Learning Objectives:

- Describe CMS reimbursement criteria for Vit D
- Define ways to determine appropriate Vit D clinical decision thresholds
- Describe experimental design strategies for method validation of Vit D assays
- Describe the impact of potential interfering substances
Vitamin D: Regulation of Ca\(^{2+}\) Homeostasis

- **Vitamin D2 (ergocalciferol)**
- **Vitamin D3 (cholecalciferol)**

**OTC supplements**
**Prescription Drug Form**

**Liver**

*25 hydroxylase*

**25-OH D3, 25-OH D2**

**(ng/mL)**

\(1\frac{1}{2} = 2-3\) wks

**Kidney**

*1-a-hydroxylase*

\(1,25 (\text{OH})_2\) D3, \(1,25 (\text{OH})_2\) D2

**(ng/mL)**

\(t\frac{1}{2} = 4\text{hr}\)

**↑↑**

- **Ca\(^{2+}\)** Absorption from Gut

**↑↑**

- **Retention of Ca\(^{2+}\)** by Kidney

**↑↑**

- **Bone Mineralization**

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Vitamin D Testing: Indications

**Endocrine Society Recommendations (2011):**

- Total 25-OHD is the marker of choice
- Meas should only be made in individuals “at risk” for deficiency
- General population screening is not recommended
- \(1,25(\text{OH})_2\)D should not be used to dx deficiency except in narrowly defined populations

**JCEM 2011; 96(7); 1911-1930**

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Vitamin D Testing: CMS Reimbursement

*The following code is the correct modifier to report the total micrograms of vitamin D (25 hydroxy) included in this visit.*

**VIT D; 25 HYDROXY, INCLUDES FRACTION(S), IF PERFORMED**

Max reimb = 4x/yr

- **General Screening** is not reimbursed
- **Max 1x/yr**


Accessed August, 2013

**VIT D; 1, 25 diHYDROXY**
Vitamin D: Reporting Guidelines

Institute of Medicine Report, 2010
Maintain populations at 20 ng/mL to reflect RDA
< 12 ng/mL "at risk of deficiency relative to bone health"
12 - 19 ng/mL "potentially at risk for inadequacy"
20 - 30 ng/mL "practically all persons are sufficient"
31 - 50 ng/mL "not consistently associated with increased benefit"
> 50 ng/mL "reason for concern"
http://www.iom.edu/Reports.aspx; Accessed August, 2013

Endocrine Society Clinical Practice Guidelines, 2011
< 20 ng/mL Deficiency
21 - 29 ng/mL Insufficiency
30 – 100 ng/mL Sufficiency
JCEM 2011; 96(7): 1911-1930

Vitamin D: Reporting Guidelines
Established in conjunction with input from VCU Division of Endocrinology and Metabolism
VCUHS Total 25-OH D Clinical Decision Thresholds
< 10 ng/mL Severe Hypovitaminosis D
10.0 – 19.9 ng/mL Moderate Hypovitaminosis D
20.0 – 29.9 ng/mL Mild Hypovitaminosis D
30.0 – 100.0 ng/mL Optimal
> 100 ng/mL Potential for Toxicity may exist
Osteoporos Int 2005; 16: 713–716
N Engl J Med 2007;357: 266-81

Why Should Vit D be measured by LC-MS/MS?
Nov 09 70yr F seen for fatigue/weakness. Osteoporosis Risk.
IA: 25 OH D = < 4.0 ng/mL
Jan 09 IA: 25-OH D = < 4.0 ng/mL = 50,000 U ergocalciferol, 2x/wk
May 09 Referred to endocrine specialty clinic
PTH 35.0 pg/mL, Ca^2+ 9.4 mg/dL, Urine Ca^2+ 190 mg/24h
ALP = 119 UL
IA: 25-OH D = < 4.0 ng/mL = 50,000 U ergocalciferol 2x/wk
June 09 IA: 25-OH D = 5.5 ng/mL
Patient was admonished for not taking her Vit D! Patient cried…
July 09 IA: 25-OH D = <4.0 ng/mL
LC-MS/MS: 25-OH D3 = 6.0 ng/mL
25-OH D2 = 56.0 ng/mL
Total D = 62.0 ng/mL
Measurement of Total 25-OH Vitamin D by LC-MS/MS Method Validation

