Key Methodological Issues to Consider for Monitoring Vitamin D Status of a Nation

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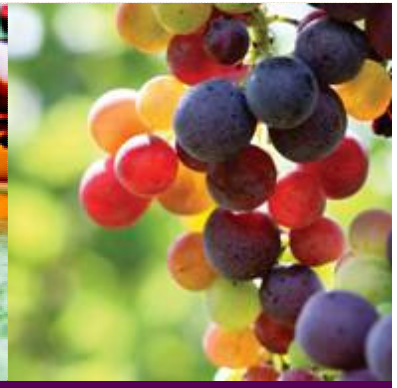
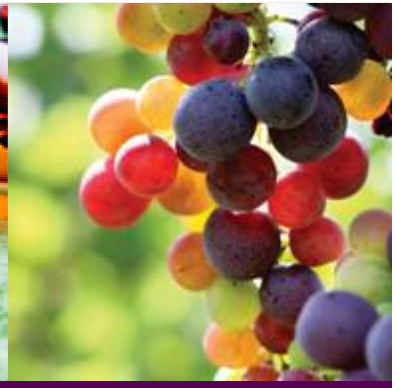
What is NHANES?

- ***National Health and Nutrition Examination Surveys***
- Conducted by the National Centers for Health Statistics (NCHS) of the CDC
- To assess the health and nutritional status of a nationally representative sample
- Data used for a variety of research and programmatic purposes

NHANES Mobile Exam Center



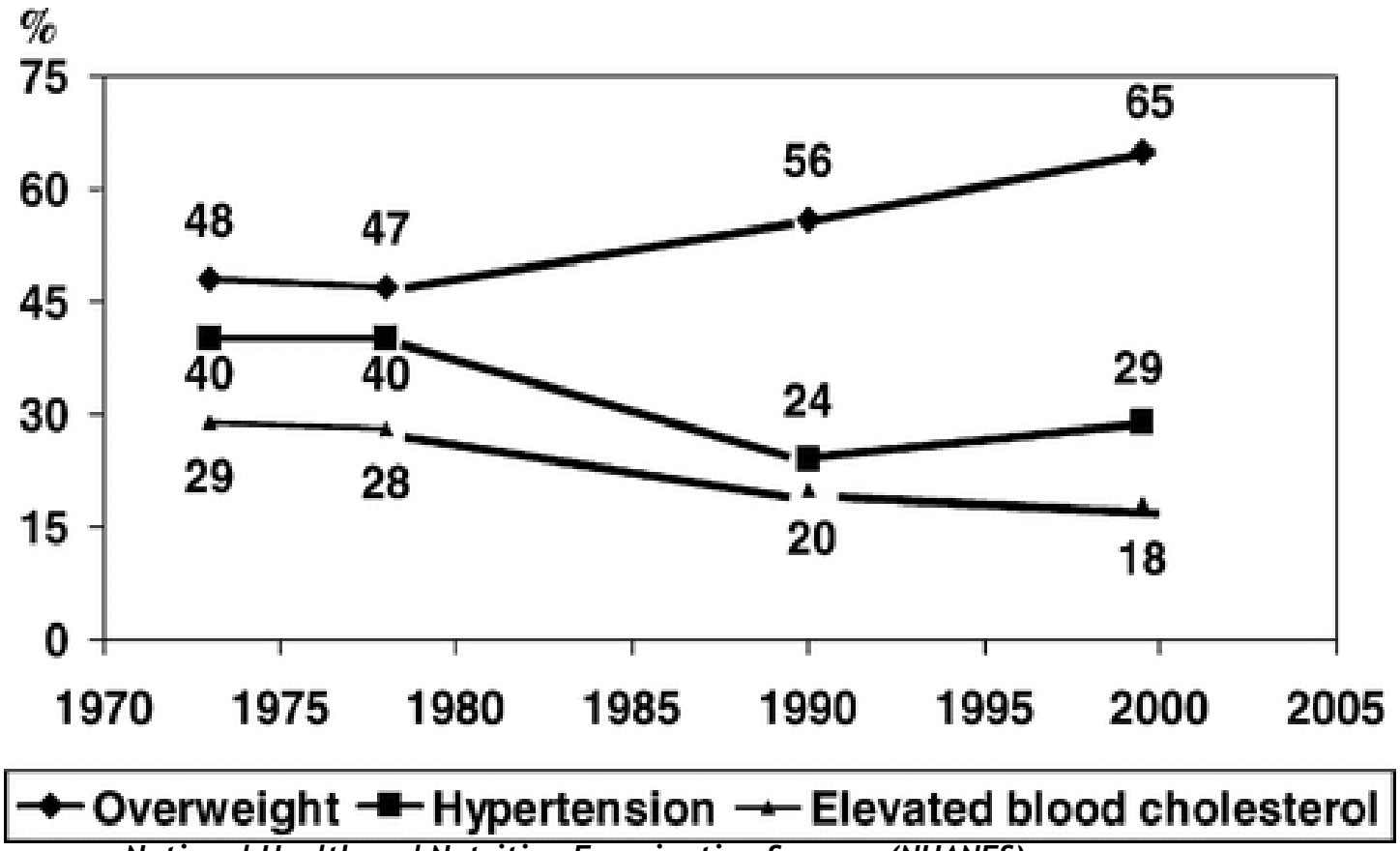
NHANES: Serum 25OHD Samples



Survey	*Ages*	*Sample*
NHANES III (1988-1994)	12+	18,158[a]
NHANES 2000	6+	4,188[a]
NHANES 2001-02	6+	7,807[a,b]
NHANES 2003-04	1+	8,294[a]
NHANES 2005-06	1+	7,402[b]

[a]Looker et al., 2007 NIH conference; [b]Pfeiffer, Roundtable handouts, 2009

Trends in the age-adjusted prevalence of diet-related health conditions in U.S. (adults age 20 to 74)



Data source: National Health and Nutrition Examination Surveys (NHANES); data are plotted at the midpoint of the NHANES survey period.

Vitamin D Status of the US Population?

