25-Hydroxyvitamin D assay performance

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Outline of Talk

1. DEQAS: Organization and Objectives
2. Mean Assay Bias and Precision
3. Bias of individual samples (major assay groups)
4. Influence of other metabolites on 25-OHD assays
5. A pre-analytical problem

DEQAS

An international External Quality Assessment Scheme for Vitamin D Metabolites:

25-hydroxyvitamin D (25-OHD)*

1,25-dihydroxyvitamin D (1,25-$(OH)_2D$)

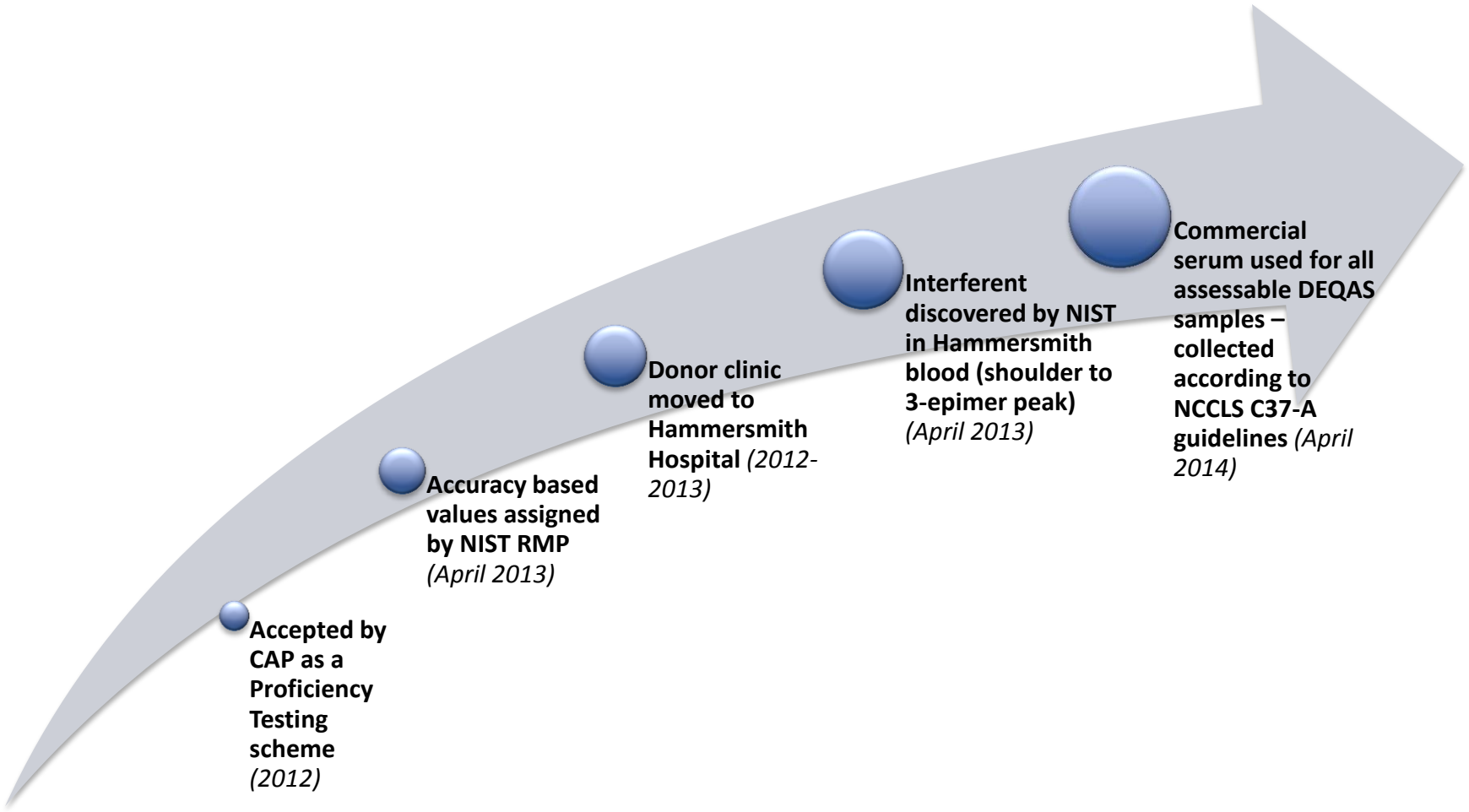
From April 2015: 24,25$(OH)_2D$ (pilot scheme)

* 5 samples of ***unadulterated*** liquid human serum distributed quarterly at ambient temperature to over 1000 participants in 53 countries

DEQAS Objectives

- Monitor the accuracy of participants' results - % Bias from the 'True' results (RMP)
- Monitor the intrinsic accuracy (% Bias) and precision of 25-OHD Methods
- Investigate aspects of 25-OHD methodology eg. specificity, matrix effects, pre-analytical
- Helping participants and manufacturers to improve assay performance
- Complement VDSP and CDC programs

DEQAS: recent milestones



Vitamin D

- Generic name for a group of anti - rachitic substances.

includes vitamin D3 (Cholecalciferol*)
vitamin D2 (Ergocalciferol**)

*synthesized in-vivo (UV on human skin)
** plants/invertebrates, in-vitro synthesis

Vitamin D

Vitamin D3 → (Liver) → 25 – OHD3 → (Kidney *) → $1,25\ (OH)_2D3$

Vitamin D2 → (Liver) → 25-OHD2 → (Kidney *) → $1,25\ (OH)_2\ D2$

* and many other tissues

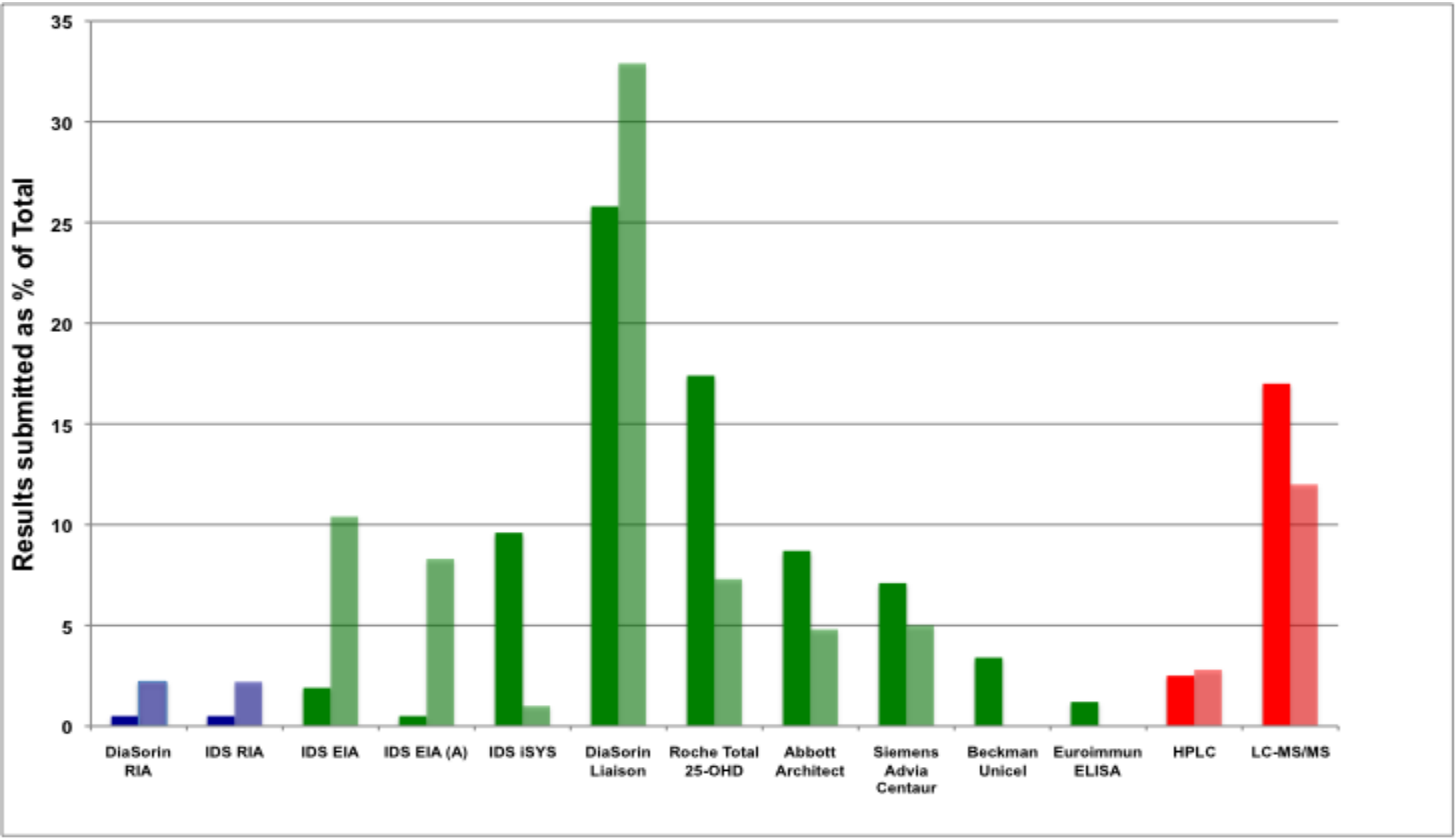
Why 25-hydroxyvitamin D?

1. Long Half-life (25-OHD3 > 25-OHD2)
   - reflects long-term vitamin D intake
   (cf. glycosylated Hb)

2. 25-hydroxylase is an unregulated enzyme
   (25-OHD levels reflect substrate conc.)

3. nmolar concentration – *should be relatively* easy to measure (but it isn't!)

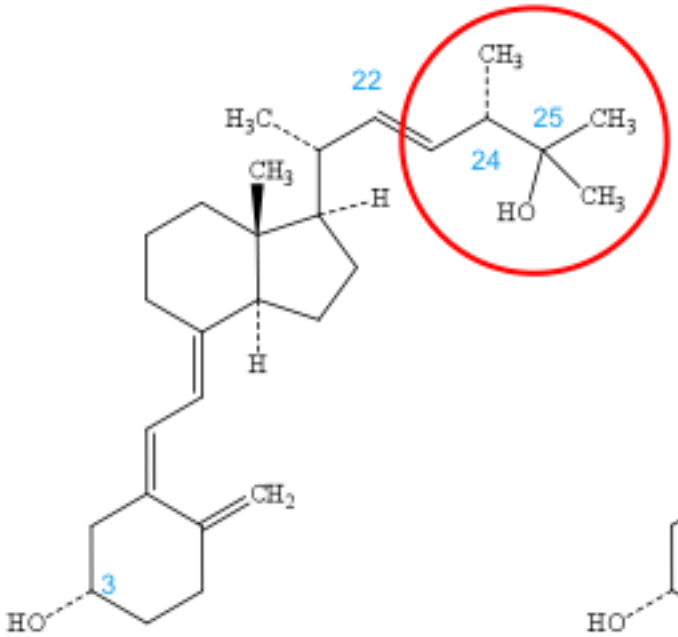
Results submitted in April 2016 (dark) and April 2012 (light)



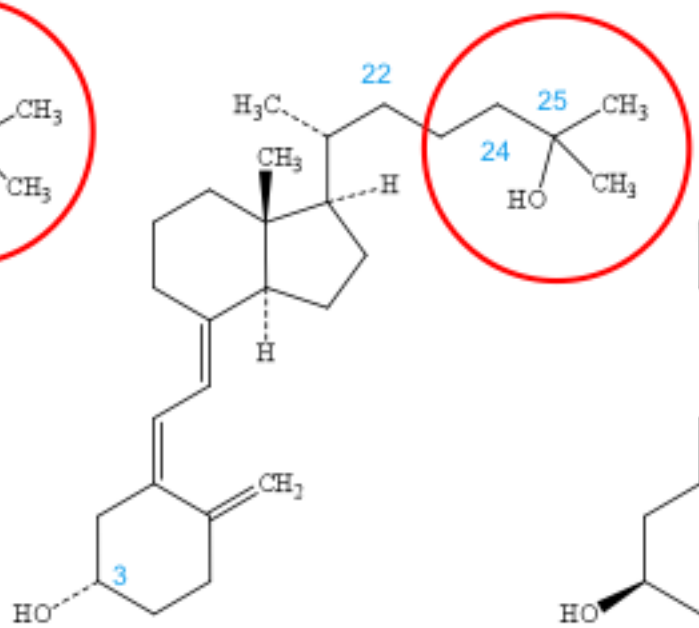
Factors influencing the accuracy of 25-OHD results

