Commutability Studies at NIST

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NIST Clinical Reference Materials

- Approximately 50 clinical SRMs including pure substance, calibration solutions, and natural matrix materials (blood, serum, plasma, urine)
- Analytes include inorganic and organic species
- Currently few nucleic acid SRMs for clinical diagnostics
- Most serum or plasma materials now fresh-frozen, not lyophilized
- Greater use of CLSI C37-A protocols in material preparation
Mechanisms for Assessing Commutability

- Commutability studies performed in accordance with CLSI C53-A or EP14-A2
- Data from interlaboratory studies
- Inclusion of routine methods in value assignment

SRM 967

Potential obstacles or limitations to commutability studies:

- Material sells out quickly
- Access to appropriate patient samples (and cost)
- Need partners to engage assay manufacturers
- End users unknown (manufacturers, clinical labs, research)
SRM 1955 Homocysteine and Folate in Human Serum

- Three level material, with two levels prepared through either dilution or spiking
- SRM issued in 2005 with certified and reference values for homocysteine, 5mTHF, and folic acid
- Commutability study performed for homocysteine using modification of CLSI EP14-A2 guidelines, collaboration with CDC
- Participating laboratories included 14 immunoassays and/or enzymatic assays
- Only 20 single donor patient samples used
- Reference assay was NIST LC-MS/MS method
Homocysteine in SRM 1955

- Statistical analysis using error-in-variables approach, based on Deming regression
- Weighted least-squares regression analysis incorporating uncertainty in x- and y-axis
- One patient sample excluded because of potentially spurious results
- Statement of commutability limited by range and number of patient samples
SRM 972a Vitamin D Metabolites in Human Serum

- Renewal material to replace SRM 972
- Four levels with varying concentrations of vitamin D metabolites
- Endogenous concentrations of all metabolites, except Level 4

- Commutability study performed as part of Vitamin D Standardization Program (VDSP)
- Additional materials from CAP and DEQAS also evaluated
- Reference method was ID LC-MS/MS (NIST and Ghent)
- Reference labs determined 25(OH)D$_2$, 25(OH)D$_3$, and 3-epi-25(OH)D$_3$
- Fifty single donor patient samples spanning range of concentrations
SRM 972a Vitamin D Metabolites in Human Serum

- Participating Labs:
  - 20 Total: 4 LC-MS/MS and 16 Immunoassay
    - 2 dropout
    - 5 – Open reporting
    - 4 – Anonymous reporting
    - 2 – Results not to be used
    - 7 – TBA

- All samples run in duplicate on 3 different days

- Total $25(\text{OH})D = 25(\text{OH})D_2 + 25(\text{OH})D_3^*$

* Does not include concentration of 3-epimers
VDSP Commutability Study: Immunoassay

Lab 1: SRM 972a Levels 1-4

Test Lab Mean Total 25(OH)D (nmol/L) vs. NIST Mean Target Total 25(OH)D (nmol/L)

- 95% PI
- Fitted Line
- Observed
- SRM 972a Sample

- n: 50
- R²: 0.92

<table>
<thead>
<tr>
<th>Term</th>
<th>Coefficient</th>
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<tbody>
<tr>
<td>Intercept</td>
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<td>Slope</td>
<td>1.15</td>
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VDSP Commutability Study: Immunoassay

Lab 2: SRM 972a Levels 1-4

Test Lab Mean Total 25(OH)D (nmol/L) vs. Ghent Mean Target Total 25(OH)D (nmol/L)

- 95% PI
- Fitted Line
- Observed
- SRM 972a Sample

- n = 50
- R^2 = 0.88

<table>
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<td>Slope</td>
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VDSP Commutability Study: Immunoassay

Lab 9: SRM 972a Levels 1-4

NIST Mean Target Total 25(OH)D (nmol/L)

Test Lab Mean Total 25(OH)D (nmol/L)

- 95% PI
- Fitted Line
- Observed
- SRM 972a Sample

<table>
<thead>
<tr>
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<td>Slope</td>
<td>1.35</td>
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</table>

n = 50
R² = 0.80
VDSP Commutability Study: LC-MS/MS

Lab 16: SRM 972a Levels 1-4

Test Lab Mean Total 25(OH)D (nmol/L) vs. Ghent Mean Target Total 25(OH)D (nmol/L)

- 95% PI
- Fitted Line
- Observed
- SRM 972a Sample

Table:

<table>
<thead>
<tr>
<th>Term</th>
<th>Coefficient</th>
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<tbody>
<tr>
<td>Intercept</td>
<td>-0.08</td>
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<tr>
<td>Slope</td>
<td>1.01</td>
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</table>

Graph:

- Linear regression line with equation:
  - Intercept: -0.08
  - Slope: 1.01
- Calculated R²: 0.998
- Sample size (n): 50
VDSP Commutability Study: Immunoassay

Lab 17: SRM 972a Levels 1-4

- Test Lab Mean Total 25(OH)D (nmol/L)
- NIST Mean Target Total 25(OH)D (nmol/L)

95% PI
Fitted Line
Observed
SRM 972a Sample

- R^2 = 0.85
- n = 49

<table>
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Conclusions from Commutability Study

- SRM 972a appears commutable with most assays
- Remaining questions about response to certain metabolites (25OHD$_2$ and 3-epimers)
- Performance of some immunoassays makes nearly any material appear commutable

- Need to require participants to be identified in any future studies
- Set minimum performance criteria for participating assays
SRM 3667 Creatinine in Frozen Human Urine

- Single level material with endogenous creatinine concentration
- Value assignment at NIST by ID LC-MS using variation of method for serum
- No previous materials for creatinine in urine
- Small comparison organized through NKDEP to compare results from routine methods to those obtained by NIST
- Samples sent to three participants, both enzymatic and Jaffe methods

<table>
<thead>
<tr>
<th>Mass fraction (µg/g)</th>
<th>Mass concentration (mg/dL)</th>
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<tr>
<td>631 ± 13</td>
<td>61.8 ± 1.3</td>
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SRM 3667 Study Results

![Graph showing the study results for various labs and methods with two runs.]

- NIST, ID-LC-MS
- Lab A, Method 1
- Lab B, Method 1
- Lab B, Method 2
- Lab C, Method 1
- Lab C, Method 2
- Lab C, Method 3
- Lab C, Method 4
### Use of Data from Interlaboratory Studies – SRM 968e

<table>
<thead>
<tr>
<th>Analyte</th>
<th>NIST LC-UV 1</th>
<th>NIST LC-UV 2</th>
<th>Study median</th>
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<tbody>
<tr>
<td>Total retinol</td>
<td>0.346 (0.016)</td>
<td>0.326 (0.008)</td>
<td>0.351</td>
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<tr>
<td>γ/β-Tocopherol</td>
<td>2.03 (0.10)</td>
<td>1.84 (0.03)</td>
<td>1.72</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>6.96 (0.34)</td>
<td>5.84 (0.10)</td>
<td>6.75</td>
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<tr>
<td>Total lutein</td>
<td>0.069 (0.004)</td>
<td>0.059 (0.003)</td>
<td>0.072</td>
</tr>
<tr>
<td>Total lycopene</td>
<td>0.173 (0.004)</td>
<td>0.294 (0.008)</td>
<td>0.236</td>
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<tr>
<td>Total β-carotene</td>
<td>0.114 (0.004)</td>
<td>0.093 (0.004)</td>
<td>0.090</td>
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<tr>
<td>Total zeaxanthin</td>
<td>0.029 (0.003)</td>
<td>0.029 (0.001)</td>
<td>0.037</td>
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</table>

Data for Level 1 of SRM 968e from NIST methods and from participants in the NIST Micronutrients Measurement QA Program (MMQAP). All results in µg/mL.
SRMs with Multiple Purposes

SRM 1950 Metabolites in Human Plasma

- Perfluorinated compounds: 6 Certified Values, 6 Reference Values, 2 Information Values
- Electrolytes/trace elements/Se species: 4 Certified Values, 6 Reference Values, 2 Information Values
- Clinical markers: 8 Certified Values, 2 Reference Values, 2 Information Values
- Vitamins/carotenoids: 11 Certified Values, 8 Reference Values, 2 Information Values
- Amino acids: 12 Certified Values, 6 Reference Values, 2 Information Values
- Lipids: 11 Certified Values, 19 Reference Values, 2 Information Values

Should commutability be assessed, and by whom?
Acknowledgments

- National Institutes of Health (NIH)
  - Office of Dietary Supplements (ODS)
  - National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
- Centers for Disease Control and Prevention (CDC)
- Ghent University
- National Kidney Disease Education Program (NKDEP)
- Vitamin D Standardization Program (VDSP)
- NIST Chemical Sciences Division