- 25(OH)D is biomarker of exposure not status:
  - UVB radiation + intakes
- Population-based status data:
  - Dietary Reference Intake Committee of the Institute of Medicine on Vitamin D and calcium
  - Dietary Guidelines Advisory Committee
- 25(OH)D collected in NHANES III and continuous NHANES since 20000

NHANES: Serum 25(OH)D Assays

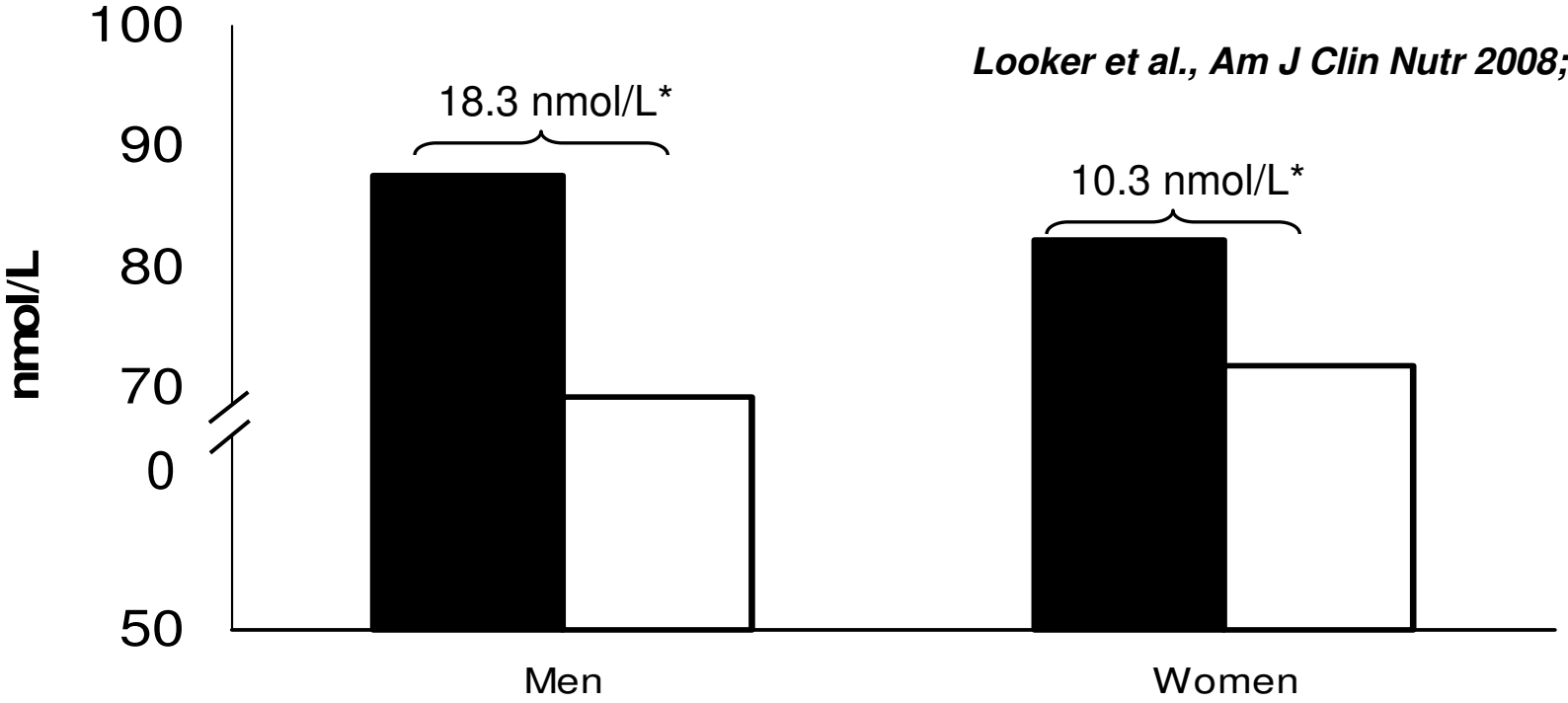
- Laboratory:
  - National Center Environmental Health, Centers for Disease Control
- Methods:
  - DiaSorin RIA
    1988-94 and 2000-2006
  - LC-MS/MS
    2007 samples and after

Initial 25(OH)D Assay Concerns (Looker et al., 2008)

- 1988-94 vs. 2000 - 2004:
  - Reformulation (introduction of antibody to improve binding) of Diasorin RIA assay kit → shifts in assay results between these two time periods
  - Adjusted for assay drifts to compare time trends in 25(OH)D and identify contributing factors

Looker et al. Am J Clin Nutr 2008;88:1519-27.

Observed differences in serum 25(OH)D concentrations (NH Whites, 20-59y, NH III vs NH 03-04)

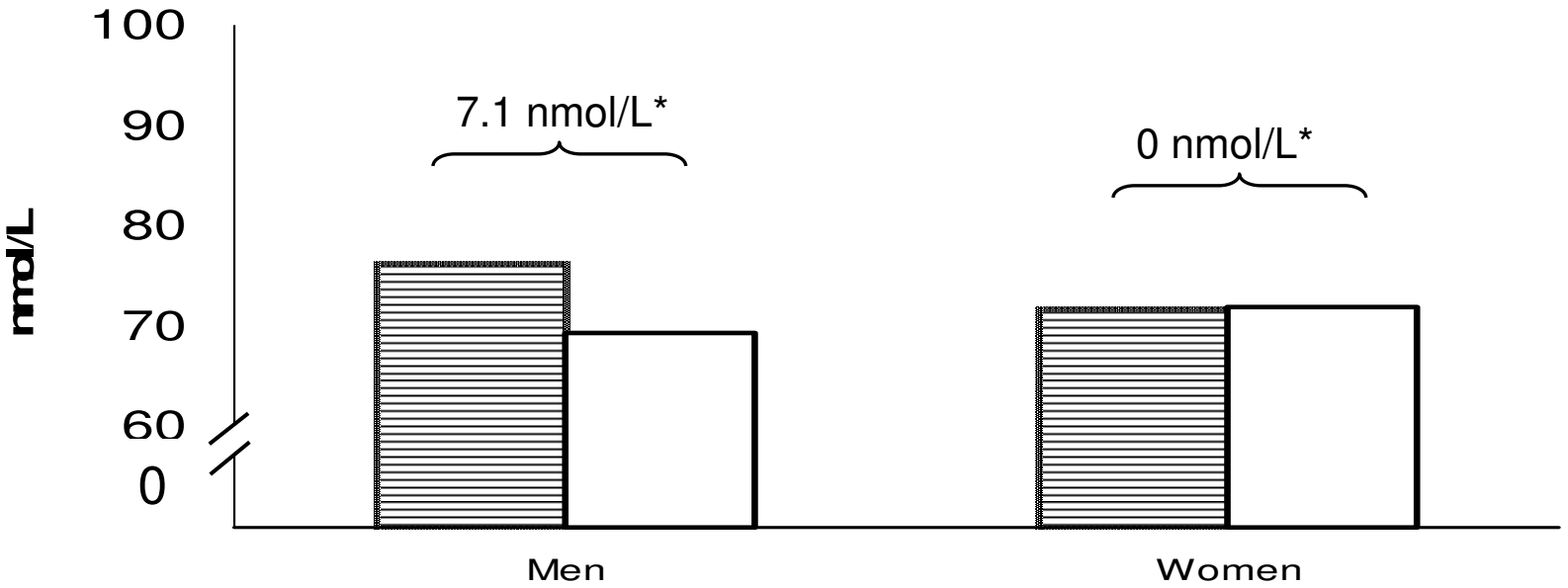


Looker et al., Am J Clin Nutr 2008;88:1519-27.