Select Examples

Notes
- Some experimental design aspects of CLSI C60 (to be renamed C62): Liquid Chromatography-Mass Spectrometry Methods; Draft Guideline (to be renamed C62) are shown
- Draft CLSI C62 experimental design strategies are still under development and may not represent the final guidelines

CLSI C62 Recommendations: Stability Assessment

C62: Assess analyte stability in native matrix under appropriate storage conditions

- Short-term stability at RT (Bench top)
- Long-term stability at 2-8°C, -20°C or -70°C
- Max # of Freeze/Thaw cycles, if applicable

FDA Guideline: Bioanalytical Method Validation
CLSI C55: Sample Stability in Chem/Tox (Draft Guideline)

- Stable for 3 days at 2-8°C (data)
- or RT (Clin Chem 1981, 27:773-774)

C62: Assess analyte stability during all phases of the analytical measurement process

- Determine max bench-top processing time (ex: extracts at RT)
- Determine max storage duration of extracts/preparations in the autosampler (evaporation effects, analyte degradation)

Vit D: Stability of Extracts Stored in Autosampler

25-OH D3
Injected extracted cal, QC and native patient samples at T = 0
Extracts re-injected after 17hr and 24hr storage in autosampler (2-8°C)

Subset of N=36 patient samples shown

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>0hr</th>
<th>1hr</th>
<th>2hr</th>
<th>Mean (ng/mL)</th>
<th>%CV</th>
<th>Diff 1hr</th>
<th>%Diff 24hr</th>
<th>Diff 24hr</th>
<th>%Diff 24hr</th>
</tr>
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<tbody>
<tr>
<td>Patient 1</td>
<td>4.6</td>
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<td>-13.3</td>
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<td>0.0</td>
<td>-0.5</td>
<td>-1.0</td>
<td>-1.0</td>
</tr>
<tr>
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<td>9.7</td>
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<tr>
<td>Patient 4</td>
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<td>-0.4</td>
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<td>-0.4</td>
<td>-0.4</td>
</tr>
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<td>-0.4</td>
</tr>
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<td>Patient 8</td>
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<td>20.2</td>
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<td>20.2</td>
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<td>0.0</td>
<td>-0.5</td>
<td>-0.5</td>
<td>-0.5</td>
</tr>
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<td>-0.5</td>
<td>-0.5</td>
<td>-0.5</td>
</tr>
<tr>
<td>Patient 12</td>
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<td>64.6</td>
<td>64.8</td>
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<td>0.0</td>
<td>-0.5</td>
<td>-0.5</td>
<td>-0.5</td>
</tr>
<tr>
<td>Patient 13</td>
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<td>76.1</td>
<td>75.9</td>
<td>76.1</td>
<td>0.0</td>
<td>0.0</td>
<td>-0.5</td>
<td>-0.5</td>
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</tr>
</tbody>
</table>
CLSI C62 Recommendations: Signal-to-Noise (S/N)

Evaluate the S/N at LLoQ

CLSI C50A Mass Spectrometry in the Clinical Laboratory: General Principles and Guidance: Approved Guideline

- Minimum S/N at LLoQ of 3:1

C62 Minimum Acceptability Criteria

- Minimum S/N at LLoQ of 10:1

C62 Best Practice Acceptability Criteria:

- Minimum S/N at LLoQ of 20:1 to ensure ruggedness

Vitamin D Method: Signal-to-Noise (S/N)

S/N degradation for 25-OH D method at LLoQ

- 2010: S/N = 6.3
- 2013: S/N = 10

Validation of a Blank Matrix for Vitamin D Method

C62: Validate a blank matrix for use in subseq validation procedures (LLoQ, AMR, recovery, cal prep... etc.)

