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

- Accepted by CAP as a Proficiency Testing scheme (2012)
- Accuracy based values assigned by NIST RMP (April 2013)
- Donor clinic moved to Hammersmith Hospital (2012-2013)
- Interferent discovered by NIST in Hammersmith blood (shoulder to 3-epimer peak) (April 2013)
- Commercial serum used for all assessable DEQAS samples – collected according to NCCLS C37-A guidelines (April 2014)
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  \[\downarrow\]  (Liver)  \[\downarrow\]  25-OHD3  \[\downarrow\]  (Kidney *)  \[\downarrow\]  1,25 (OH)\(_2\)D3

Vitamin D2  \[\downarrow\]  25-OHD2  \[\downarrow\]  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-Hydroxyvitamin D October 2015 - Bias from NIST Target Value for Individual Methods
25-Hydroxyvitamin D April 2016 - Bias from NIST Target Value for Individual Methods
Abbott Architect

R² = 0.15423

% Bias from NIST assigned value

Total 25-OHD nmol/L (NIST RMP)
DEQAS samples
24,25(OH)$_2$D$_3$ vs 25-OHD$_3$

$R^2 = 0.90598$
DEQAS samples
3-epi-25-OHD₃ vs 25-OHD₃

R² = 0.65793
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

![Bar Chart]

- Abbott Architect DiaSorin Liaison (p=0.528)
- IDS iSYS (p=0.268)
- Roche Total (p=0.275)
- Siemens Advia Centaur (p=0.0047)

Legend:
- Glass
- Bags
Conclusions

1. Participate in an accuracy-based EQA scheme
2. Performance of 25-OHD assays has improved
3. Inter-sample variability of bias is problematic
4. Matrix effects particularly affect non-extraction assays
5. Commutability of EQA samples essential
6. Be critical!
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