Black bars: Observed mean for NHANES III 1988-1994
Open bars: Observed mean for NHANES 2003-2004
*Calculated as NHANES III mean minus NHANES 2003-04 mean

Differences in 25(OHD) after accounting for assay difference**

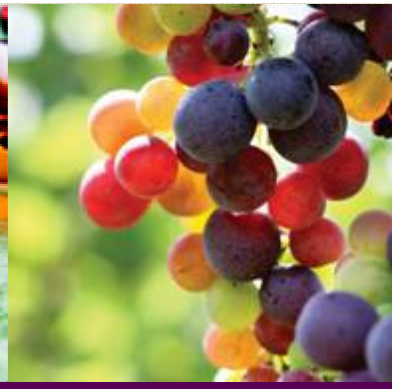
(NH Whites, 20-59y, NH III vs NH 03-04)

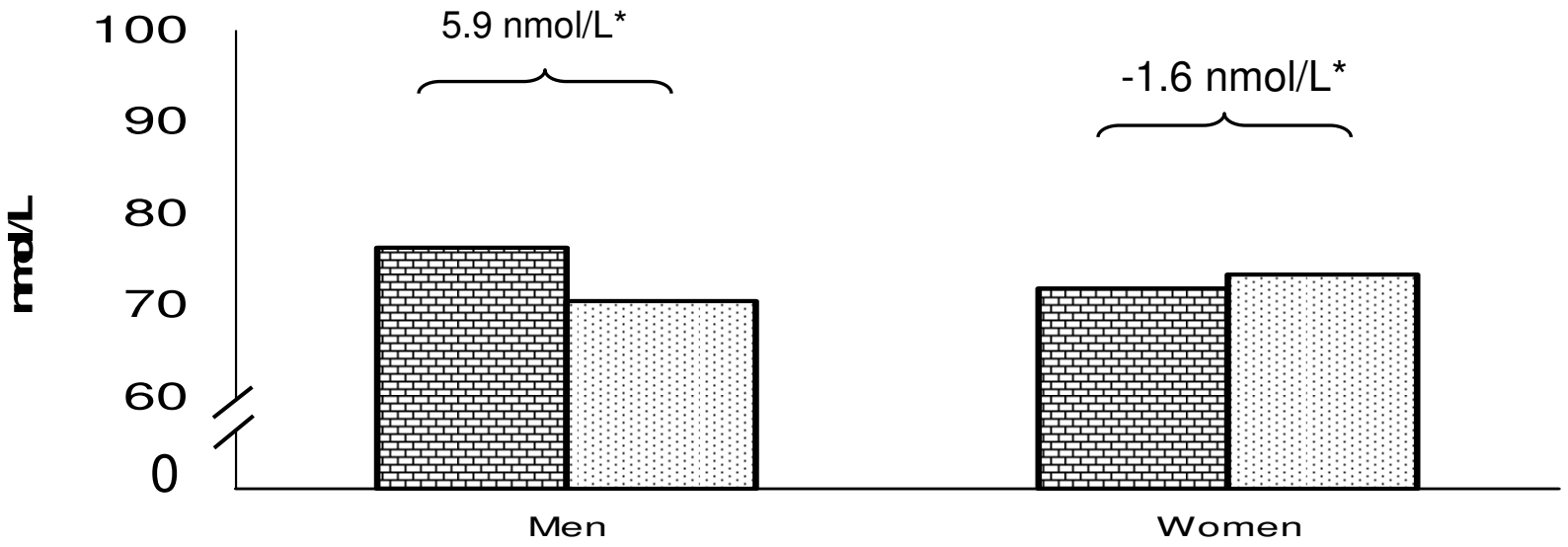


"Brick" pattern: Predicted mean for NHANES III 1988-1994 assuming current RIA used
Clear bar: Observed mean for NHANES 2003-2004
*Calculated as NHANES III 1988-1994 minus NHANES 2003-04 mean
**Mean predicted for NHANES III 1988-1994 if current RIA was used.

Looker et al., Am J Clin Nutr 2008;88:1519-27.

Differences in 25(OH)D after accounting for assay difference and biological/behavioral factors*** (NH Whites, 20-59y, NH III vs NH 03-04)