- Standardisation of assays (affects inter-assay variability) – province of the VDSP
- Cross reactivity of other vitamin D metabolites
- Interference from other sample constituents – matrix effects (inter-sample variability)

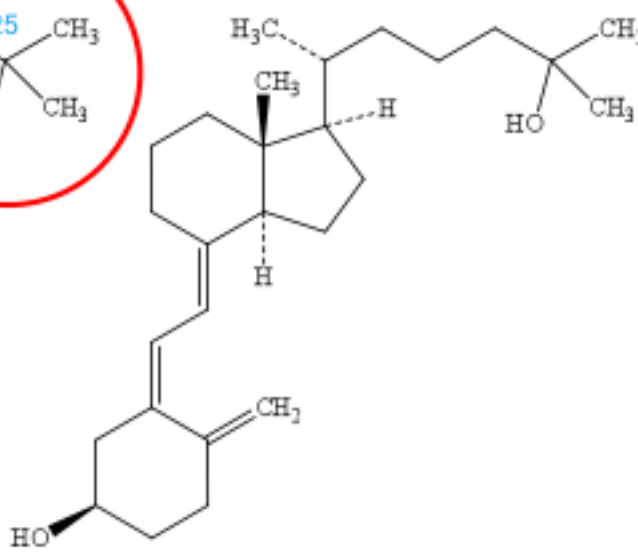
Structures of 25(OH)D metabolites



25-OHD_2



25-OHD_3

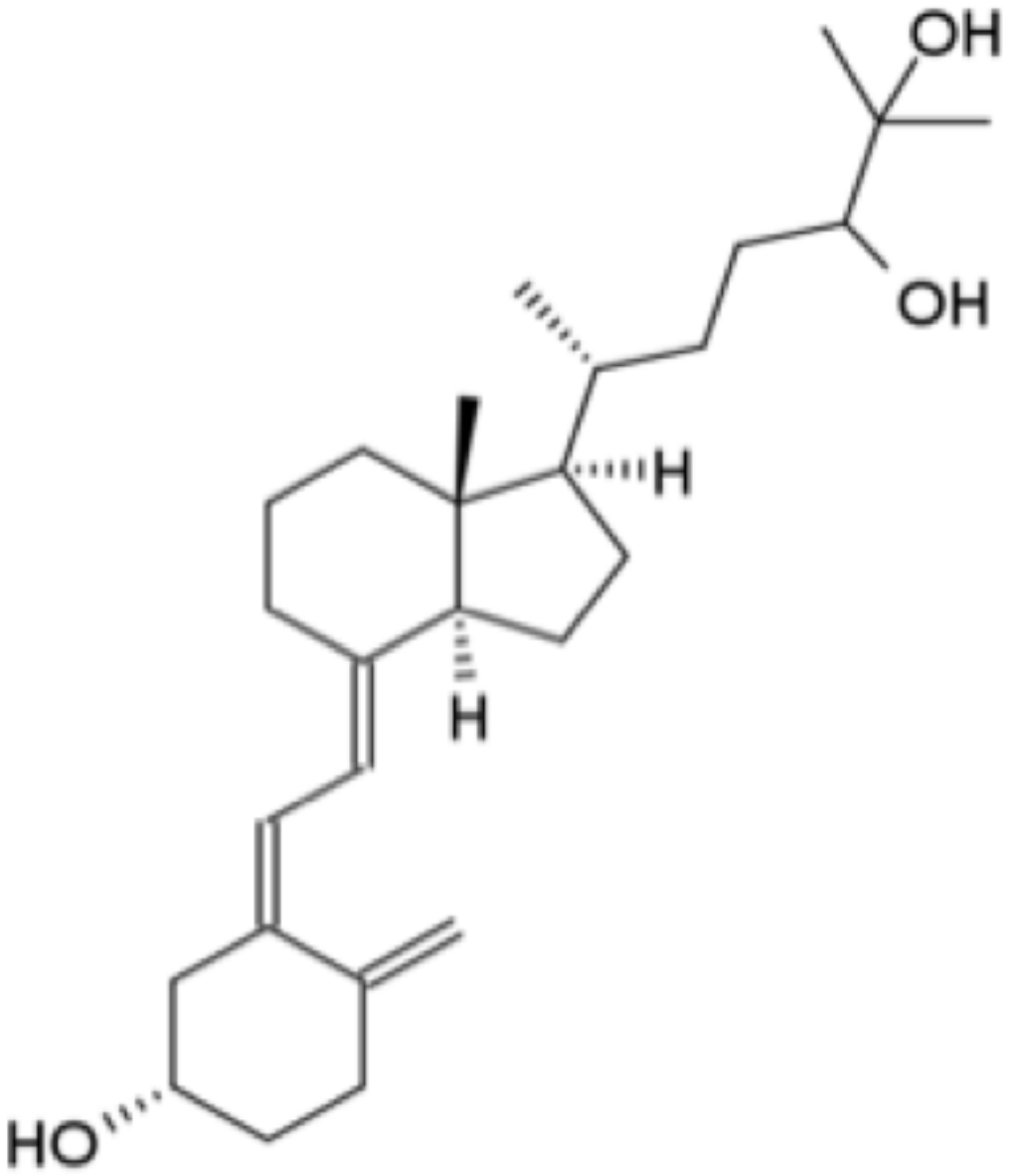


3-epi-25-OHD_3

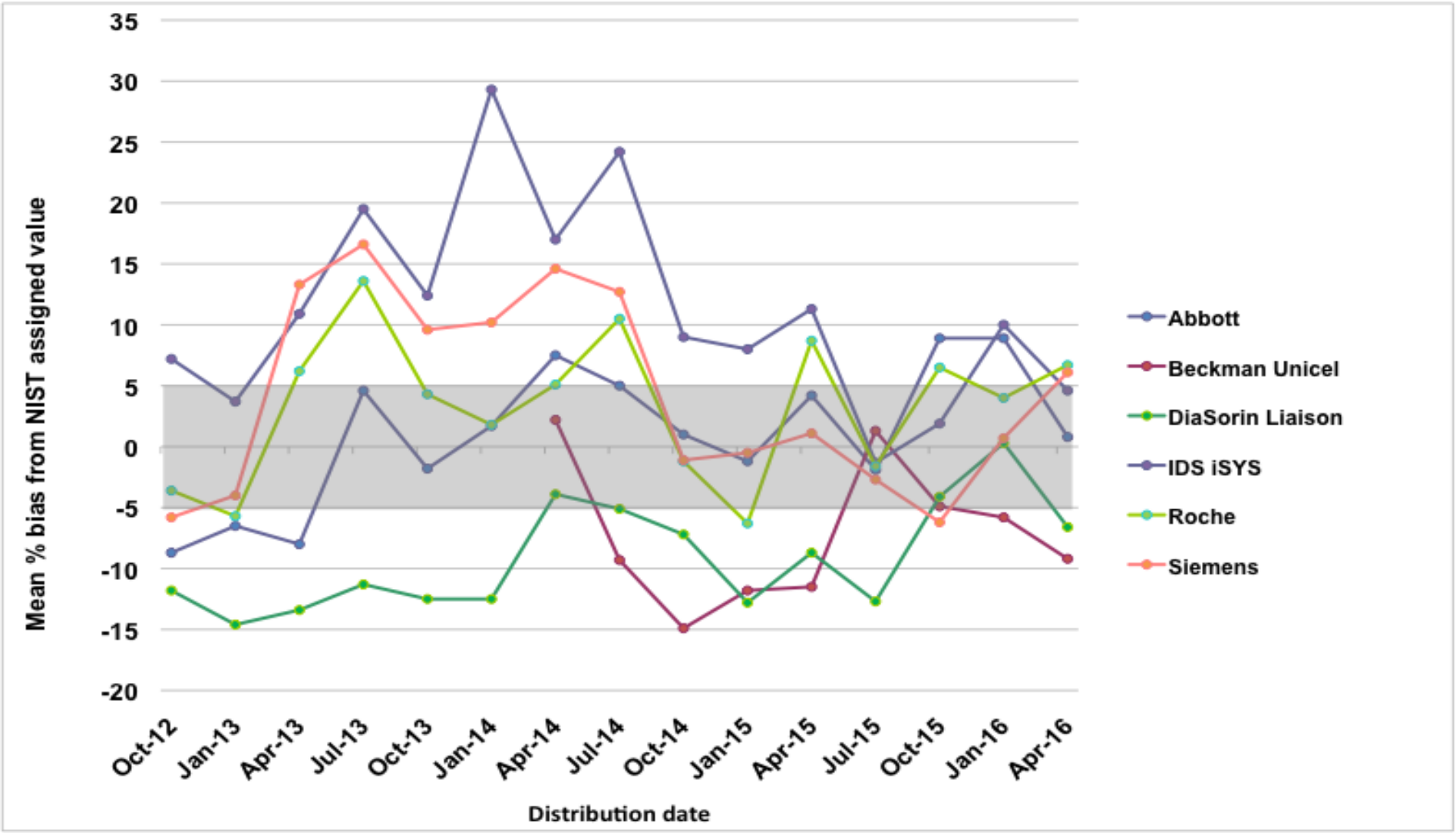
Molecular Weight:

412.6 400.6 400.6

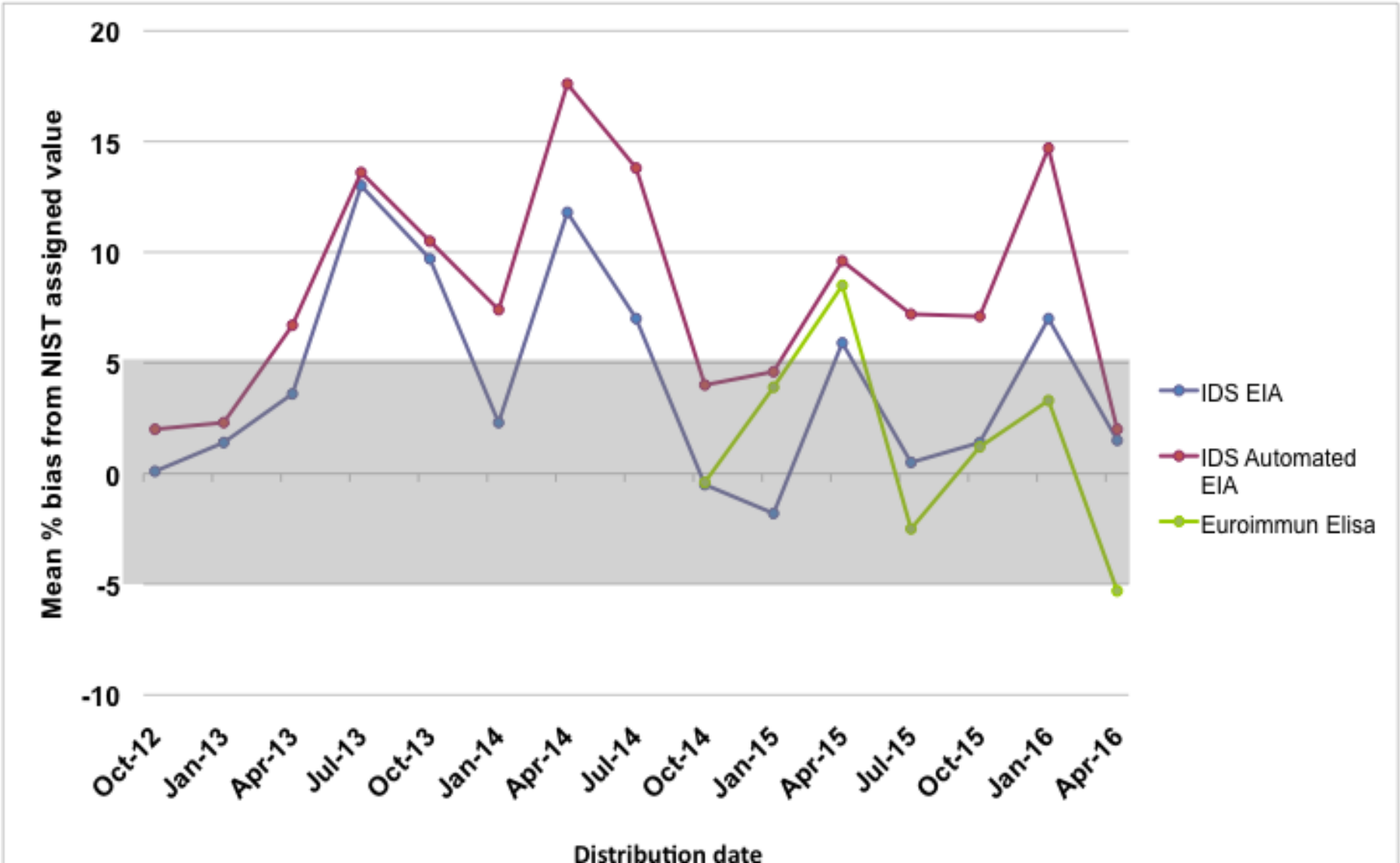
24,25 –dihydroxyvitamin D3



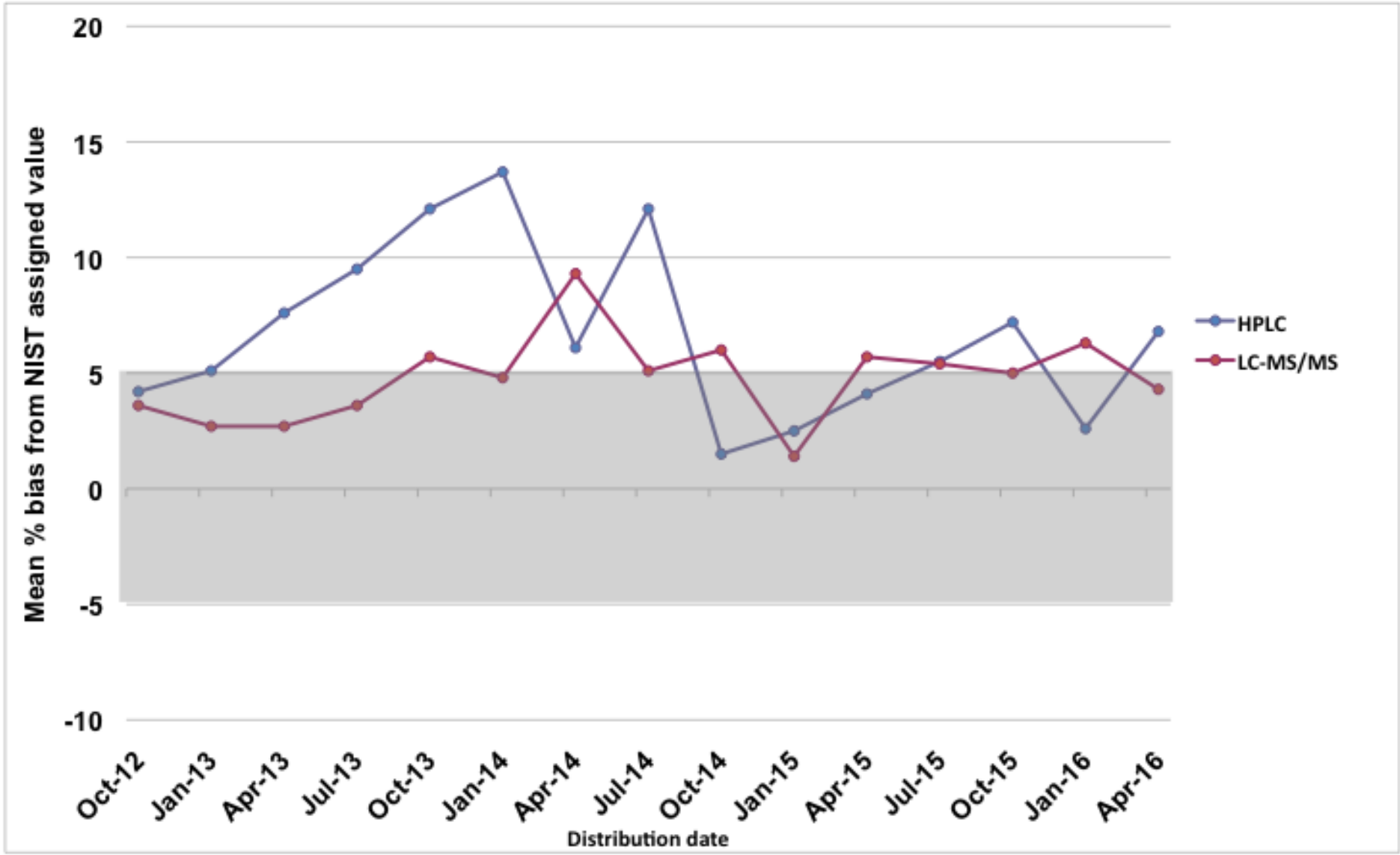
25-OHD Automated Assays; Mean % Bias from NIST Target Values Oct 2012 to April 2016



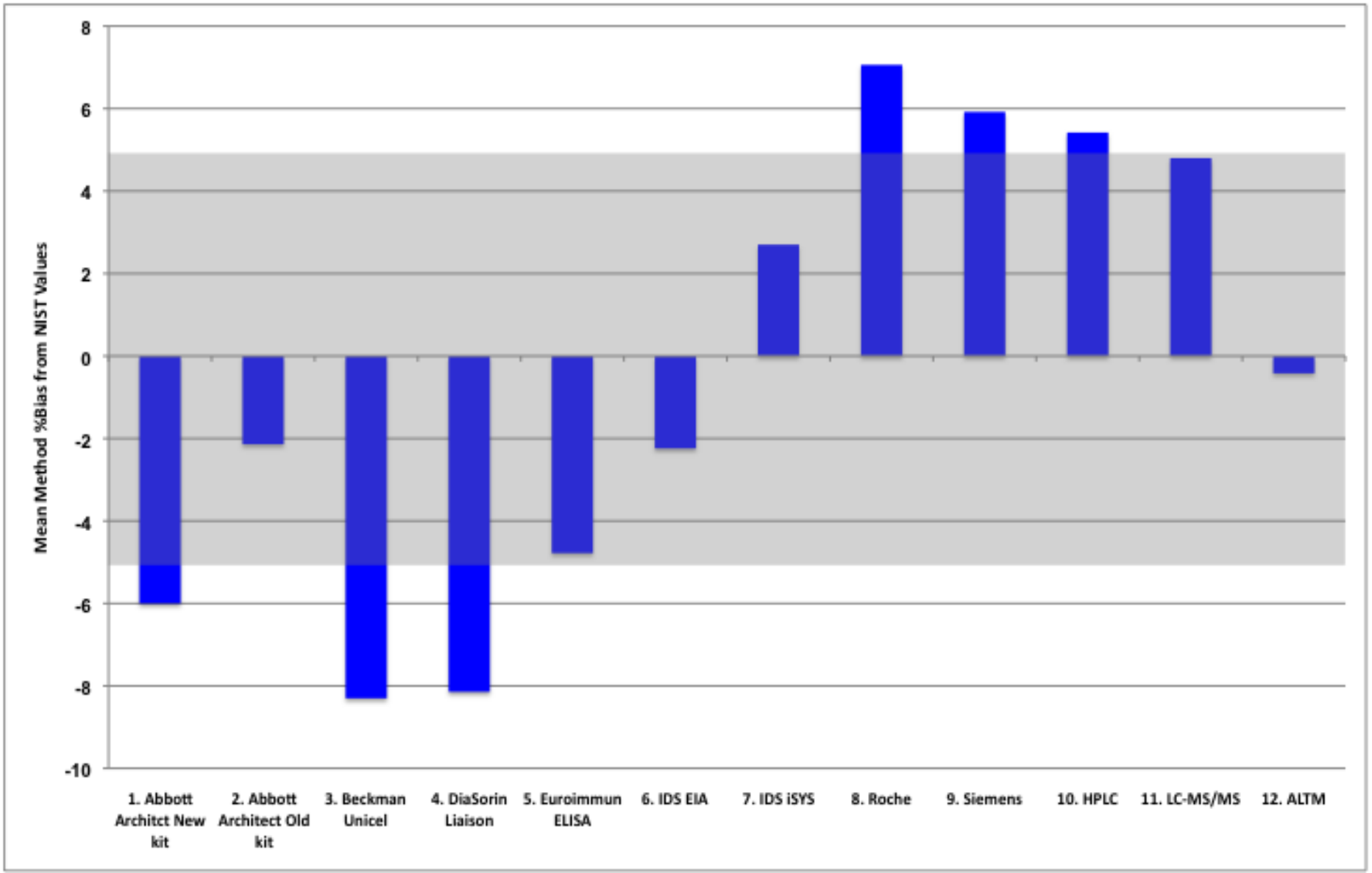
25-OHD Manual Assays; Mean % Bias from NIST Target Values Oct 2012 to April 2016



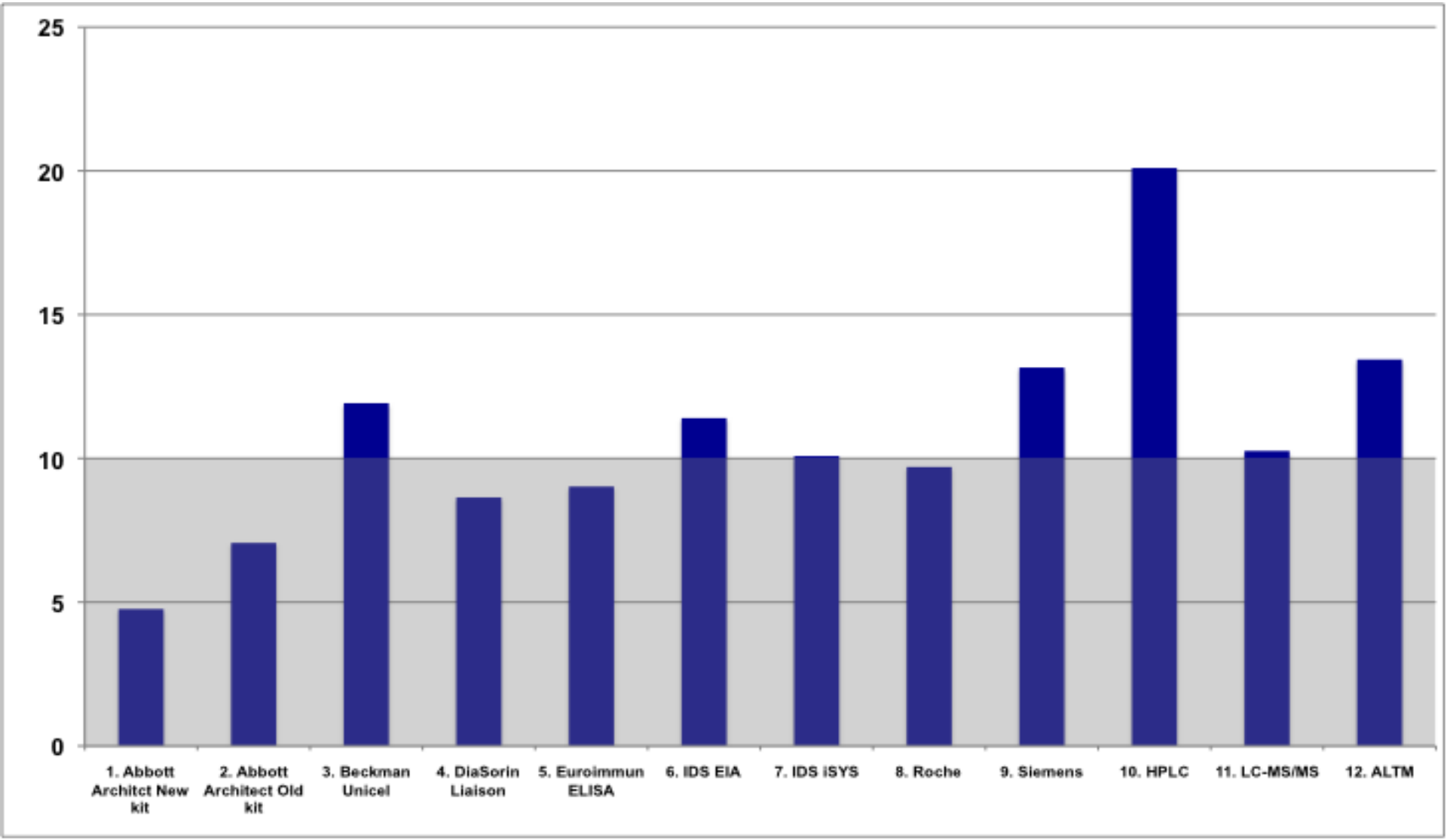
25-OHD HPLC & LC-MS/MS Assays; Mean % Bias from NIST Target Values Oct 2012 to April 2016



