Vitamin D Standardization Program's (VDSP) Commutability Study 2: Overview

Commutability of NIST SRMs and PT/EQA Test Materials in Clinical and Research Vitamin D Assays

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Vitamin D Status Measurement

Total 25-Hydroxyvitamin D or 25(OH)D in serum or plasma

- Total 25(OH)D is defined as:

Total 25(OH)D = 25(OH)D_2 + 25(OH)D_3*

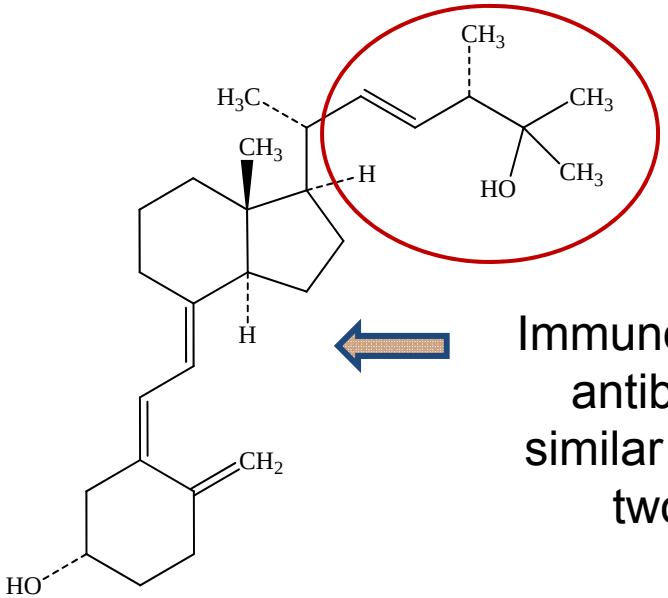
- Units: ng/mL or nmol/L where: ng/ml * 2.5 ≈ nmol/L

** Assumes that Vitamin D_2 and D_3 are of equal biological value.*

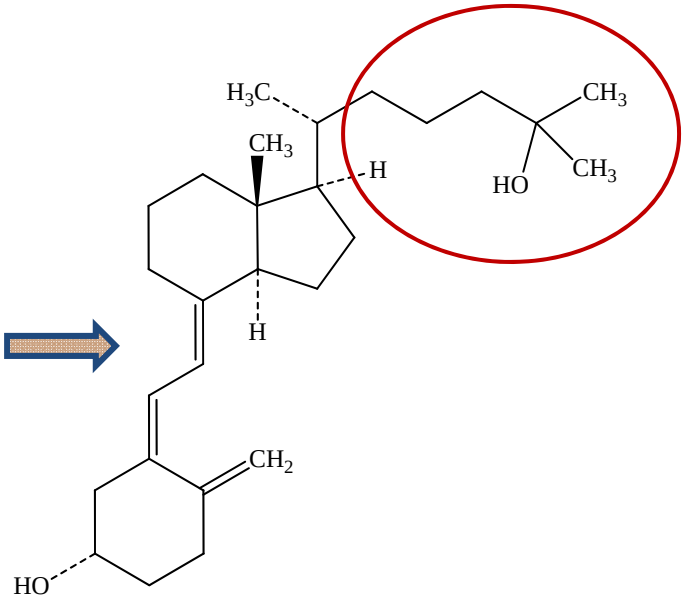
NB: Total 25(OH)D does *not* include epimer concentrations!

Structural Forms for 25(OH)D

The two primary metabolites of interest differ only in the side chains



Immunoassays utilize antibody against similar regions of the two analytes



25-hydroxyvitamin D_2

Molecular Formula: $C_{28}H_{44}O_2$
Molecular Weight: 412.65 g/mol

25-hydroxyvitamin D_3

$C_{27}H_{44}O_2$
400.63 g/mol

VDSP Commutability Study 2

Purpose

To promote standardized measurement of total 25-hydroxyvitamin D [25(OH)D] by evaluating commutability of NIST SRMs used as "Trueness" Controls and the materials used in the major PT/EQA programs.