- Measure peak area of a double blank matrix (no analyte/no IS)
- Can use BSA or stripped serum (we could not find a SS w/o 25OHD)

C62 Best Practice Acceptability Criteria:

- No peak or peak area < 20% of LLoQ and <5% of IS in 5-6 lots of blank material
Vit D Method Imprecision Profile

Imprecision profile generated to determine %CV across AMR
Analyzed in quadruplicate/run for 2 runs

- 6% BSA spiked with D3
- Stripped Serum spiked with D3
- Native Patient Serum Pools with endogenous D3

Vitamin D Method: Accuracy

C62: Assess accuracy using multiple approaches

C62 Best Practice:
Validate “trueness” using a “reference of higher order” (CLSI X5, ISO 17511) listed by JCTLM (approved RMPs, ref labs and RMs)
(www.bipm.org/jctlm/)

- 1) Method Comp vs. a JCTLM-approved RMP
- 2) Matrix-Approp CRMs (pref commutable)
- 3) Spike and Recovery – only if RMP or RMs are unavail

C62 Alternative Approaches:
- 4) Accuracy-Based PT Materials
- 5) Method Comp vs. Previous Method using CLSI EP9-A2
  (Bias vs. prev method only, not trueness)

Accuracy Approach 1) Method Comp with RMP – VDSP; EP 9

- ODS/CDC VDSP
  (www.cdc.gov/Research/VitaminD.aspx#vsp)
- CDC HoST
  (http://www.cdc.gov/labstandards/hs.html)
Calibration/Calibration Verification

40 single donor serum samples with reference values known to participant

Challenge 1 (1st Quarter)

Challenge 2 (2nd Quarter)

Challenge 3 (3rd Quarter)

Challenge 4 (4th Quarter)

Performance Assessment

Performance criteria:
Mean bias: 5%
Imprecision: 10%

CDC assesses bias and imprecision and issues certificates of performance

http://www.cdc.gov/labstandards/hs.html

Slide prepared by Hubert Vesper, CDC

Accuracy Approach 2) Certified Reference Material (CRM)

Assessment of traceability to NIST SRM 972a

<table>
<thead>
<tr>
<th>TEST LEVEL</th>
<th>HPLC METHOD</th>
<th>NIST SRM 1</th>
<th>NIST SRM 2</th>
<th>NIST SRM 3</th>
<th>NIST SRM 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIST SRM 1</td>
<td>30.64 µg/mL</td>
<td>30.64 µg/mL</td>
<td>30.64 µg/mL</td>
<td>30.64 µg/mL</td>
<td>30.64 µg/mL</td>
</tr>
<tr>
<td>NIST SRM 2</td>
<td>30.64 µg/mL</td>
<td>30.64 µg/mL</td>
<td>30.64 µg/mL</td>
<td>30.64 µg/mL</td>
<td>30.64 µg/mL</td>
</tr>
<tr>
<td>NIST SRM 3</td>
<td>30.64 µg/mL</td>
<td>30.64 µg/mL</td>
<td>30.64 µg/mL</td>
<td>30.64 µg/mL</td>
<td>30.64 µg/mL</td>
</tr>
<tr>
<td>NIST SRM 4</td>
<td>30.64 µg/mL</td>
<td>30.64 µg/mL</td>
<td>30.64 µg/mL</td>
<td>30.64 µg/mL</td>
<td>30.64 µg/mL</td>
</tr>
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</table>

Acceptability Criteria:
±10% or 2 ng/mL

Accuracy Approach 3) Spike and Recovery

25-OH D3 Spiked into 8% BSA (Low Conc)

25-OH D3 Spiked into Stripped Serum with back-calculation

N = 10 reps/sample over 2 runs

<table>
<thead>
<tr>
<th>8% BSA</th>
<th>Stripped Serum</th>
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</thead>
<tbody>
<tr>
<td>Mean (ng/mL)</td>
<td>SD</td>
</tr>
<tr>
<td>2.8</td>
<td>0.1</td>
</tr>
<tr>
<td>2.8</td>
<td>0.1</td>
</tr>
<tr>
<td>2.8</td>
<td>0.1</td>
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<tr>
<td>2.8</td>
<td>0.1</td>
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<td>2.8</td>
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<tr>
<td>2.8</td>
<td>0.1</td>
</tr>
<tr>
<td>2.8</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Acceptability Criteria: 10% or 2 ng/mL
Accuracy Approach 4) Accuracy-Based PT Programs