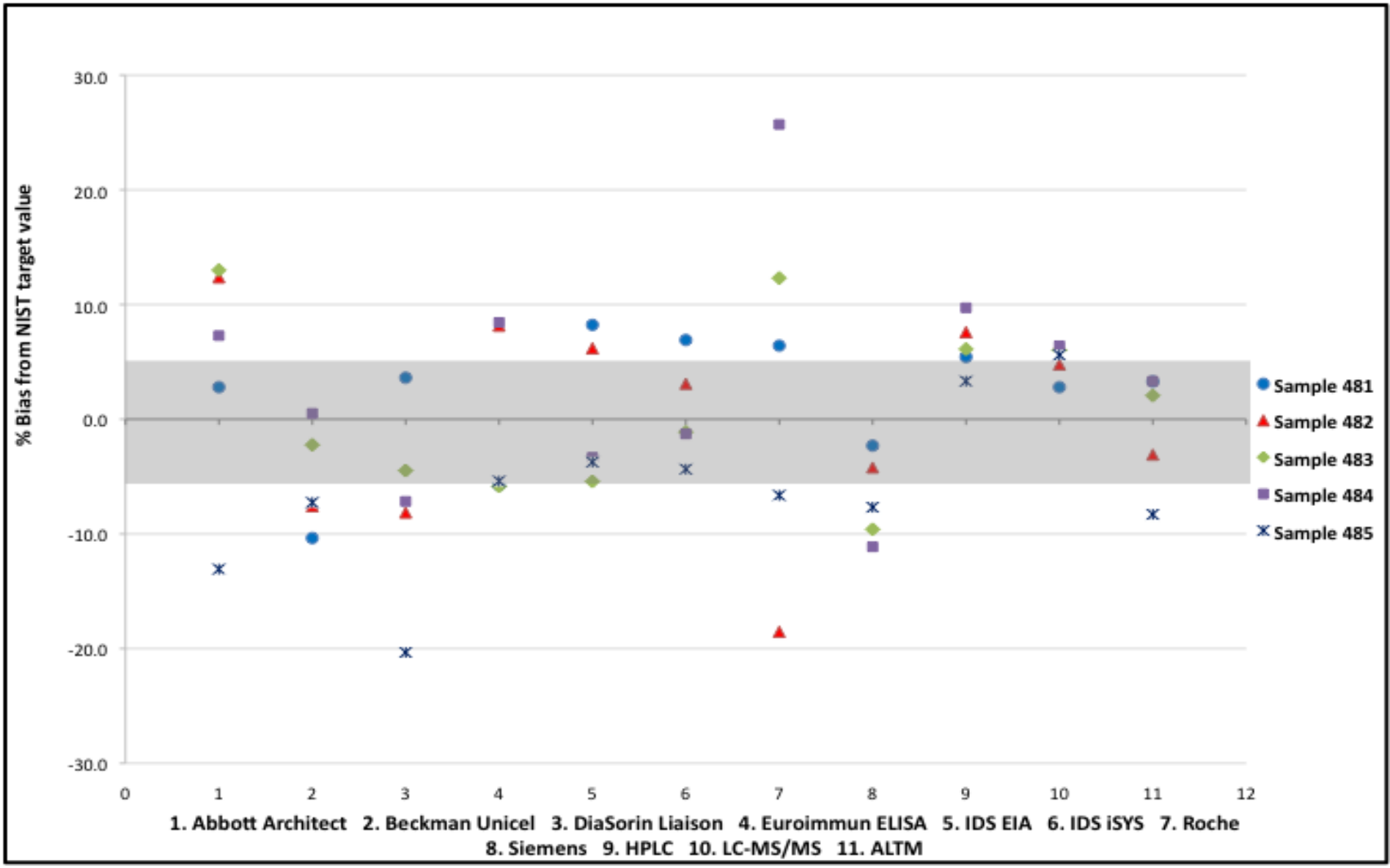
25-OHD April 2016; Mean % Bias by Method



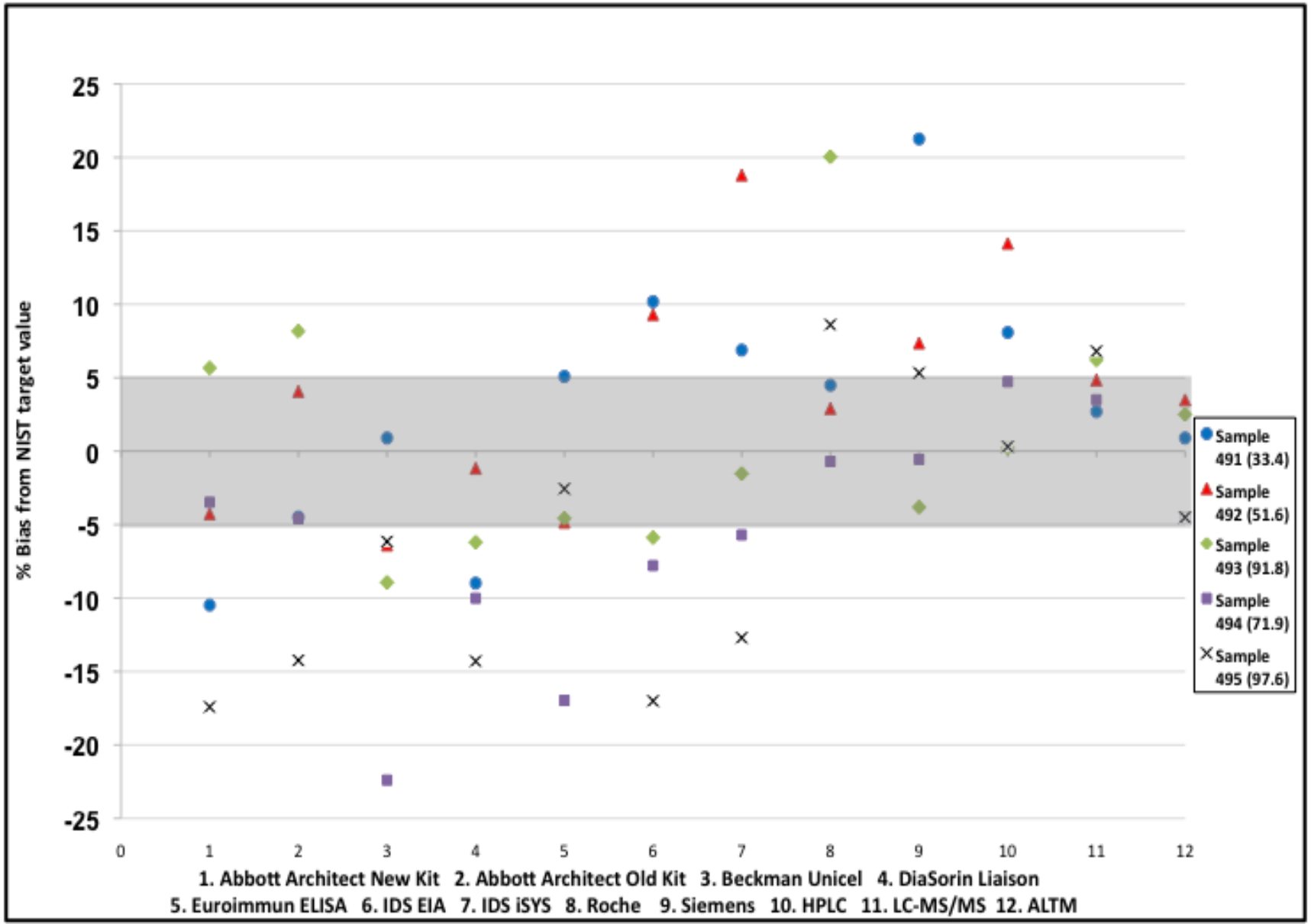
25-OHD April 2016; Mean CV % by Method



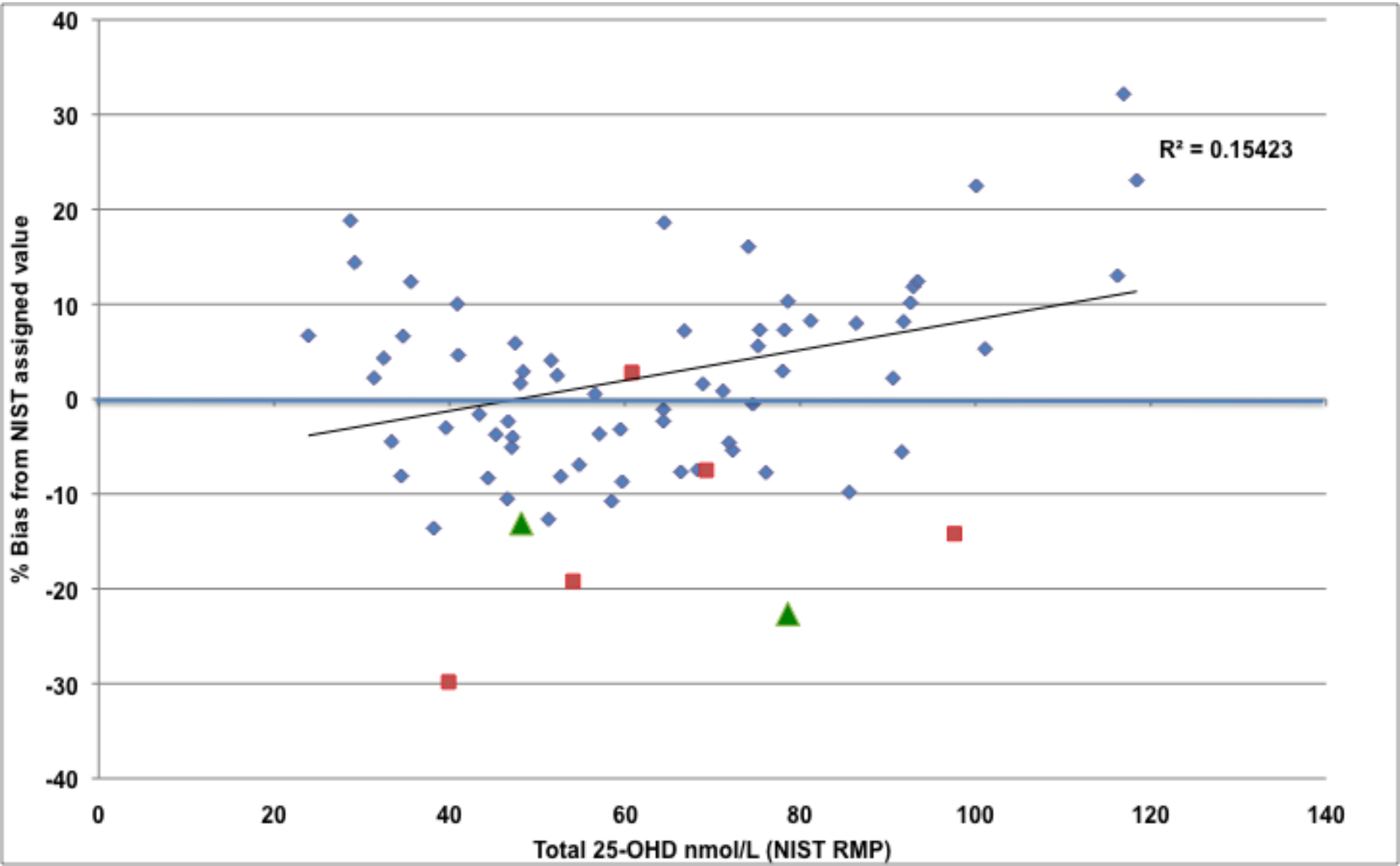
25-Hydoxyvitamin D October 2015 - Bias from NIST Target Value for Individual Methods



25-Hydoxyvitamin D April 2016 - Bias from NIST Target Value for Individual Methods