Objectives

1. To evaluate commutability of:
   - NIST SRMs
   - PT/EQA materials from CAP, DEQAS, and possibly other sources
2. In the following:
   - Currently marketed commercial vitamin D assays
   - National and subnational nutrition surveys
   - Selected laboratory developed assays

Protocol Overview: # 1

- 50 Single donor patient samples, blinded. Samples collected and prepared by Solomon Park (Seattle, WA, USA) using CLSI 37A guidelines
- Non-blinded test materials for NIST SRM 972a (4 levels), NIST SRM 2973 (1 level), CAP ABVD and DEQAS
- NIST reference measurement procedures (RMP) used to determine “Target Values” for patient samples and test materials (duplicate injections of duplicate preparations)
- Target Values provided for:
  - 25(OH)D2 (RMP)
  - 25(OH)D3 (RMP)
  - Total 25(OH)D (RMP)
  - 3-epi-25(OH)D3 (ID-LC-MS/MS)
  - 24R,25(OH)$_2$D3 (ID-LC-MS/MS)

Vitamin D Samples Specifications

- 5 units containing total 25(OH)D <20 nmol/L
- 3 units containing total 25(OH)D 20-24 nmol/L
- 7 units containing total 25(OH)D 25-44 nmol/L
- 7 units containing total 25(OH)D 45-59 nmol/L
- 7 units containing total 25(OH)D 60-74 nmol/L
- 7 units containing total 25(OH)D 75-119 nmol/L
- 5 units containing total 25(OH)D 120-129 nmol/L
- 5 units containing total 25(OH)D 130-139 nmol/L
- 4 units containing total 25(OH)D ≥140 nmol/L

NB: **5** units shall contain 25(OH)D2 10-14 nmol/L &
5 shall contain 25(OH)D2 15-20 nmol/L

Protocol Overview: # 2

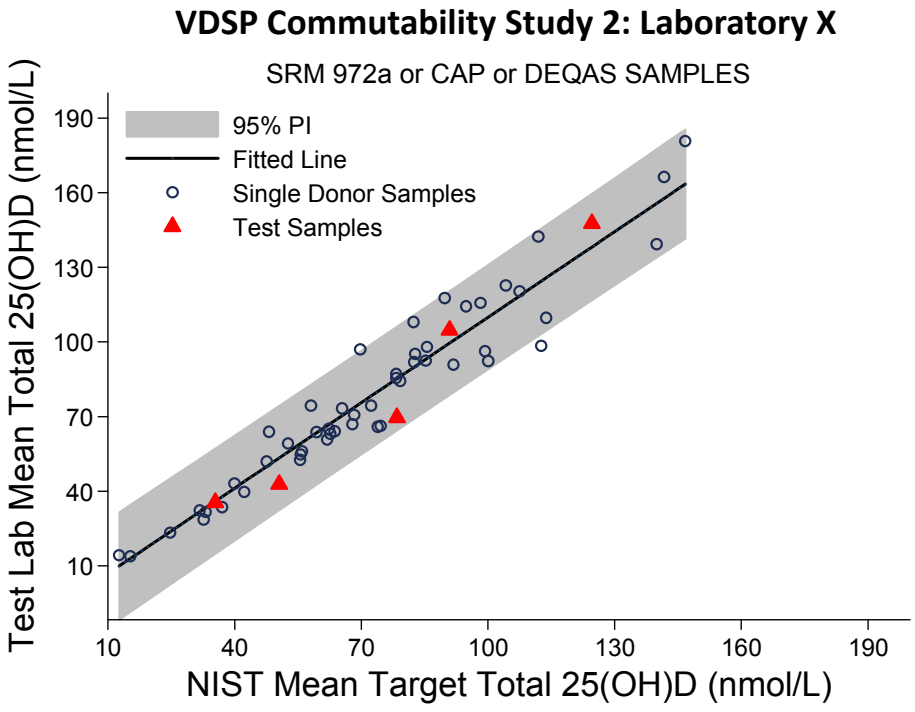
- DEQAS, CAP and NIST test materials shall be analyzed together with patient samples using a specified run order to minimize effects that can confound commutability assessment such as lot-to-lot variability, variability in specimen preparation, etc.
- All samples run in duplicate on a single day according to run order protocol
- Statistical assessment of commutability will be based on first value and conducted using CLSI EP-14 and EP30A guidelines

Approach to Assessing Commutability

Prepare file with initial values for each **patient sample**

Plot patient sample values (Y) vs. NIST target values (X)

Calculate and plot OLS regression line, & 95% prediction interval



Prepare file with initial values for each **test material**

Plot test material means over target values in same graph

Results for test materials, i.e. SRM 972a (Levels 1-4), & CAP & DEQAS CTM, are shown separately vs. NIST RMP Target Values

Test materials with values inside the 95% prediction interval are considered commutable

Recruitment of Participants

- All commercial assay manufacturers
  - Focus on commercially-available assay platforms
  - Will consider requests for assays in development
- Clinical and research laboratories using a commercial assay platform
- Laboratories for national/subnational nutrition surveys
- Laboratories with in-house developed assays