<table>
<thead>
<tr>
<th>CAP Accuracy Based Survey April 2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAP Accuracy by Criteria x %</td>
</tr>
<tr>
<td>AB-D-20</td>
</tr>
<tr>
<td>AB-D-07</td>
</tr>
<tr>
<td>AB-D-26</td>
</tr>
<tr>
<td>AB-D-10</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>DEQAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB-D-20</td>
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<tr>
<td>AB-D-07</td>
</tr>
<tr>
<td>AB-D-26</td>
</tr>
<tr>
<td>AB-D-10</td>
</tr>
</tbody>
</table>

Total 25-OHD, D2, D3 and D3-epi ref values provided
Note that 3-epi D3 is NOT included in the Target Value

- CAP Accuracy Based Vitamin D Survey
- New Accuracy Based Program – DEQAS (www.deqas.org)

Vitamin D Proficiency Testing

**CAP BGS (Peer Group)**
- VCUHS does not participate (historical matrix problems)

**CAP Vitamin D Accuracy Based**
- 2x/yr
- Uses donor samples, some supplemented with vitamin D2
- Values assigned using the CDC RMP
- Total 25-OHD is graded
- Total 25-OH D is graded
- 25-OHD3 and 25-OH D2 provided but "educational grade"

**DEQAS (www.deqas.org)**
- 4x/yr, now approved by CAP
- Becoming an accuracy-based survey

**NY STATE (www.wadsworth.org)**
- 25-OH D3 and 25-OH D2 peer values avail

**NIST QAP (http://www.nist.gov/mml/csd/vitdqap.cfm)**
- 2x/yr
- Ongoing traceability to NIST RMP

Accuracy Approach 5) Bias vs. Previous Method (IA); CLSI EP-9

Total 25-OH D: LC-MS/MS vs. IA
25-OH D3: LC-MS/MS vs. 2 independent LC-MS/MS Methods

Accuracy Approach 5 continued) Bias vs. LC-MS/MS method

- Compare to a RMP, if possible

Linearity (CLSI EP6-A) – Polynomial Regression Approach

- Nonlinearity should be assessed for clinical significance

Vit D Carryover Assessment – CLSI EP10

- CLSI EP10 Protocol

- Low: 24.3 ng/mL
  Mid: 65.3 ng/mL
  High: 154.3 ng/mL

- Carryover

<table>
<thead>
<tr>
<th>Run</th>
<th>% Carryover</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-0.6</td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>4</td>
<td>0.1</td>
</tr>
<tr>
<td>MED</td>
<td>-0.8</td>
</tr>
<tr>
<td></td>
<td>0.00</td>
</tr>
</tbody>
</table>
C62 Best Practice Acceptability Criteria:

- No peak in blank or < 20% of LLoQ

Matrix Effect 1) Pre vs. Post-Extract Spike and Rec (CLSI-C50)

10 ng/mL D3 spiked into Low Patient Pool (~1 ng/mL)

<table>
<thead>
<tr>
<th>Spiked Pre-Extraction</th>
<th>Spiked Post-Extraction</th>
<th>BL in Spiked</th>
<th>BL in Matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td>EU spiked into Serum (ng/mL)</td>
<td>Endogenous D3 in Serum (ng/mL)</td>
<td>Diff (ng/mL)</td>
<td>EU spiked into Matrix (ng/mL)</td>
</tr>
<tr>
<td>Rep 1</td>
<td>10000</td>
<td>2600</td>
<td>13400</td>
</tr>
<tr>
<td>Rep 2</td>
<td>10000</td>
<td>2600</td>
<td>12000</td>
</tr>
<tr>
<td>Rep 3</td>
<td>11000</td>
<td>2500</td>
<td>16700</td>
</tr>
<tr>
<td>Rep 4</td>
<td>10000</td>
<td>2600</td>
<td>10300</td>
</tr>
<tr>
<td>Mean</td>
<td>10400</td>
<td>2700</td>
<td>11888</td>
</tr>
</tbody>
</table>

