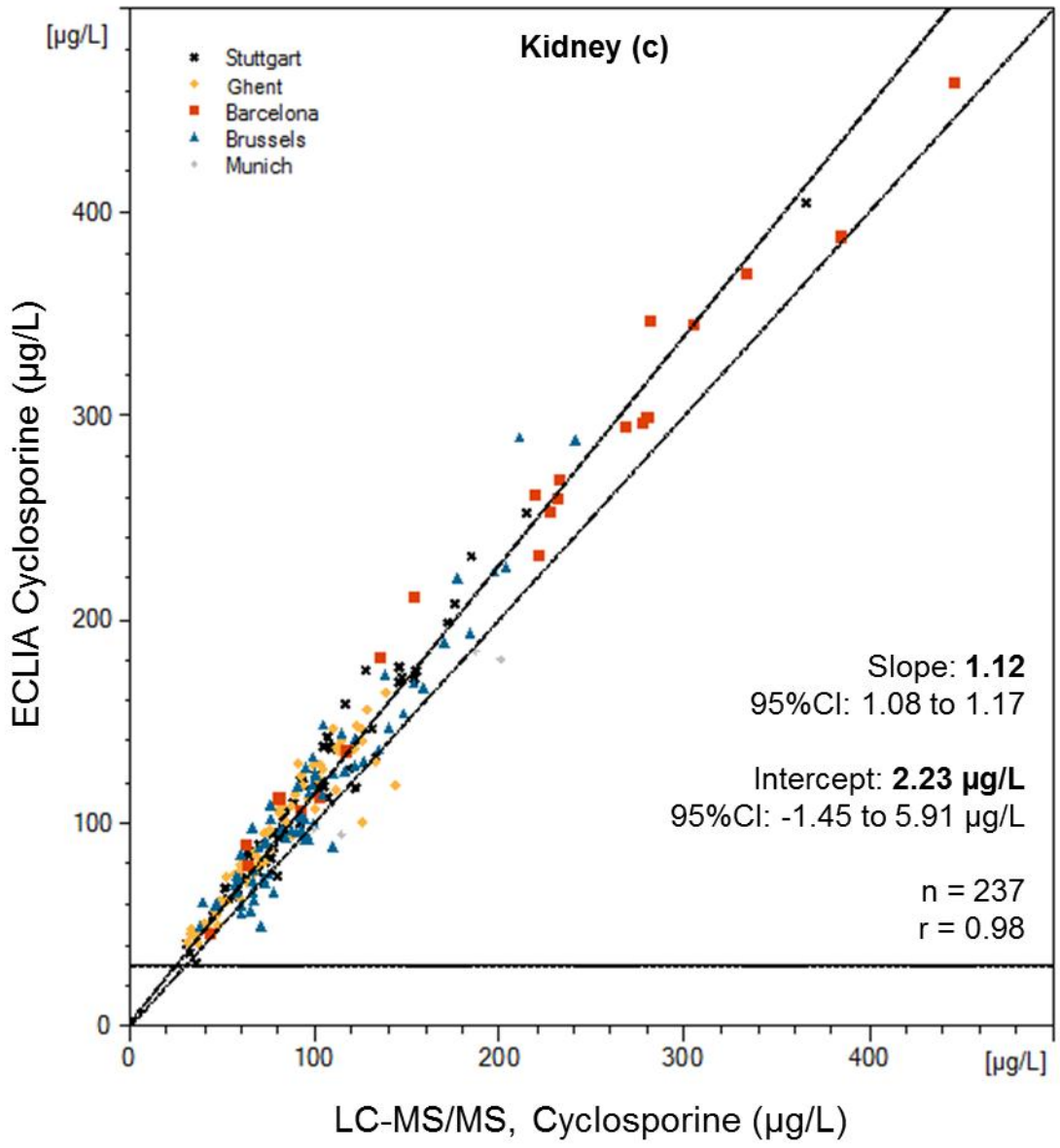
c) ECLIA vs ACMIA, Deming regression

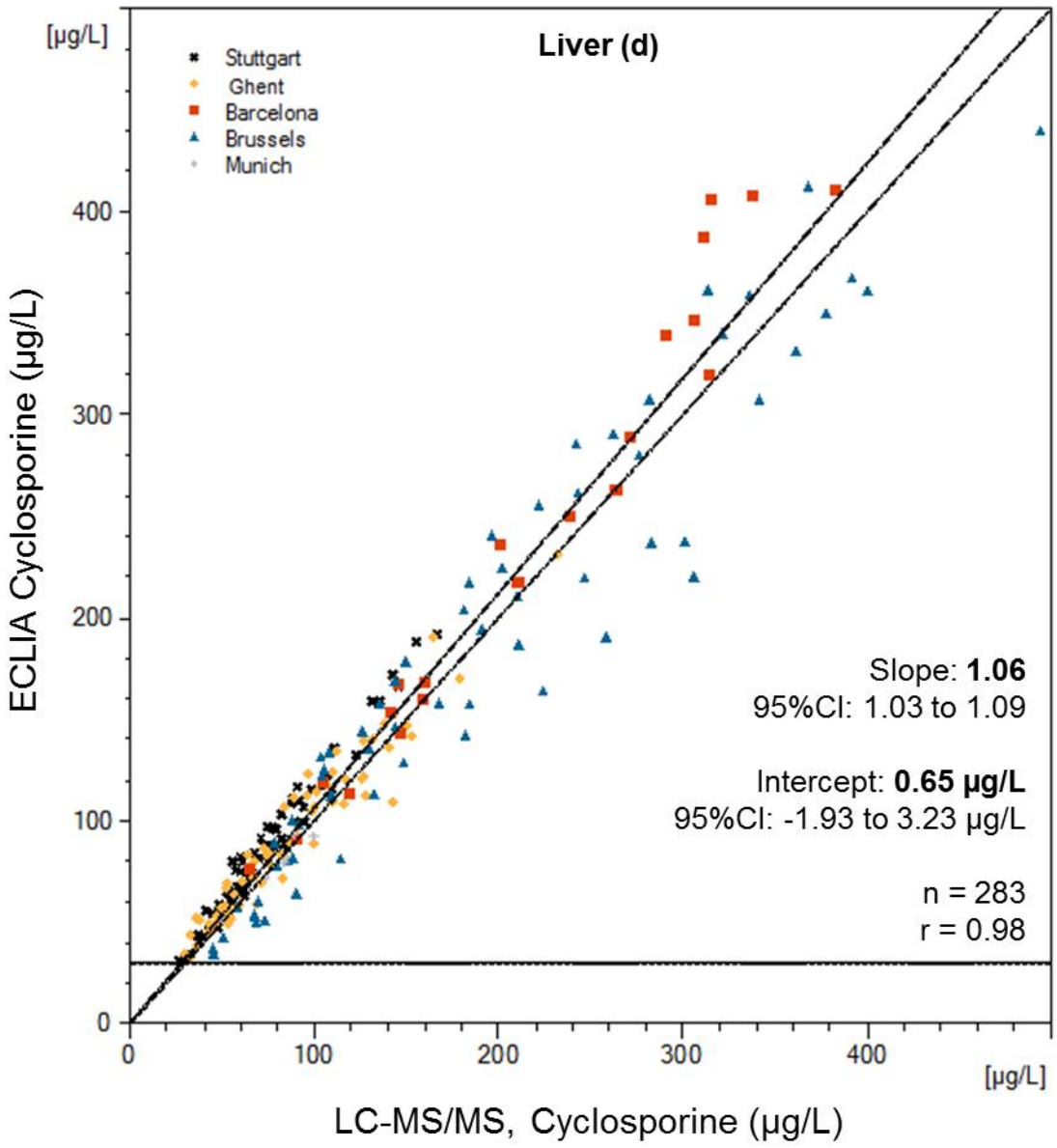


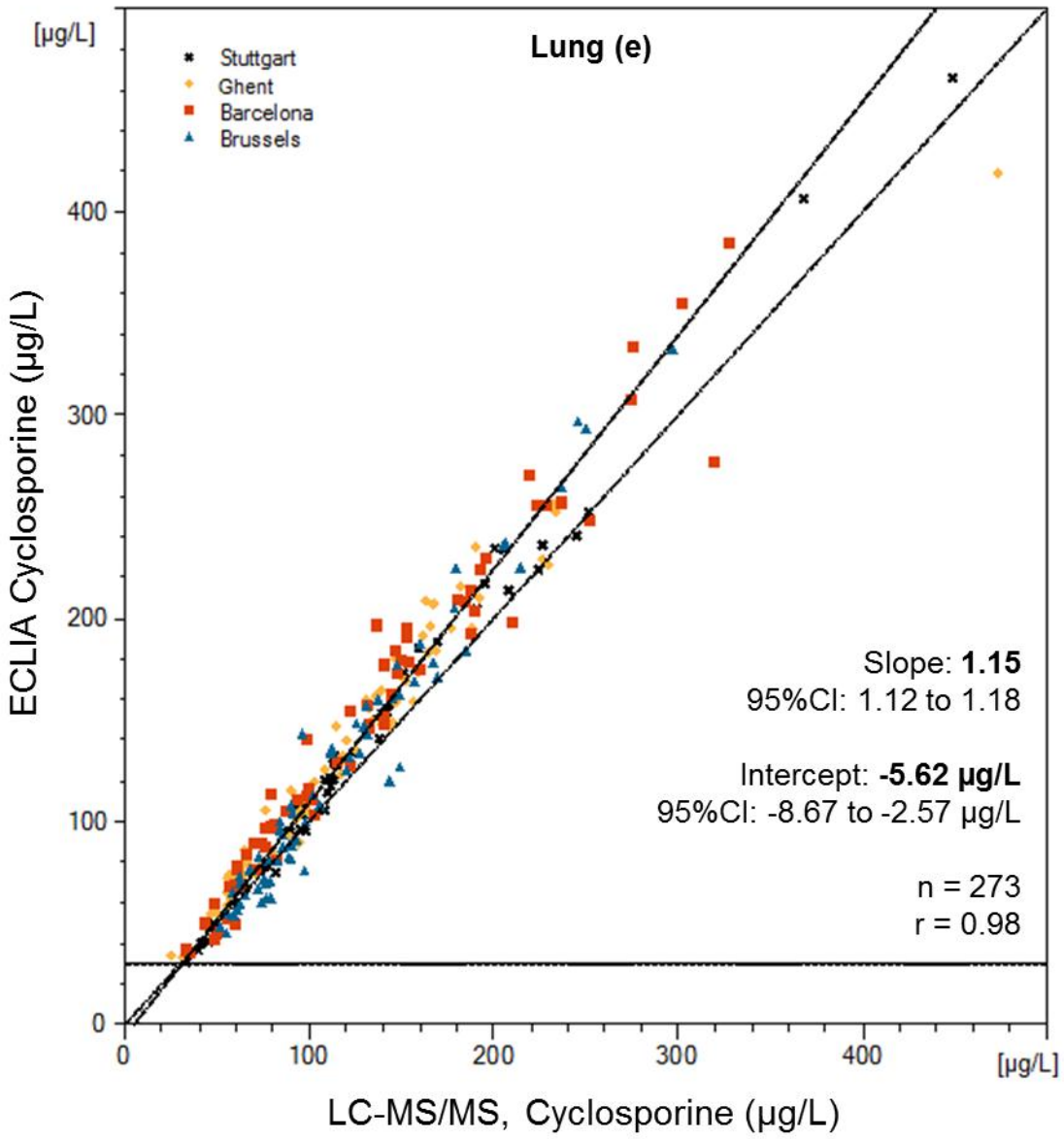
Supplemental Figure 4a-e. Method Comparison of ECLIA versus LC-MS/MS for each transplant group: bone marrow (a), heart (b), kidney (c), liver (d), and lung (e). Method comparison of ECLIA against LC-MS/MS for each transplant group was performed separately using weighted Deming regression. Data points from 0 to 500 µg/L are displayed due to the highest concentration of data points being in this region. Five transplant cohorts and commercially obtained samples of unknown transplant