Abbott Architect



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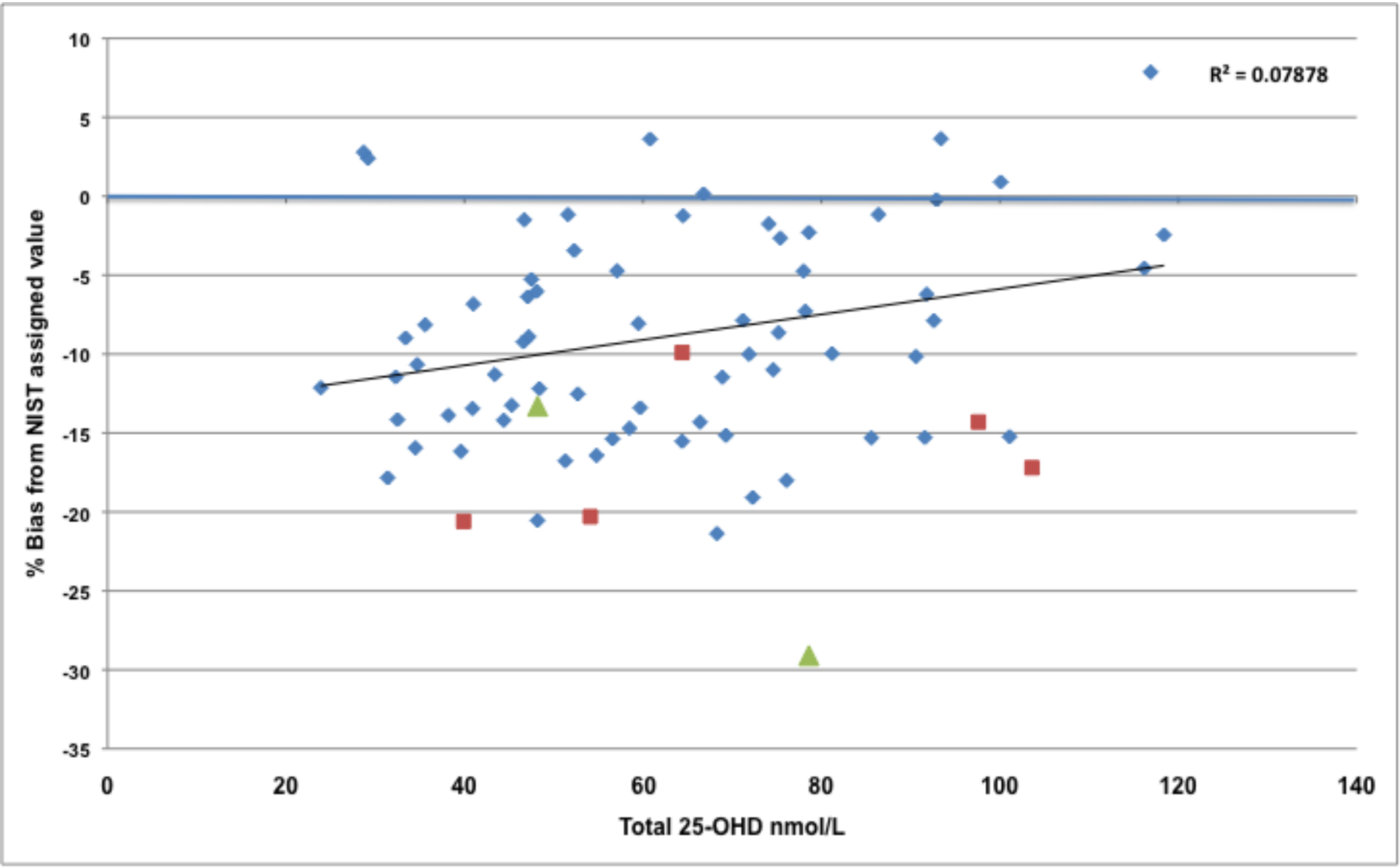

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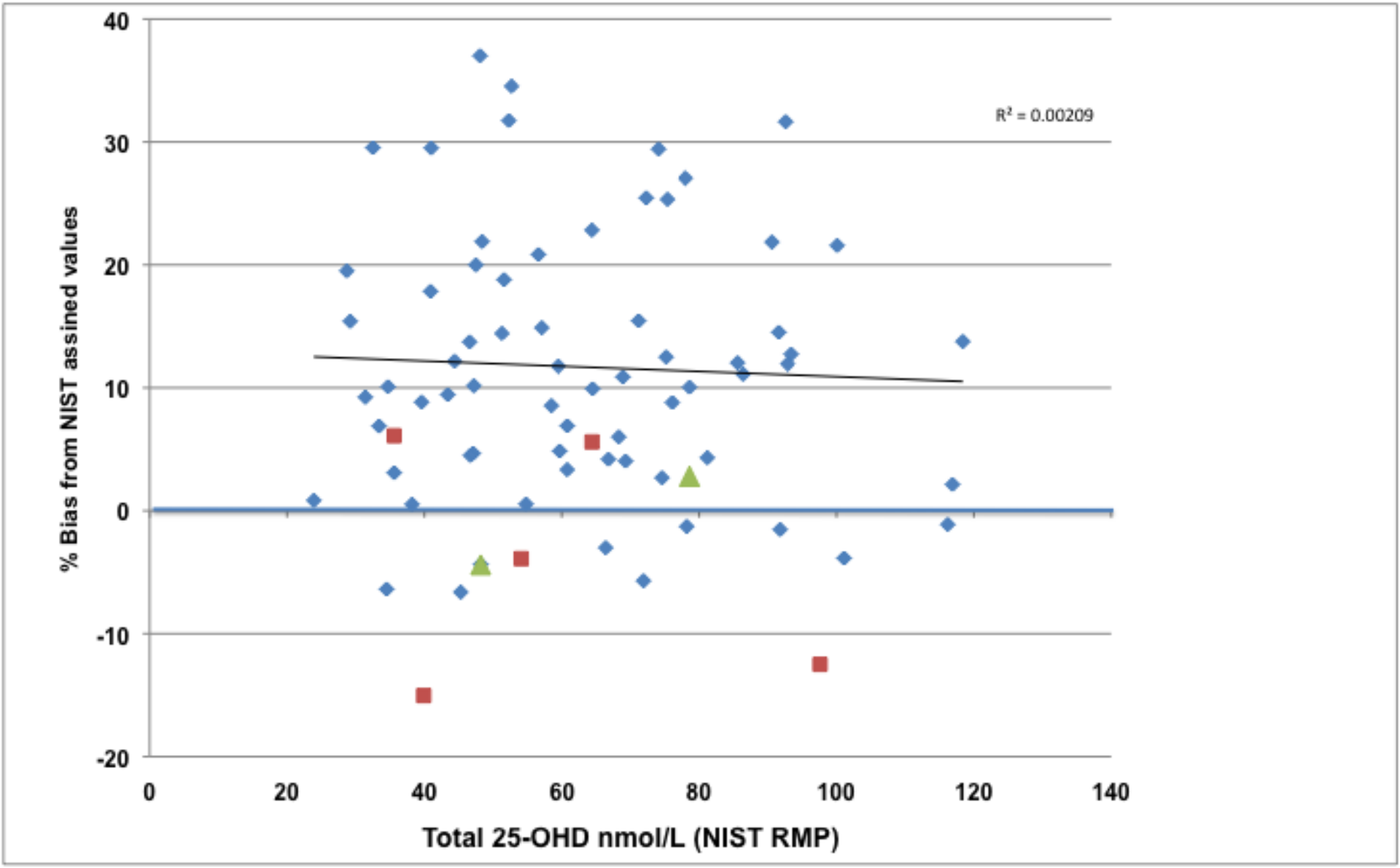
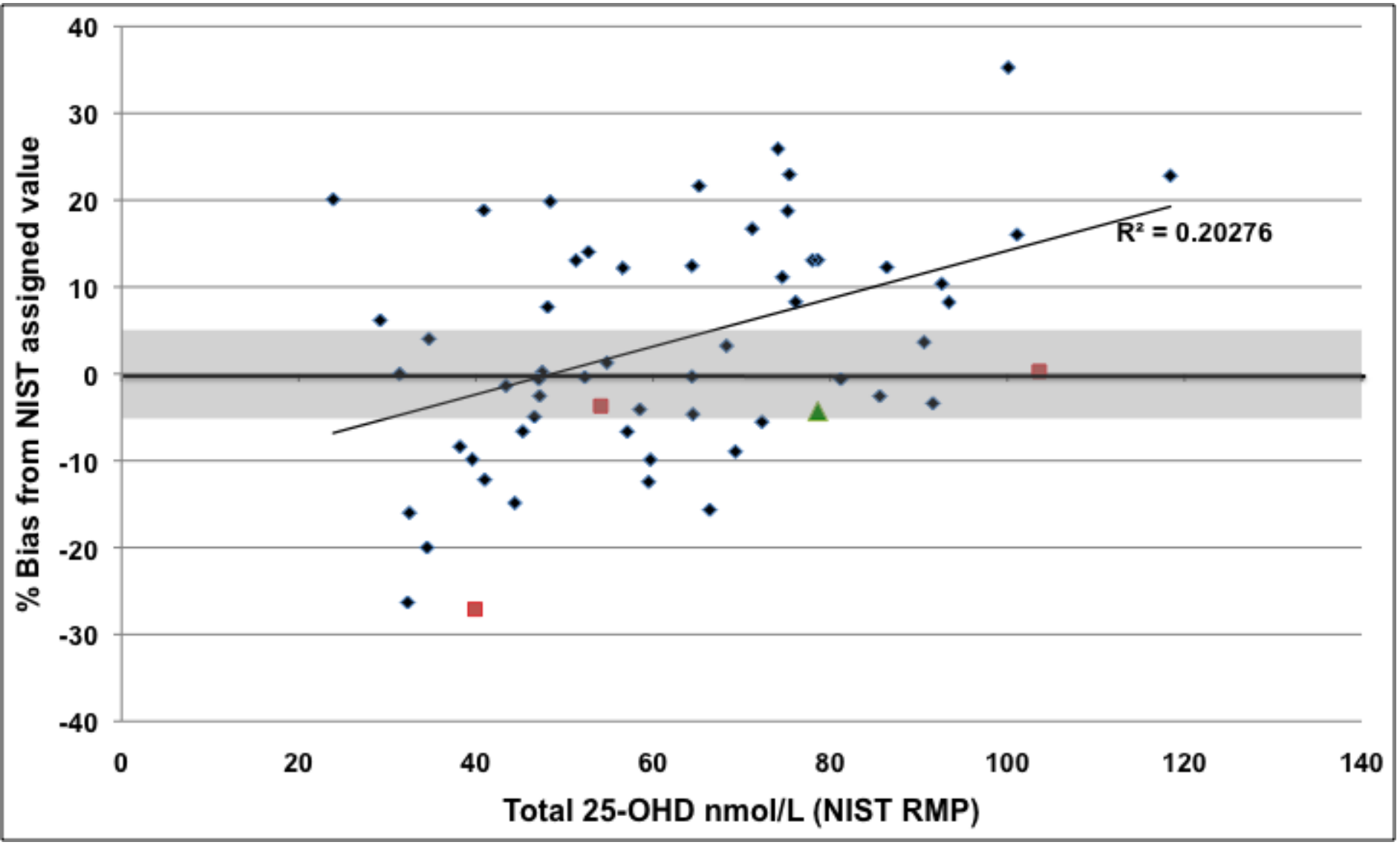
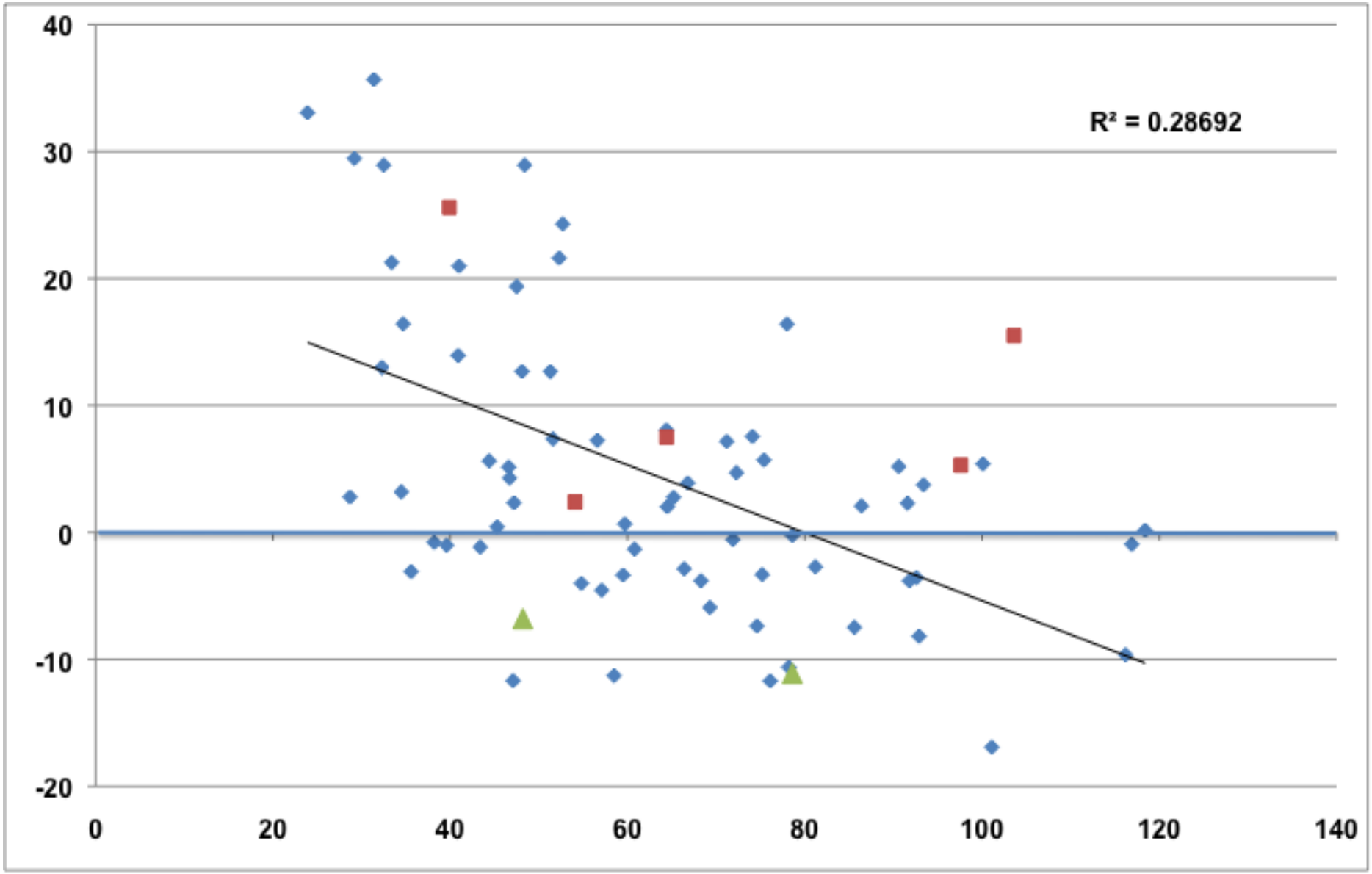