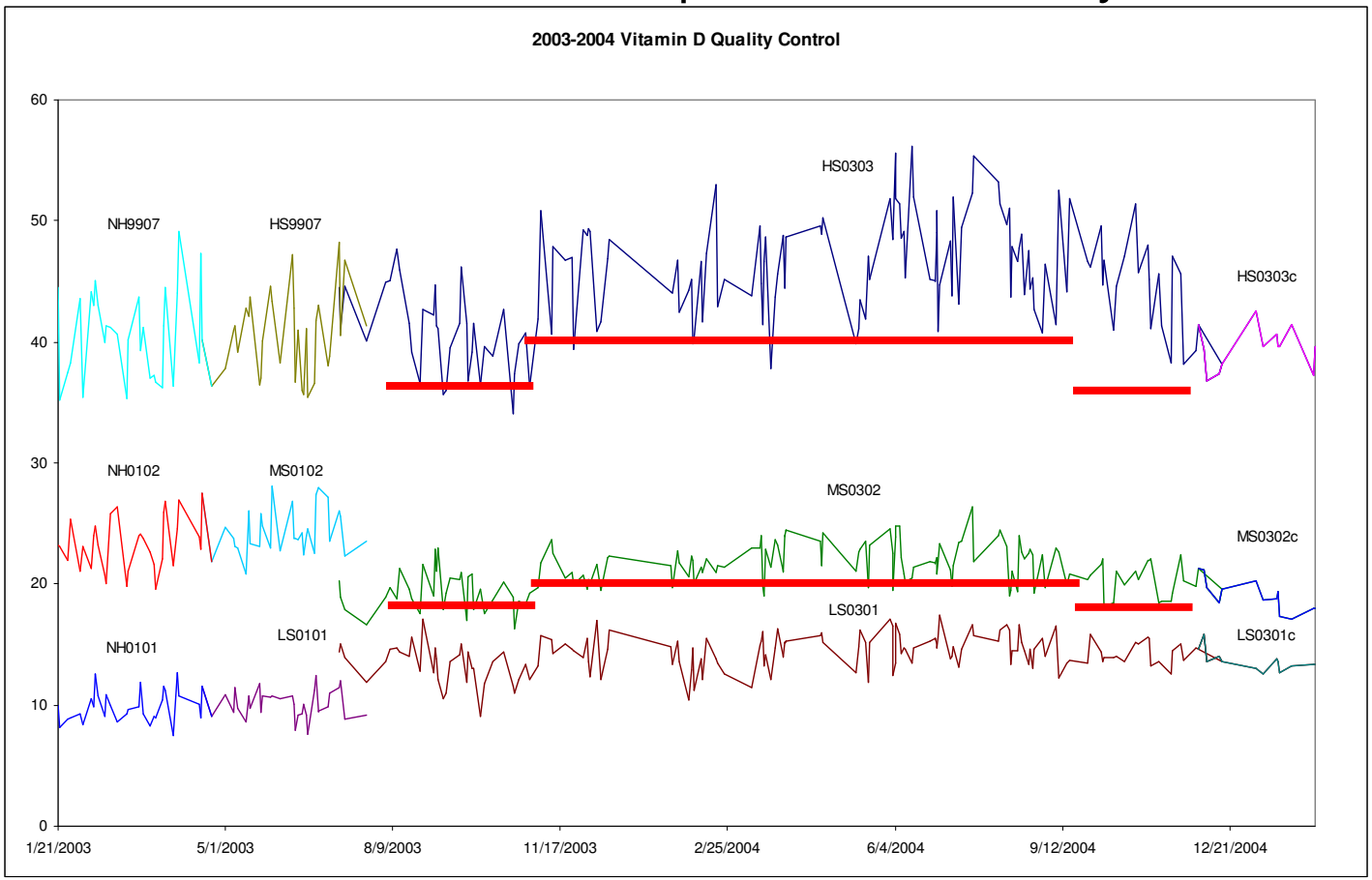


"Brick" design: Predicted mean for NHANES III 1988-1994 assuming current RIA used
"Dot" design: Predicted mean for NHANES 2003-04 after adjusting for BMI, milk intake and sun protection changes
*Calculated as NHANES III mean minus NHANES 2003-04 mean
*** Mean predicted for NHANES III 1988-94 assuming current RIA was used and mean predicted for NHANES 2003-2004 if **mean BMI, milk consumption, and sun protection** applied.

Looker et al., Am J Clin Nutr 2008;88:1519-27.

Review of 2004 QC Performance

- DiaSorin RIA assay was performing on the high side of the QC limits during 2004, particularly for the high pool
  - Late 2003 – new lot number for standards, tracer, other reagents
  - Late 2004 – new lot number of tracer, antiserum
- Conclusion: Conduct a 150 sample crossover study for QC shift



3. 2004 assay shift

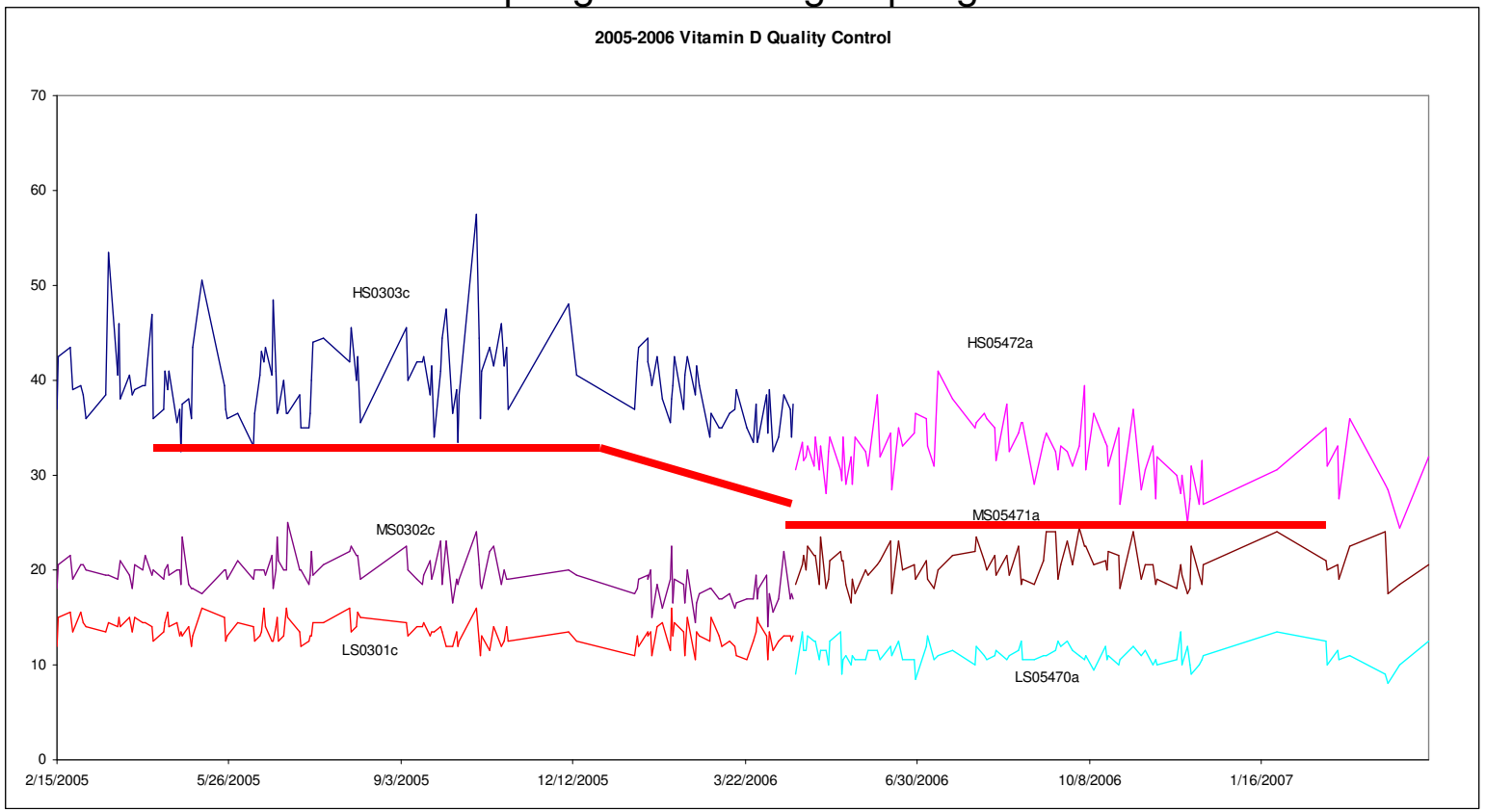
Letter to the Editor (2009)

- Effects of drifts on population data not fully recognized until after publication

- Analyses repeated after excluding data of concern:
  - Effects were minimal
  - CDC to address methodological drift issues