% Matrix Effect (B/A*100) | 85.7
% Extr Eff (C/B*100) | 76.4
% Process Eff (C/A*100) | 65.5

% Matrix Bias | -14.3
% Matrix Bias Corr for IS | -2.3

Approach 2) Eval of Matrix Effects: Matrix Mixing (CLSI EP7)

Experiment for purpose of validating surrogate matrices
But, this approach can also be used to validate ME in patient samples

Native Patient Serum mixed in proportion with Stripped Serum or 8% BSA
N=4 reps over a single run

Native Patient Serum + Stripped Serum:

<table>
<thead>
<tr>
<th>% Dil Patient Serum</th>
<th>Expected (pg/mL)</th>
<th>Rep1 (pg/mL)</th>
<th>Rep2 (pg/mL)</th>
<th>Rep3 (pg/mL)</th>
<th>Rep4 (pg/mL)</th>
<th>Mean (pg/mL)</th>
<th>SD</th>
<th>% DIFF (pg/mL)</th>
<th>% DIFF</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>31.1</td>
<td>31.0</td>
<td>31.3</td>
<td>31.2</td>
<td>31.0</td>
<td>31.2</td>
<td>0.5</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>20%</td>
<td>24.9</td>
<td>24.7</td>
<td>24.6</td>
<td>24.7</td>
<td>24.6</td>
<td>24.7</td>
<td>0.3</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>40%</td>
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<td>18.8</td>
<td>18.8</td>
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<td>18.8</td>
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<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>60%</td>
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<td>12.7</td>
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<td>12.7</td>
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<tr>
<td>80%</td>
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<td>6.6</td>
<td>6.6</td>
<td>6.6</td>
<td>6.6</td>
<td>6.6</td>
<td>0.0</td>
<td>0.0</td>
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</tr>
</tbody>
</table>

- contribution from endogenous D3 in stripped serum removed

Native Patient Serum + 8% BSA:

<table>
<thead>
<tr>
<th>% Dil Patient Serum</th>
<th>Expected (pg/mL)</th>
<th>Rep1 (pg/mL)</th>
<th>Rep2 (pg/mL)</th>
<th>Rep3 (pg/mL)</th>
<th>Rep4 (pg/mL)</th>
<th>Mean (pg/mL)</th>
<th>SD</th>
<th>% DIFF (pg/mL)</th>
<th>% DIFF</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>30.6</td>
<td>30.5</td>
<td>30.5</td>
<td>30.5</td>
<td>30.5</td>
<td>30.5</td>
<td>0.0</td>
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<td>0.0</td>
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<tr>
<td>20%</td>
<td>24.5</td>
<td>24.4</td>
<td>24.4</td>
<td>24.4</td>
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<tr>
<td>40%</td>
<td>18.4</td>
<td>18.4</td>
<td>18.4</td>
<td>18.4</td>
<td>18.4</td>
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<td>12.3</td>
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</tr>
<tr>
<td>80%</td>
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<td>6.2</td>
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<td>6.2</td>
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<td>6.2</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>
Approach 3) Evaluation of Matrix Effects: T - Infusion

D3 in MeOH infused + injection of analyte-free serum

3-epimer Vit D interference

3-epi 25-OH Vitamin D
- Elevated in serum of infants and adults
- Clinical significance unknown
- Not recognized by most IAs
- Not resolved by most routine LC-MS/MS methods – source of discrepancies
- VDSP LC-MS/MS methods do resolve the 3-epimer