40
30
20
10
0
-10
-20
0
20
40
60
80
100
120
140
% Bias from NIST assined values
Total 25-OHD nmol/L (NIST RMP)
R² = 0.00209

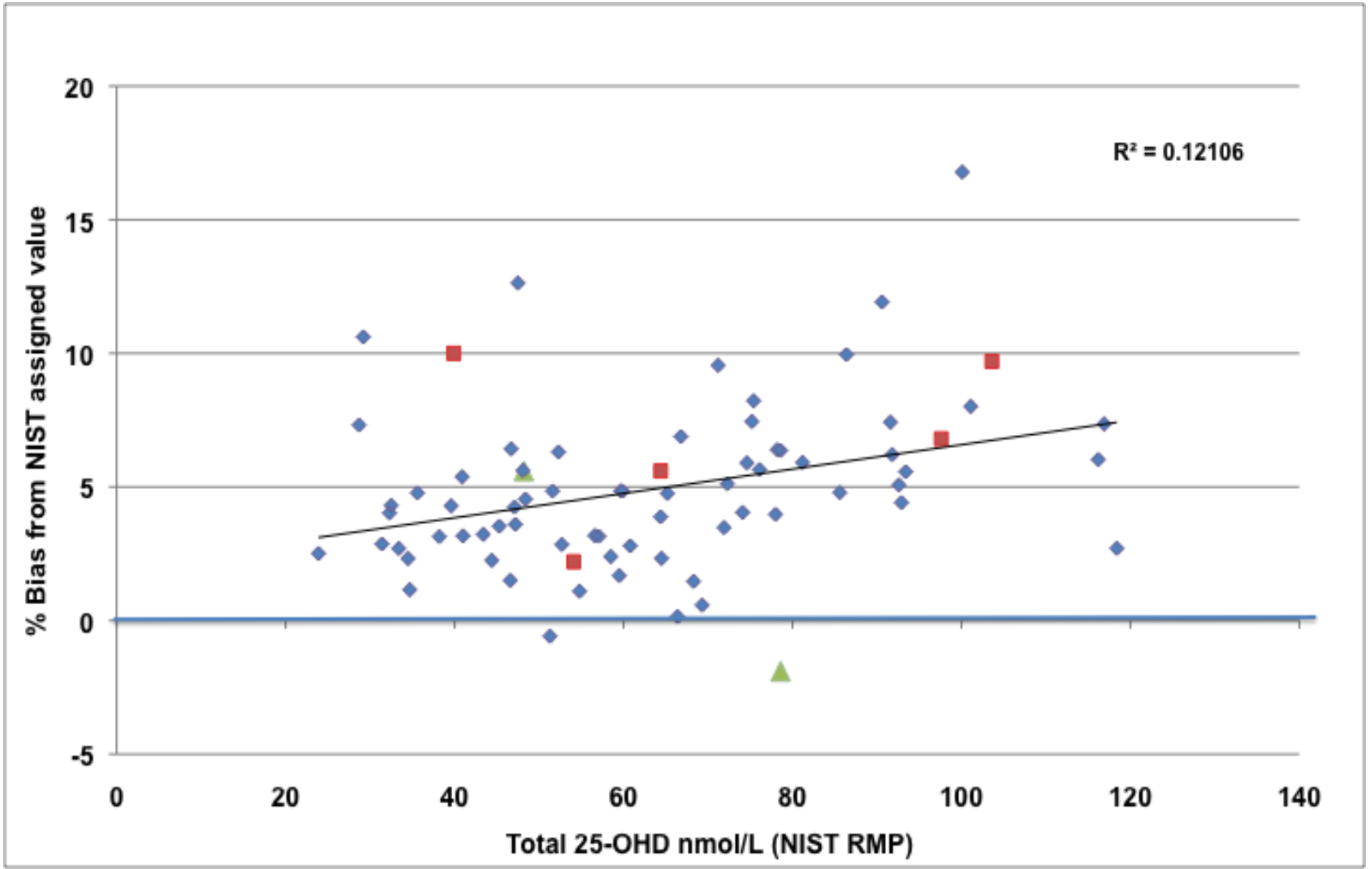
Roche Total 25-OHD



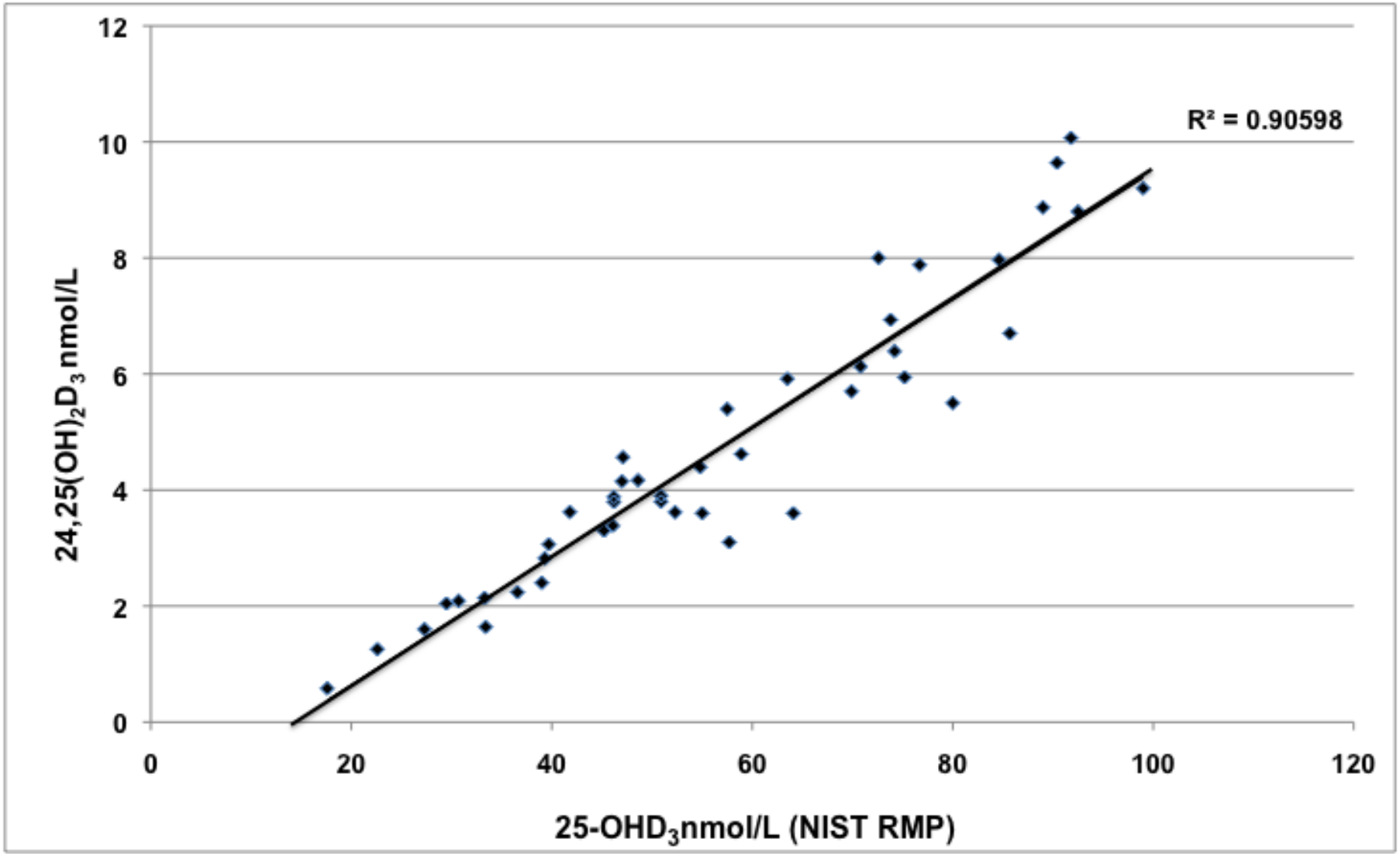
Siemens Advia Centaur



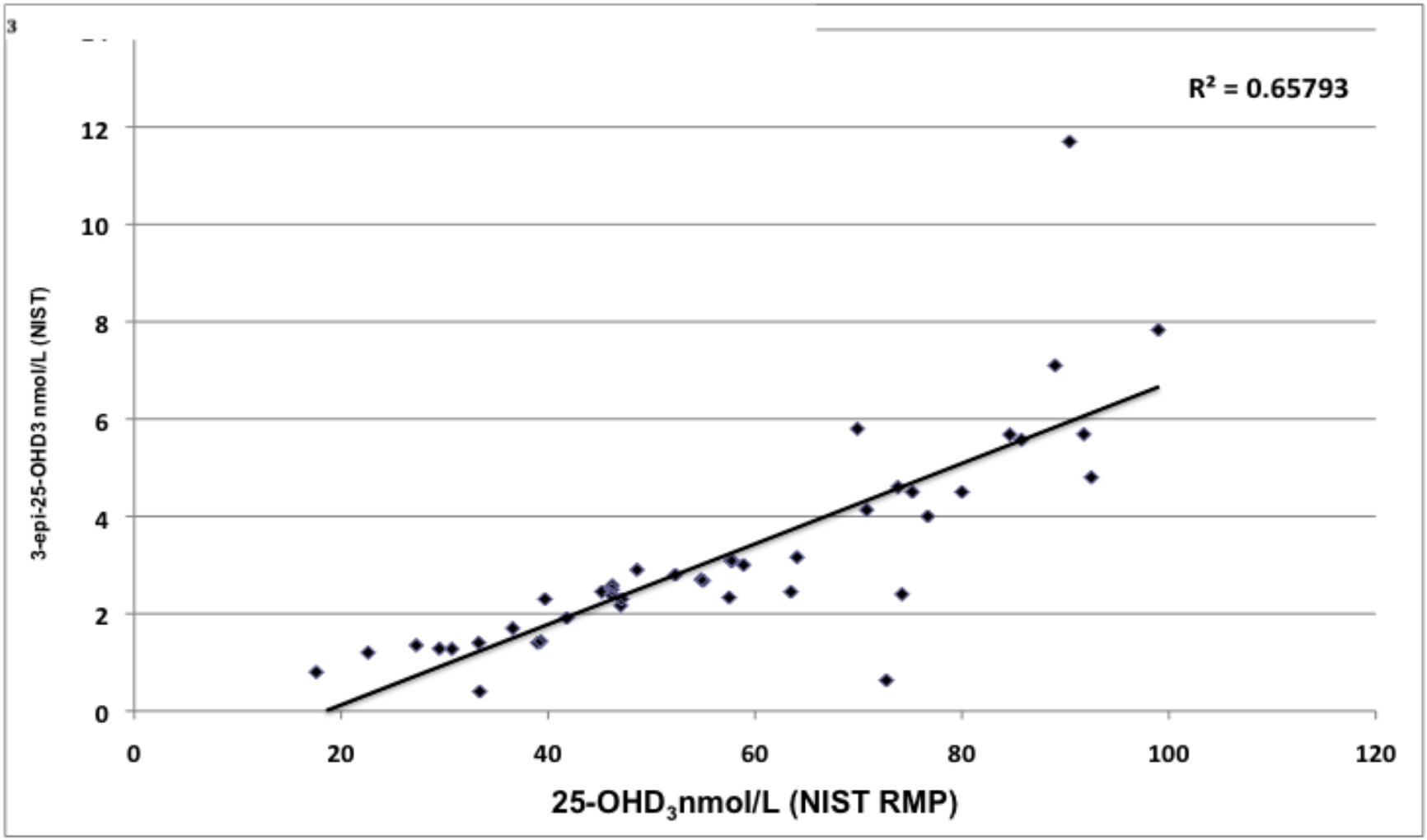
LC-MS/MS


20
15
10
5
0
-5
% Bias from NIST assigned value
R² = 0.12106
0
20
40
60
80
100
120
140
Total 25-OHD nmol/L (NIST RMP)