type are included, and the investigational site that is the source of each data point is indicated with different symbols in the graph. The dashed line in the regression plot parallel to the x-axis represents the lower limit of the ECLIA assay measuring range. The unity line and the fitted line are also indicated with dashed lines.




Heart (b)
[µg/L]
Stuttgart
Ghent
Barcelona
Brussels
Munich
ECLIA Cyclosporine (µg/L)
LC-MS/MS, Cyclosporine (µg/L)
0
100
200
300
400
[µg/L]
Slope: 1.15
95%CI: 1.09 to 1.20
Intercept: -0.69 µg/L
95%CI: -4.50 to 3.13 µg/L
n = 238
r = 0.97


Kidney (c)
[µg/L]
Stuttgart
Ghent
Barcelona
Brussels
Munich
ECLIA Cyclosporine (µg/L)
LC-MS/MS, Cyclosporine (µg/L)
0
100
200
300
400
[µg/L]
Slope: 1.12
95%CI: 1.08 to 1.17
Intercept: 2.23 µg/L
95%CI: -1.45 to 5.91 µg/L
n = 237
r = 0.98


Liver (d)
[µg/L]
Stuttgart
Ghent
Barcelona
Brussels
Munich
ECLIA Cyclosporine (µg/L)
0
100
200
300
400
[µg/L]
LC-MS/MS, Cyclosporine (µg/L)
Slope: 1.06
95%CI: 1.03 to 1.09
Intercept: 0.65 µg/L
95%CI: -1.93 to 3.23 µg/L
n = 283
r = 0.98


Lung (e)
Stuttgart
Ghent
Barcelona
Brussels
[µg/L]
ECLIA Cyclosporine (µg/L)
LC-MS/MS, Cyclosporine (µg/L)
[µg/L]
0
100
200
300
400
Slope: 1.15
95%CI: 1.12 to 1.18
Intercept: -5.62 µg/L
95%CI: -8.67 to -2.57 µg/L
n = 273
r = 0.98