Participant Recruitment Steps

1. Questionnaire will be sent to all interested laboratories asking:
   - Assay system description
   - Assay performance data: CV (%) and Mean Bias (%)
2. Acceptance of requirement that their laboratory's study results, including the identification of assay platform and their laboratory will be included, as appropriate, in the papers that are eventually published
3. Selection of laboratories for each commercially available assay platform, as feasible
4. Selection of national nutrition survey labs, as feasible
5. Selection of labs with in-house assays, as feasible

Requirement of Participants

- Assay must meet minimum VDSP performance guidelines: **CV ≤ 10% and Mean Bias ≤ 5%**
- A requirement for participation in the commutability study will be a laboratory's agreement that study results, including the identification of assay platform and laboratory of analysis will be included, as appropriate, in the papers that are eventually published

Benefits: All Participants

- Access to free set of 50 single donor serum samples
- Receive Final Report
  - NIST Target values for single donor serum samples and all SRM and CTM test materials
  - Evaluation of Commutability results
  - Evaluation of intra-batch Bias vs. NIST target values
- Direct participation in international effort to standardize vitamin D measurement

Benefits: Assay Manufacturers

- Knowledge of SRMs and PT/EQA programs to recommend to their customers
- Provided with information as to gaps in their surveys, e.g. due to cross reactivity
- Estimates of within-batch bias in clinical setting

Benefits: NIST, CAP and DEQAS

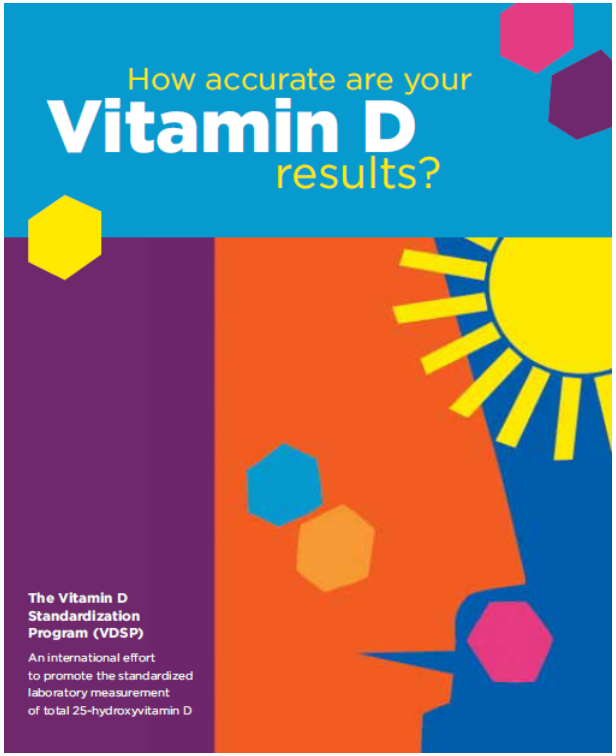
- Knowledge of commutability of SRMs and PT/EQA materials in different measurement systems
- Information to improve current materials and gaps in metabolite concentration ranges of materials
- Tools to promote the standardized measurement of total 25(OH)D around the world by clinical and research laboratories

Proposed Timelines

Task	Target Date
Submit procurement paperwork to NIST Acquisition Management Division (completed)	April, 2014
Designation of Contractor (completed)	September, 2014
Participant recruitment begins (ongoing)	October, 2014
Official call for participation	March, 2015
NIST receipt of samples from Contractor	April, 2015
NIST RMP measurements of 25(OH)D2 and 25(OH)D3 begin (4 months)	April, 2015
Send out samples to participants	June, 2015
NIST measurements of 3-epi-25(OH)D3 begin (2 months)	August, 2015
NIST receipt of participant results	September, 2015
Complete data set preparation	October, 2015
Data analyses completed	January, 2016
Results reported to participants	March, 2016
Submission of Commutability manuscript	September, 2016
Post study results on ODS and NIST websites	October, 2016

Current Status

- Contract for Vitamin D Commutability Samples successfully awarded through NIST acquisitions process
  - Contract awarded to Solomon Park Research Lab on September 24, 2014
  - Labeling scheme has been sent to Contractor
  - Contractor has 180 days to deliver samples to NIST
- Have started discussions with PT/EQA providers to discuss samples that may be part of the study
- Investigating NIST resources for shipping and data acceptance
- Shipping will be according to current PT/EQA practice



Thank you!

Join us in this effort!

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