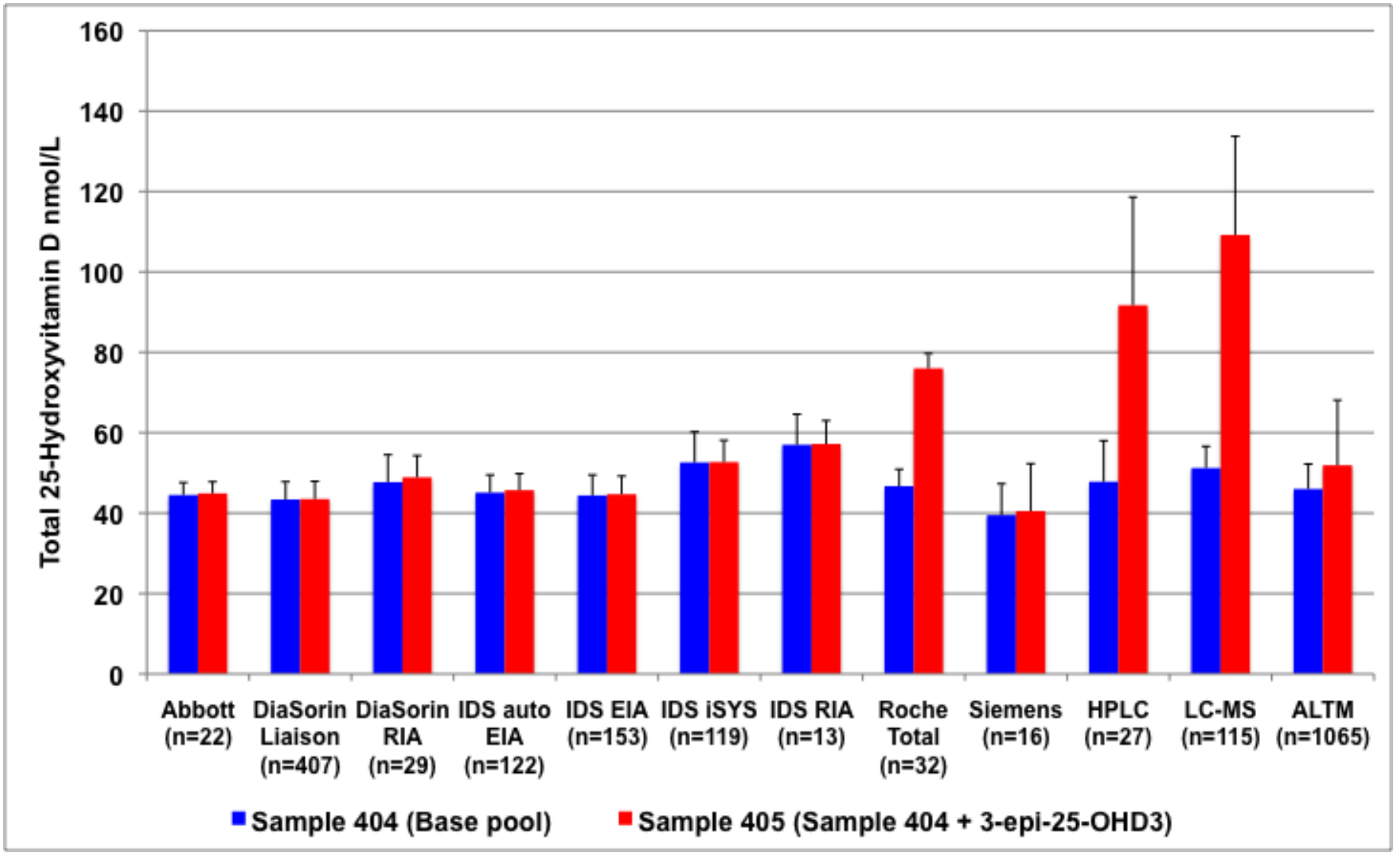
DEQAS samples
$24,25(OH)_2D_3$ vs 25-OHD_3



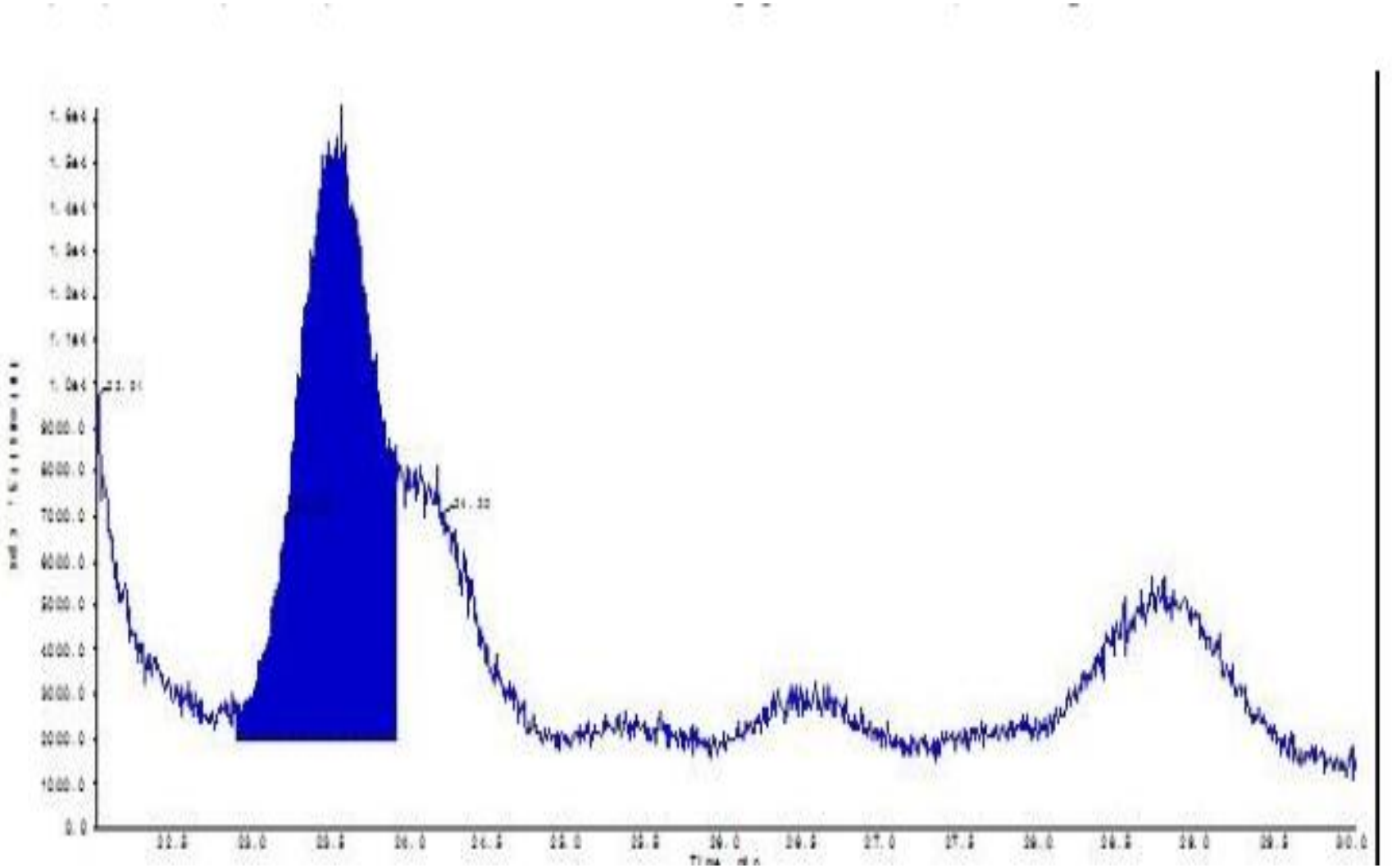
DEQAS samples
3-epi-25-OHD$_3$ vs 25-OHD$_3$