Used for NHANES measurements to establish dietary recommendations

J Clin Endocrinol Metab 2012, 97:163–168
Clinica Chimica Acta 2012, 413: 203–206

NIST SRM 972a and 3-epimer Vit D3

<table>
<thead>
<tr>
<th>Level</th>
<th>25OH D3 (ng/mL)</th>
<th>3-epi 25OH D3 (ng/mL)</th>
<th>25OH D2 (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>28.8 ± 1.1</td>
<td>1.84 ± 0.08</td>
<td>0.54 ± 0.06</td>
</tr>
<tr>
<td>Level 2</td>
<td>18.1 ± 0.4</td>
<td>1.29 ± 0.06</td>
<td>0.81 ± 0.06</td>
</tr>
<tr>
<td>Level 3</td>
<td>19.8 ± 0.5</td>
<td>1.18 ± 0.13</td>
<td>13.3 ± 0.3</td>
</tr>
<tr>
<td>Level 4</td>
<td>29.4 ± 0.9</td>
<td>26.4 ± 2.1</td>
<td>0.55 ± 0.10</td>
</tr>
</tbody>
</table>

If using to value-assign calibrators or verify traceability of method

Your method does not resolve 3-epimer

Need to add 3-epi concentration to NIST value assignments

*Caveat: 3-epi values are not certified

If using to value-assign calibrators or verify traceability of method

Your method does not resolve 3-epimer

Need to add 3-epi concentration to NIST value assignments

*Caveat: 3-epi values are not certified
Vit D: Interference Testing

C62: Check for interferences from reagents & disposables

New lot MeOH

![Graph showing interference testing](image)

C62: Check for potential endogenous interferences

†† Bile Salts in PBC patient

![Graph showing interference testing](image)

Exogenous and Other Endogenous Interferences

- Reagents and Disposables (major interference from new lot MeOH)
- Collection Tube Additives (ex: SST interference in some testo methods)
- Physiological or Disease-Associated Interferences (ex: PBC patients)
- Isobaric/Isotopomeric – Interferences resulting in shared product ions or a precursor and product ion (3-epi 25-OH D)
- Drugs/Metabolites (Vit D metabolites, use of T2/T1 ratios)

Vit D QA Monitoring – Run Acceptability and Sample Acceptability

- Slope & Intercept PASS Range
- % Calibrator Recovery (ex: ±10%)
- r² or SE of Calibrator Curve
- Run-to-run IS Peak Area Recovery Criteria (ex: ±30%)
- IS Recovery Criteria for each sample (ex:±20%)
- RT, Peak Resolution, Peak Symmetry
- Presence of Interfering Peaks or Absent Peaks
- T2/T1 Ratio Criteria (CLSI-C50)
- For multiplexing, each column/stream is considered a separate method
1,25 (OH)₂ Vitamin D – To Test or Not To Test?

Test name changed from 1,25 dihydroxyvitamin D to Calcitriol
### C60: Reagent Lot Change – Patient Comps and Linearity (CAP)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>Conc (ng/mL)</th>
<th>CLSI EP-6</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1</td>
<td>4.5</td>
<td>4.8</td>
<td>Fail</td>
</tr>
<tr>
<td>2</td>
<td>0.2</td>
<td>4.5</td>
<td>4.8</td>
<td>Fail</td>
</tr>
<tr>
<td>3</td>
<td>0.3</td>
<td>4.5</td>
<td>4.8</td>
<td>Fail</td>
</tr>
</tbody>
</table>

**Plus chromatography acceptability criteria**

**Plus QC acceptability criteria (native pt based)**

### C62: Periodic Accuracy Monitoring

**Using Patient Pools traceable to NIST SRM 972 (25-OHD)**

1. Create Patient Pools near NIST SRM values
2. Calc Method Bias vs. NIST SRM
3. Apply a Correction Factor to Patient Pools to trace their values to NIST-assigned values

**Table: Patient Pool Results**

<table>
<thead>
<tr>
<th>Pool</th>
<th>Method Bias</th>
<th>Correction Factor</th>
<th>Assigned Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.2</td>
<td>0.8</td>
<td>19.0</td>
</tr>
<tr>
<td>2</td>
<td>1.5</td>
<td>0.8</td>
<td>19.0</td>
</tr>
<tr>
<td>3</td>
<td>1.8</td>
<td>0.8</td>
<td>19.0</td>
</tr>
</tbody>
</table>

**Diagram: CLSI EP-6**

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