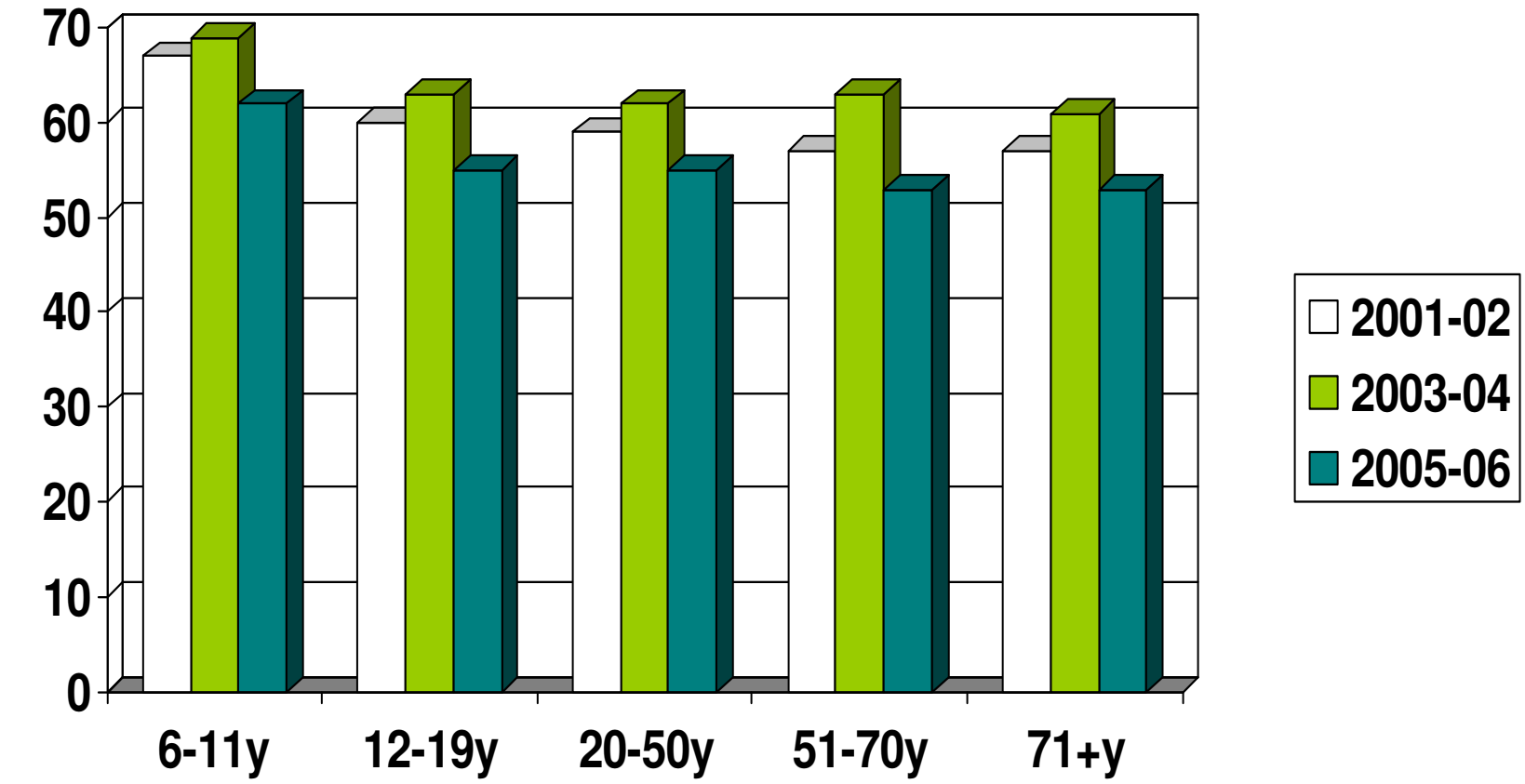
Review of 2006 QC Performance

- DiaSorin RIA assay was performing on the low side of the QC limits during 2006, particularly for the high pool
  - Early 2006 – new lot number for tracer, antiserum and other reagents
  - End date? Stopped using 0301/0302/0303 series QC in Spring 2006; new 05470/05471/05472 series QC (characterized during Spring 2006) performed on the same level from Spring 2006 through Spring 2007