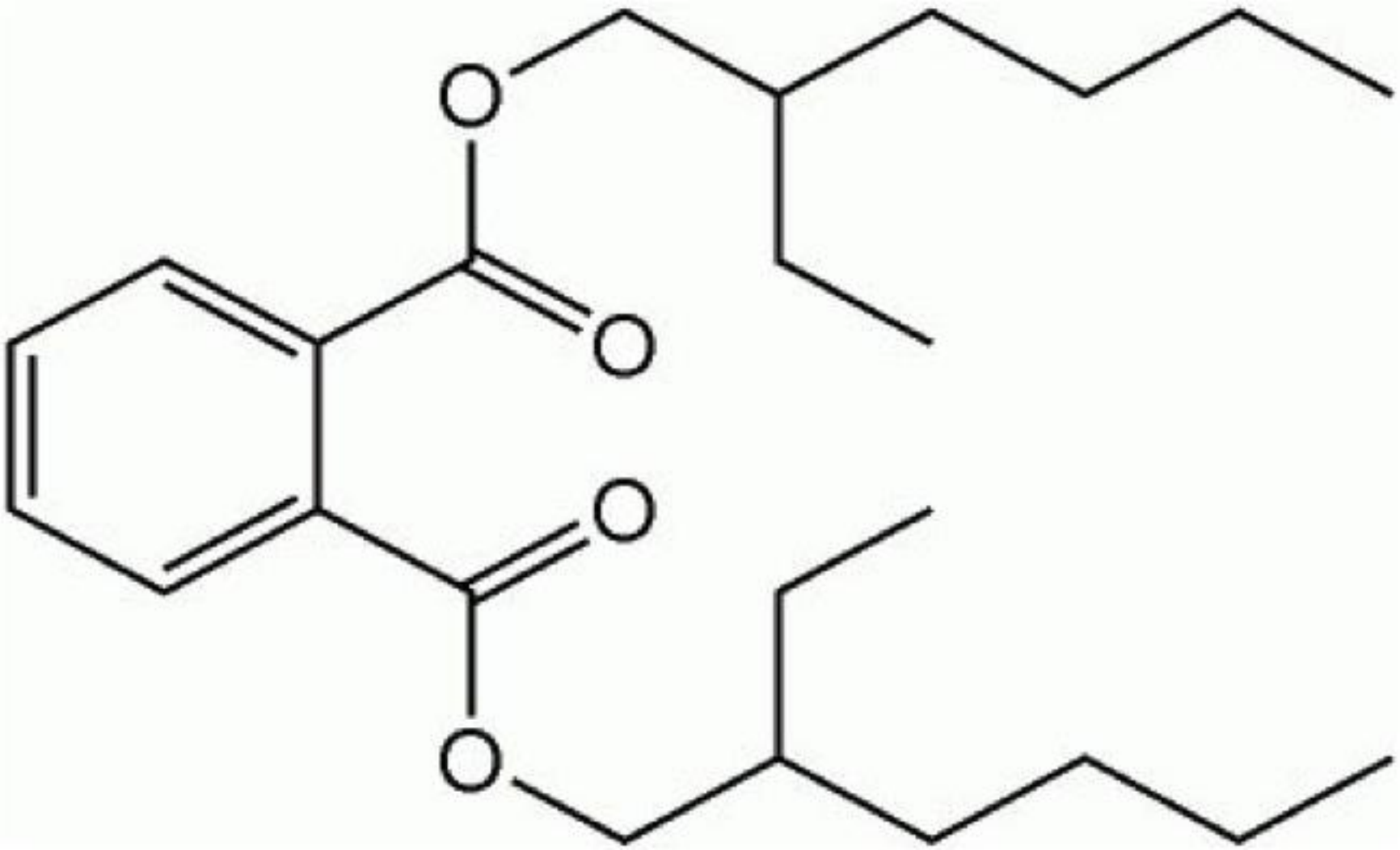
Interference from 3-epi-25-OHD_3



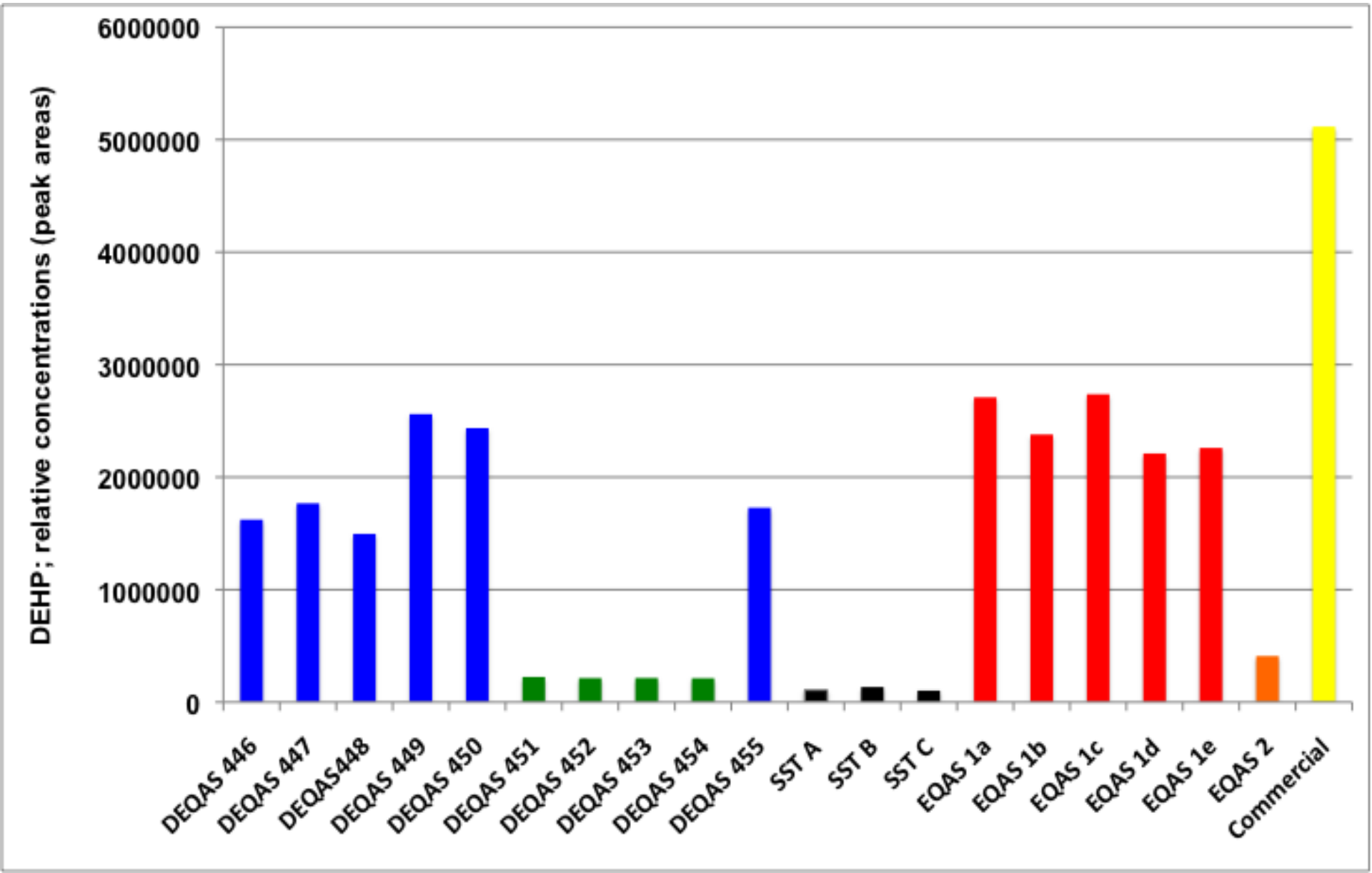
NIST: Selected ion chromatogram by LC-MS/MS for 3-epi-25(OH)D3 at a concentration of 11.7 nmol/L from a DEQAS sample (Hammersmith bags).



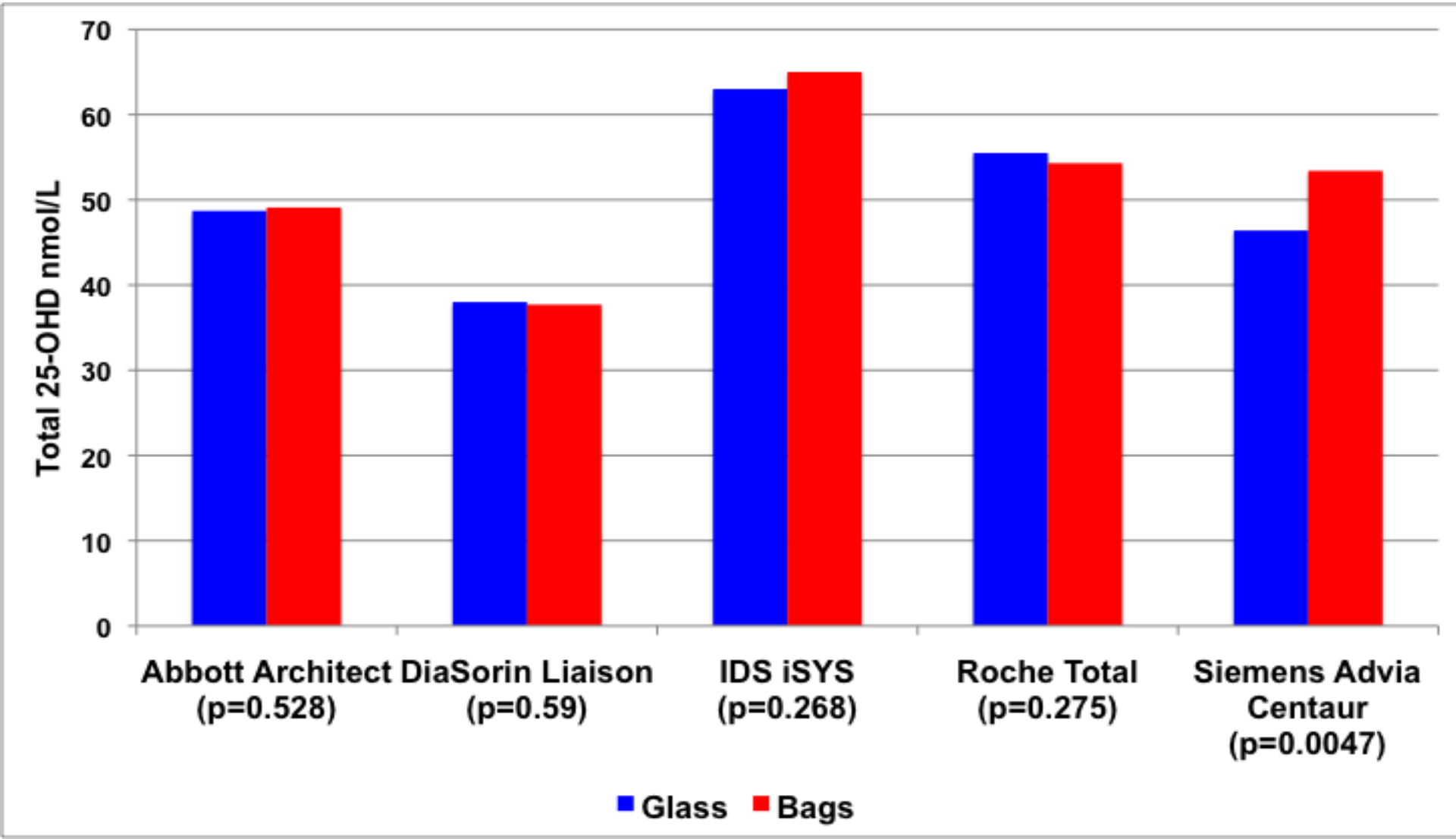
Di (2-ethylhexyl) phthalate (DEHP)



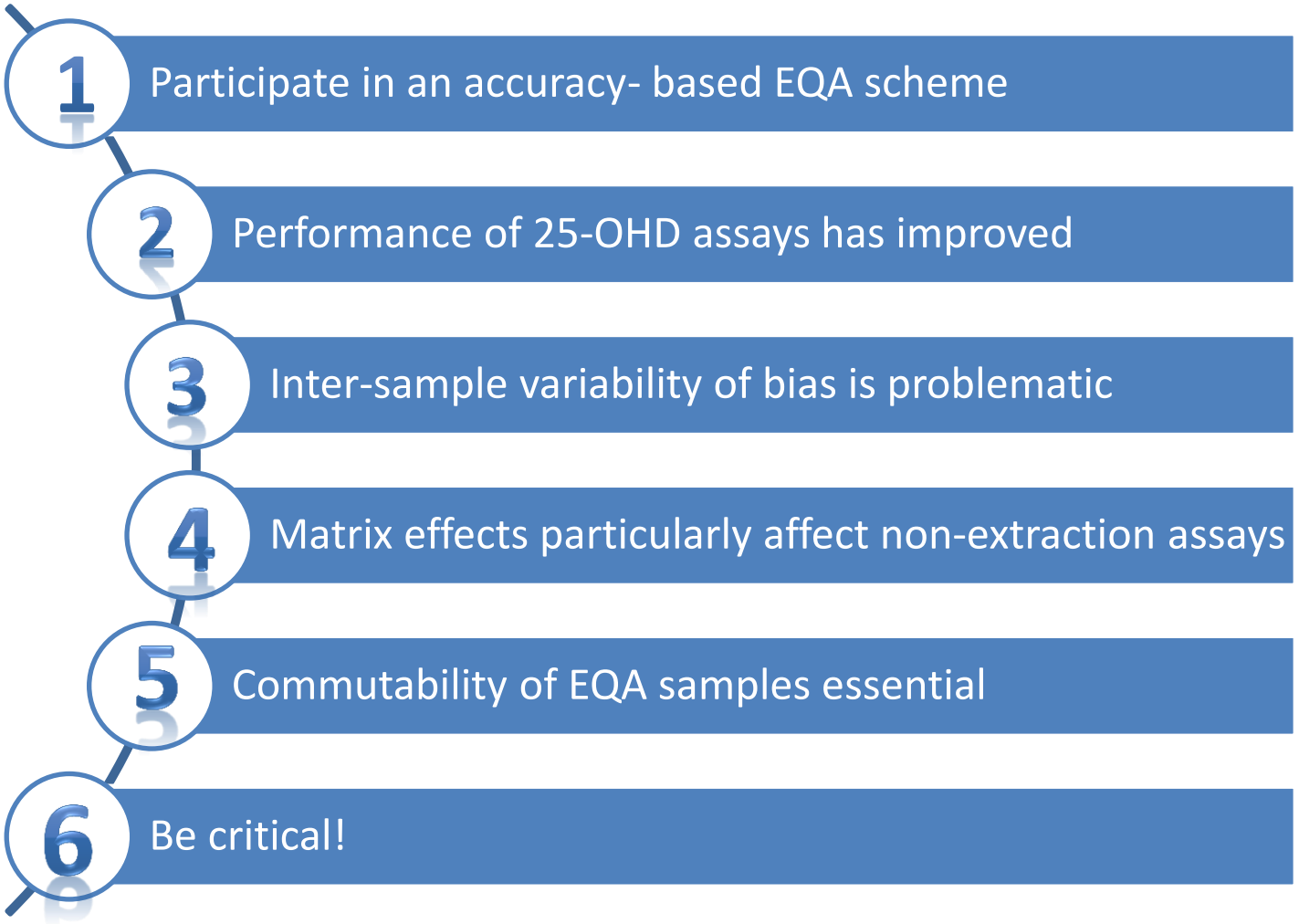
DEHP concentrations in EQA samples



25-OHD in Glass and plastic bags



Conclusions



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Thank You !