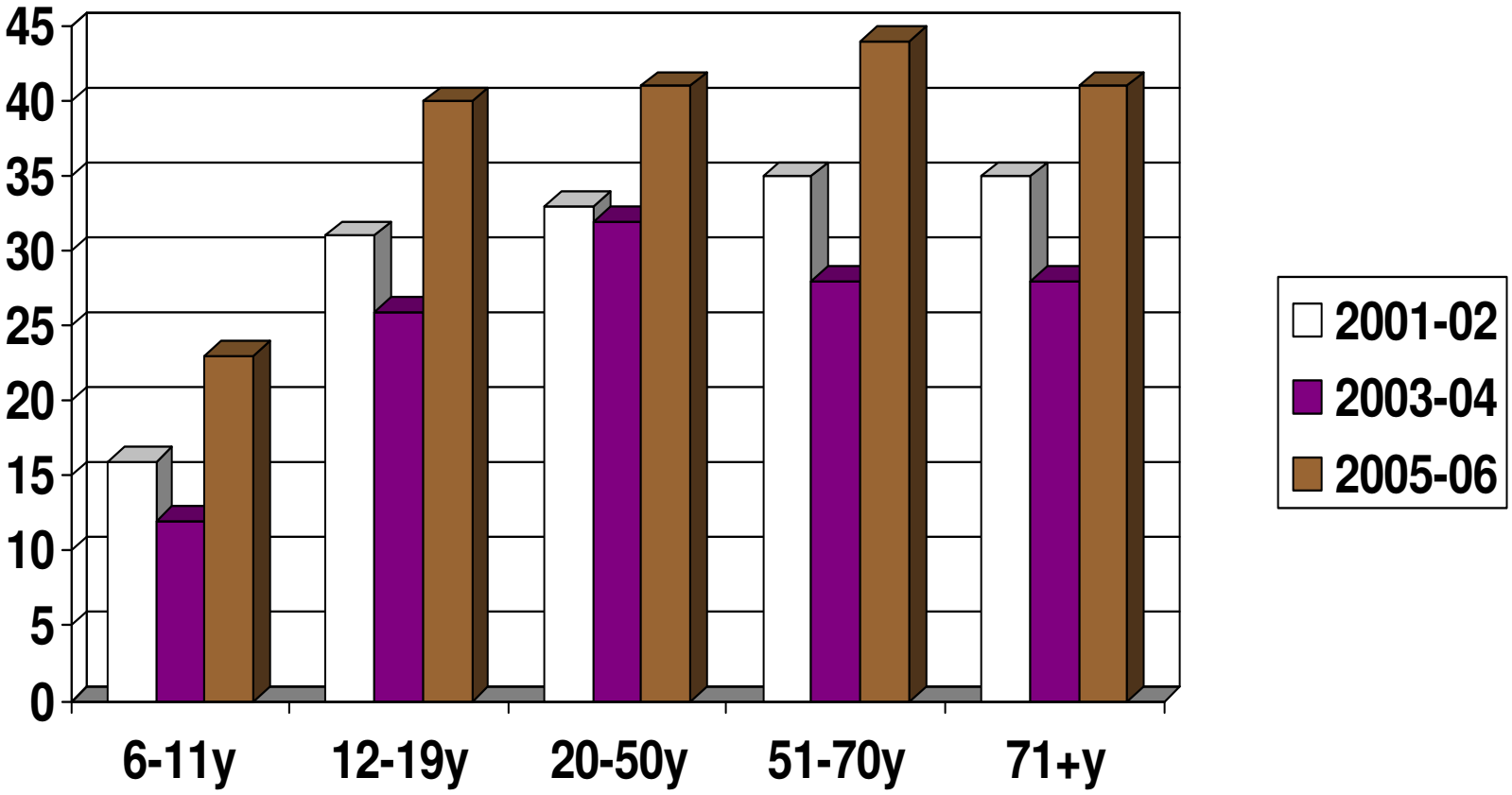
5. 2006 assay shift

NHANES: Mean 25(OH)D (nmol/L)*



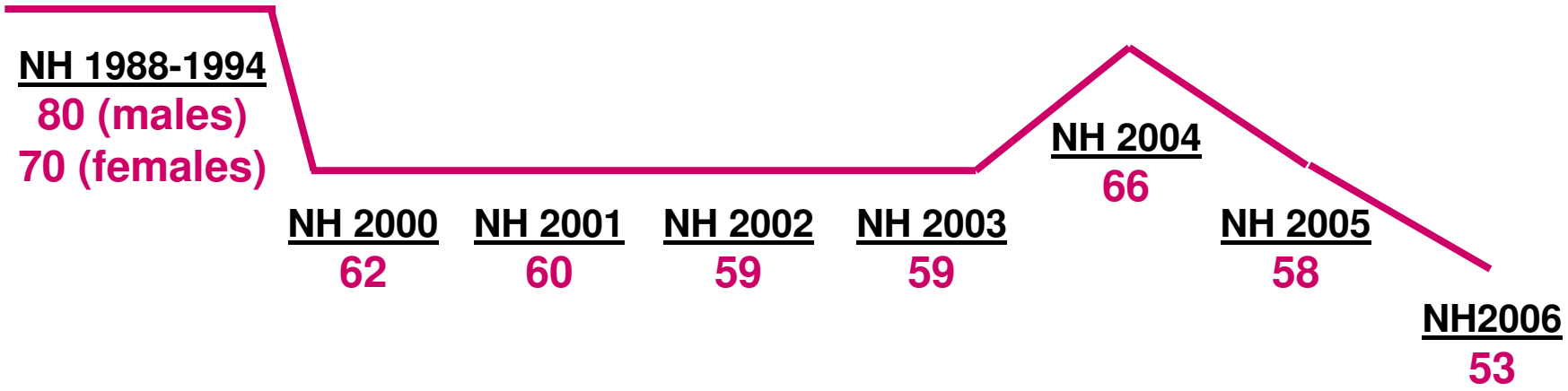
*Not adjusted for assay drift

NHANES: % <50 nmol/L*



*Not adjusted for assay drift

25OHD (nmol/L) NHANES weighted means track well with trends seen in QC data



For methods comparison only and not for publication. Not adjusted for reformulation or assay shifts

NCHS Analytic Note (2009

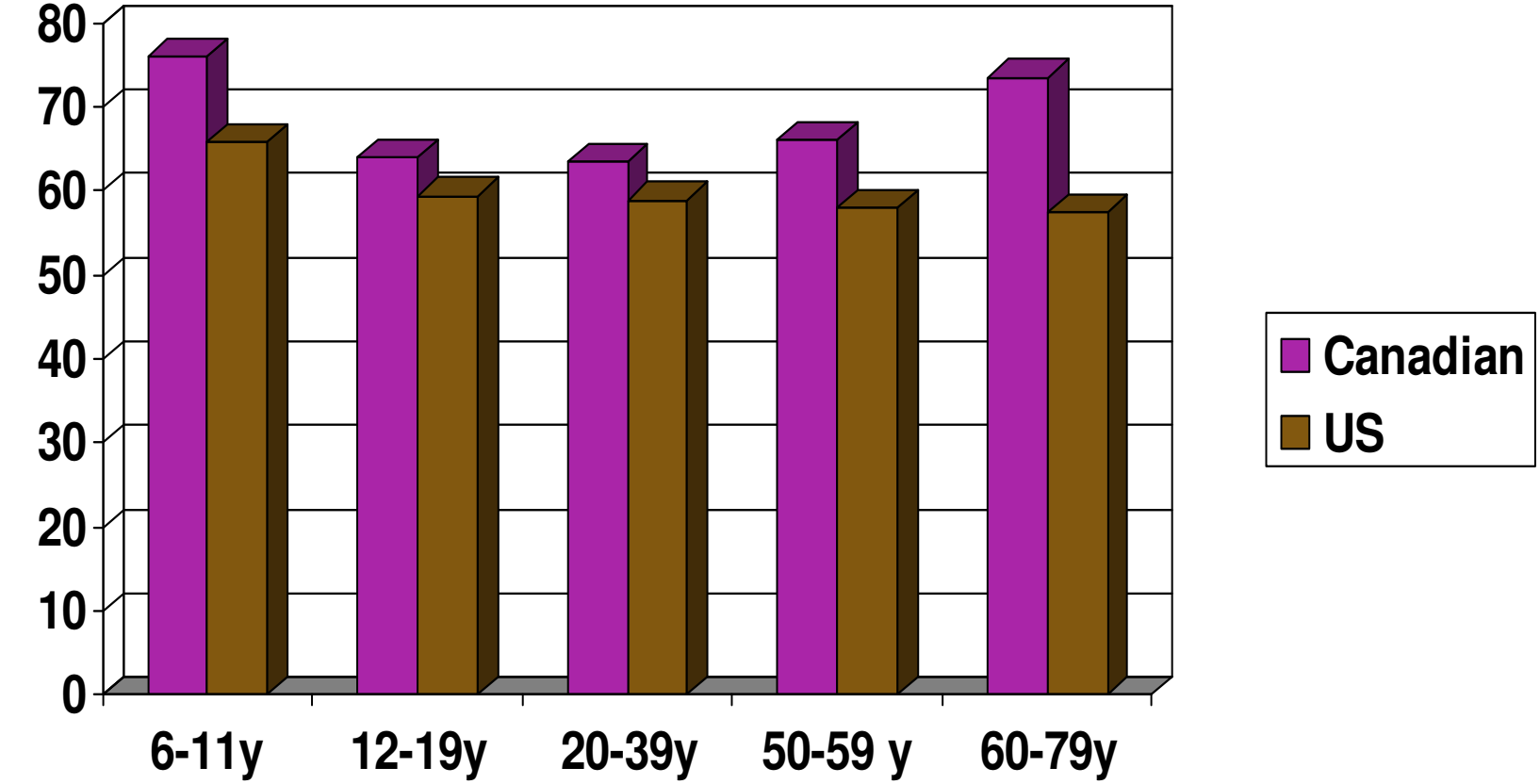
- Published data 2000 – 2004:
  - Effect of drifts in assay comparability for the serum 25(OH)D data collected in this time period not fully recognized
- Additional drifts in assay comparability for the 2005 – 2006 results
- Cautions users when assessing time trends

http://www.cdc.gov/nchs/data/nhanes/nhanes3/VitaminD_analyticnote.pdf

Adjustment Affects Policy Conclusions

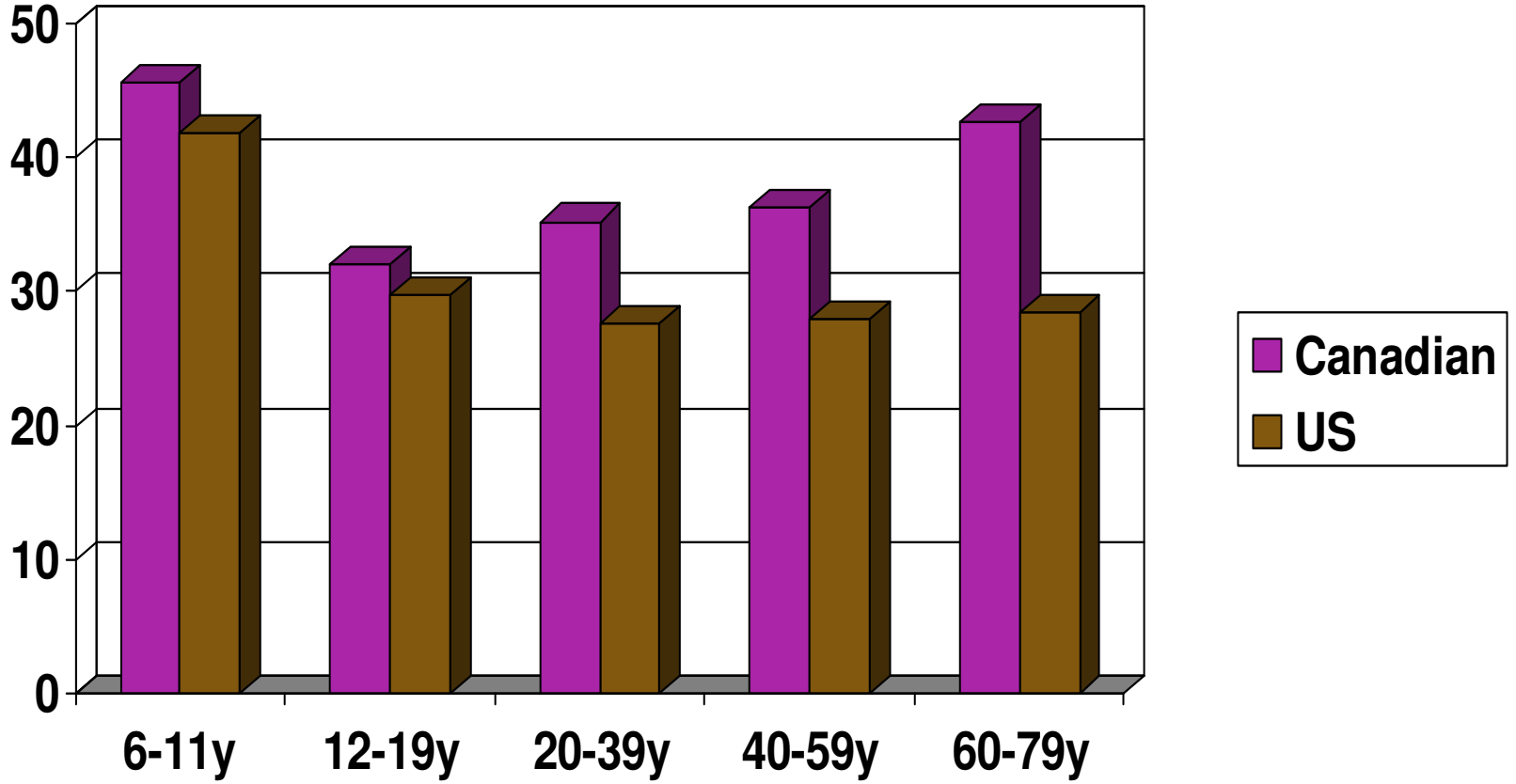
- **Adjustments for assay differences**:
  - Looker et al. (2008):
    - Overall mean 25(OH)D lower in 2000-2004 than in 1988-94
    - Assay changes accounted for much of the difference
- **No adjustment for assay differences:**
  - Ginde et al. (2009): Marked decrease in 25(OH)D in 2000-2004 compared to 1988-1994
    - Growing epidemic of vitamin D insufficiency
  - Saintonge 2009: Vitamin D deficiency is increasing from 1988-1994 to 2006
    - Implementation of national fortification and public health strategy

Mean 25(OH)D (nmol/L) Canada vs. US.



Canadian data: Diasorin Liaison 2007-2008
US data: Diasorin RIA, NHANES 2001-2006 (unadjusted for assay drift)

10th % 25(OH)D (nmol/L) Canada vs. U.S.



Canadian data: Diasorin Liaison 2007-2008
US Data: Diasorin RIA NHANES 2001-2006 (unadjusted for assay drift)

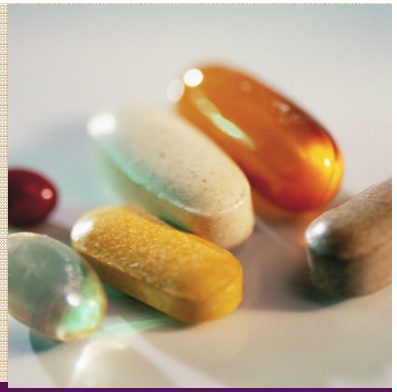
Challenges

- "Old":
  - Appropriate adjustments to 1988-94 values?
- Recently identified:
  - How to resolve QC drifts for 2004 and 2006?
- Future (>2006):
  - Diasorin RIA → LC-MS/MS
  - Integration of standard reference material for vitamin D in human serum (SRM 972)

Roundtable of Experts in Vitamin D, Laboratory Methodology and Statistics Convened

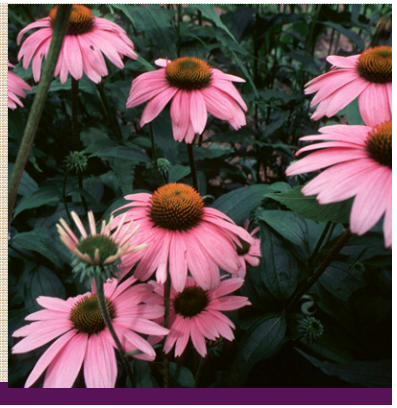
- Convened: July 27-28, 2009
- Identify issues and discuss their pros and cons
  - Not – to make recommendations
- To think constructively about next steps
- Focus on NHANES needs
  - Methods used in NHANES – Diasorin RIA and LC-MS/MS
  - Not on larger concerns about comparisons across all available methods

Roundtable Objectives



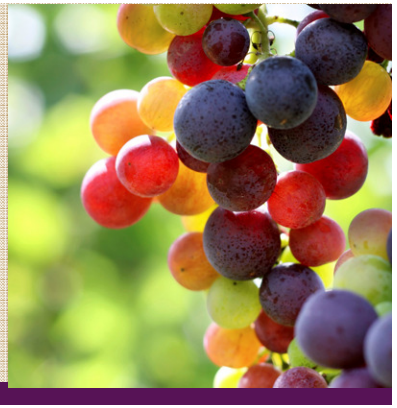
1. Laboratory QC procedures:
   - Identify key issues for ensuring accurate and reliable estimates of 25(OH)D
   - Discuss pros and cons of approaches for their successful implementation

Roundtable Objectives



2. Harmonizing across all Diasorin-based NHANES surveys:
   - Identify the key issues
   - To adjust the data across surveys to facilitate trend analyses of summary statistics and prevalence estimates of both high and low vitamin D status

Roundtable Objectives



3. Future efforts:
   - Integrating the SRM 972 into the NHANES data analysis
   - Bridging the LC-MS/MS data with the Diasorin data
   - Analyzing for the Vitamin D **epimer**
4. Identify research and education needs

Standard Reference Material 972
25(OH) D in Human Serum

https://www-s.nist.gov/srmors/certificates/view_cert2gif.cfm?certificate=972

National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 972

Vitamin D in Human Serum

Standard Reference Material (SRM) 972 is intended for use as an accuracy control in the critical evaluation of methods for determining the amount of substance concentration of vitamin D metabolites in human serum. This SRM can also be used as a quality assurance tool for assigning values to in-house control materials for these constituents. A unit of SRM 972 consists of four vials (Levels 1 through 4) of frozen serum with different concentration levels of 25-hydroxyvitamin D [25(OH)D]. Measurement of 25(OH)D in serum is generally considered a reliable indicator of vitamin D status. Each vial of SRM 972 contains approximately 1 mL of serum.

Each of the four levels of SRM 972 was prepared with specific target levels of vitamin D metabolites. While some measurement methods might be applicable to each of the four levels of SRM 972, it is recognized that some specific levels may not be applicable to a given method. Individual users will need to assess which level or levels best suit their particular needs. Level 1 of SRM 972 was prepared from "normal" human serum and has not been altered. Level 2 was prepared by diluting Level 1 with horse serum to achieve a lower 25(OH)D concentration. Level 3 contains "normal" human serum that has been fortified with 25-hydroxyvitamin D_2, and Level 4 contains "normal" human serum that has been fortified with 3-epi-25-hydroxyvitamin D_3.

Certified Concentration Values: The certified concentration values for 25-hydroxyvitamin D_3 [25(OH)D_3], 25-hydroxyvitamin D_2 [25(OH)D_2], and 3-epi-25-hydroxyvitamin D_3 [3-epi-25(OH)D_3] are provided in Table 1. Structures of these compounds are provided in Figure 1. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account [1]. The certified concentration values for these analytes are based on the agreement of results from isotope dilution liquid chromatography mass spectrometry (ID-LC-MS), and isotope dilution liquid chromatography tandem mass spectrometry (ID-LC-MS/MS) procedures performed at NIST, and from results provided by the Centers for Disease Control and Prevention (CDC), Atlanta, GA.

Reference Concentration Values: Reference concentration values for 25(OH)D_2 and 3-epi-25(OH)D_3 are provided in Table 2. Reference values are noncertified values that are the best estimate of the true values based on available data; however, the values do not meet the NIST criteria for certification, and are provided with associated uncertainties that may reflect only measurement precision, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods [1]. The reference values for 3-epi-25(OH)D_3 are based on LC-MS/MS measurements performed at NIST.

Expiration of Certification: The certification of **SRM 972** is valid, within the measurement uncertainty specified, until **30 September 2015,** provided the SRM is handled in accordance with the instructions given in this certificate (see "Instructions for Use"). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of SRM Certificate: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

Support for the development of SRM 972 was provided in part by the National Institutes of Health (NIH) Office of Dietary Supplements (ODS). Technical consultation was provided by J.M. Betz and M.F. Picciano (NIH-ODS).

The overall direction and coordination of the preparation and analytical measurements leading to the certification of this SRM were performed by K.W. Phinney and S.A. Wise of the NIST Analytical Chemistry Division.

Stephen A. Wise, Chief
Analytical Chemistry Division

Gaithersburg, MD 20899
Certificate Issue Date: 9 June 2009

Robert L. Watters, Jr., Chief
Measurement Services Division

Roundtable: Review Draft Fact Sheet on SRM 972

- What is vitamin D in human serum?
- What is a Standard Reference Material?
- What is SRM 972?
- Why was SRM 972 developed?
- Who is likely to use SRM 972?
- What process was used to develop SRM 972?

Next Steps

- Finalize Fact Sheet for the SRM 972 on 25(OH) D in Human Serum
  - Incorporate comments from Roundtable participants, Office of Dietary Supplements (ODS), and National Institute of Standards and Technology
  - Quick review by Roundtable participants
  - Post on ODS Website with linkages to NIST Website

Next Steps

- Conduct follow-up analyses to evaluate and compare several approaches for adjusting Quality Control (QC) drifts during 2000-2006:
  - Use QC data to make adjustments
  - Apply a statistical model identified by Roundtable statisticians
  - Pool 2000-2006 data

Next Steps

- Publish Roundtable Report:
  - Supplement to *Journal of Nutrition*
  - Manuscript due to Journal Jan 31, 2010
  - Co-authored by Roundtable members and Federal Working Group (Planners & Presenters)