2010 Add Health Users Conference

Wave IV Biomarker Data Update

UNC
CAROLINA
POPULATION
CENTER

Wave IV Biomarkers – Overview

- Types + updates
- Measures & Classification + updates
- Collection Sequence
- Collection Methods
- Central Labs
- QA/QC + updates
- Discussion

Wave IV Biomarkers – Types

- Cardiovascular
- Anthropometric
- Metabolic
- Immune
- Inflammatory
- Genetic
- Hematologic
- [Pharmacologic]

Wave IV Biomarkers – Measures

- Cardiovascular
  - Primary
    - systolic blood pressure (SBP)
    - diastolic blood pressure (DBP)
    - pulse rate (PR)
  - Secondary
    - pulse pressure (PP = SBP - DBP)
    - mean arterial pressure (MAP = [SBP + 2 DBP] 3)

Wave IV Biomarkers – Measures

- Cardiovascular
  - Classified according to Joint National Committee VII guidelines*
    - < 120/80 mm Hg — Normal
    - 120-139/80-89 mm Hg — Pre-Hypertension
    - 140-159/90-99 mm Hg — Stage 1 Hypertension
    - ≥ 160/100 mm Hg — Stage 2 Hypertension

*The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure (JNC 7). Hypertension 2003;42:1206.

Wave IV Biomarkers – Measures

- Anthropometric
  - Primary
    - weight
    - height
    - waist circumference (waist)
  - Secondary
    - body mass index (BMI = weight in kg / height in m^2)

Wave IV Biomarkers – Measures

- Anthropometric
  - Classified according to NHLBI Evidence Report*
    - < 18.5 kg/m^2 Underweight
    - 18.5-24.9 kg/m^2 Normal
    - 25.0-25.9 kg/m^2 Overweight
    - 30.0-34.9 kg/m^2 Obesity, Stage I
    - 35.0-39.9 kg/m^2 Obesity, Stage II
    - ≥ 40.0 kg/m^2 Obesity, Stage III

*Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults – the evidence report. Obesity Res 1998;6(S2);51S-210S.

Wave IV Biomarkers – Measures

- Metabolic, lipids*
  - Primary
    - total cholesterol (TC)
    - high density lipoprotein cholesterol (HDL-C)
    - triglycerides (TG)
  - Secondary
    - low density lipoprotein cholesterol (LDL-C = TC - HDL-C - TG   5)†
    - TC:HDL-C ratio (TC:HDL-C = TC / HDL-C)
    - non-HDL-C (= TC - HDL-C)

*Random. †The Friedwald (1972) equation applies when TG < 400.

Wave IV Biomarkers – Measures

- Metabolic, lipids*
  - Classified according to National Cholesterol Education Panel Adult Treatment Panel III guidelines†

• TC (mg/dL)	< 200	desirable
	200-239	borderline high
	≥ 240	high
• HDL-C (mg/dL)	< 40	low
	≥ 60	high
• LDL-C (mg/dL)	< 100	optimal
	100-129	near optimal
	130-159	borderline high
	160-189	high
	≥ 190	very high
• TG‡ (mg/dL)	< 150	normal
	150-199	borderline high
	200-499	high
	≥ 500	very high

*Random. †Third Report of the National Cholesterol Education Program (NCEP) on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). ‡Spuriously high in the non-fasting state.

Wave IV Biomarkers – Measures

- Metabolic, glucose homeostasis*
  - Primary
    - glucose
    - glycosylated hemoglobin (HbA1c)
  - Secondary
    - mean plasma glucose (MPG = 35.6 HbA1c – 77.3)†
    - estimated average glucose (EAG = 28.7 HbA1c – 46.7)‡

*Random. †Rohlfing et al. Diabetes Care 2002;25(2):275-278. ‡Nathan et al. Diabetes Care 2008;31(8):1-6.

Wave IV Biomarkers – Measures

- Metabolic, glucose homeostasis*
  - Classified according to American Diabetes Association and Endocrine Society guidelines†
    - fasting glucose

≥ 126 mg/dL	diabetes
100-125 mg/dL	impaired
≤ 99 mg/dL	normal

    - random glucose

≥ 200 mg/dL	diabetes

    - HbA1c

≥ 6.5%	diabetes
5.7-6.4%	increased risk for diabetes

*Random. †American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diab Care 2010;33(S1):S62-S69. http://www.endo-society.org/advocacy/upload/TES-Statement-on-A1C-Use.pdf

Wave IV Biomarkers – Measures

- Immune / Inflammatory
  - Epstein-Barr virus antibody titer (EBV)
  - high sensitivity C-reactive protein (hsCRP)
    - classified according to Centers for Disease Control / American Heart Association guidelines*
      - low < 1 mg/L
      - average 1-3 mg/L
      - high > 3 mg/L

*Pearson et al. Markers of inflammation and cardiovascular disease. Application to clinical and public health practice. A statement for healthcare professionals from the CDC and AHA. Circualtion 2003;107:499-511.

Wave IV Biomarkers – Measures

- Hematologic
  - Hemoglobin (Hb)
    - gender-specific classification

	Females	Males
– low	< 12.0 g/dL	13.5 g/dL
– average	12.0-16.0 g/dL	13.5-17.5 g/dL
– high	> 16.0 g/dL	17.5 g/dL

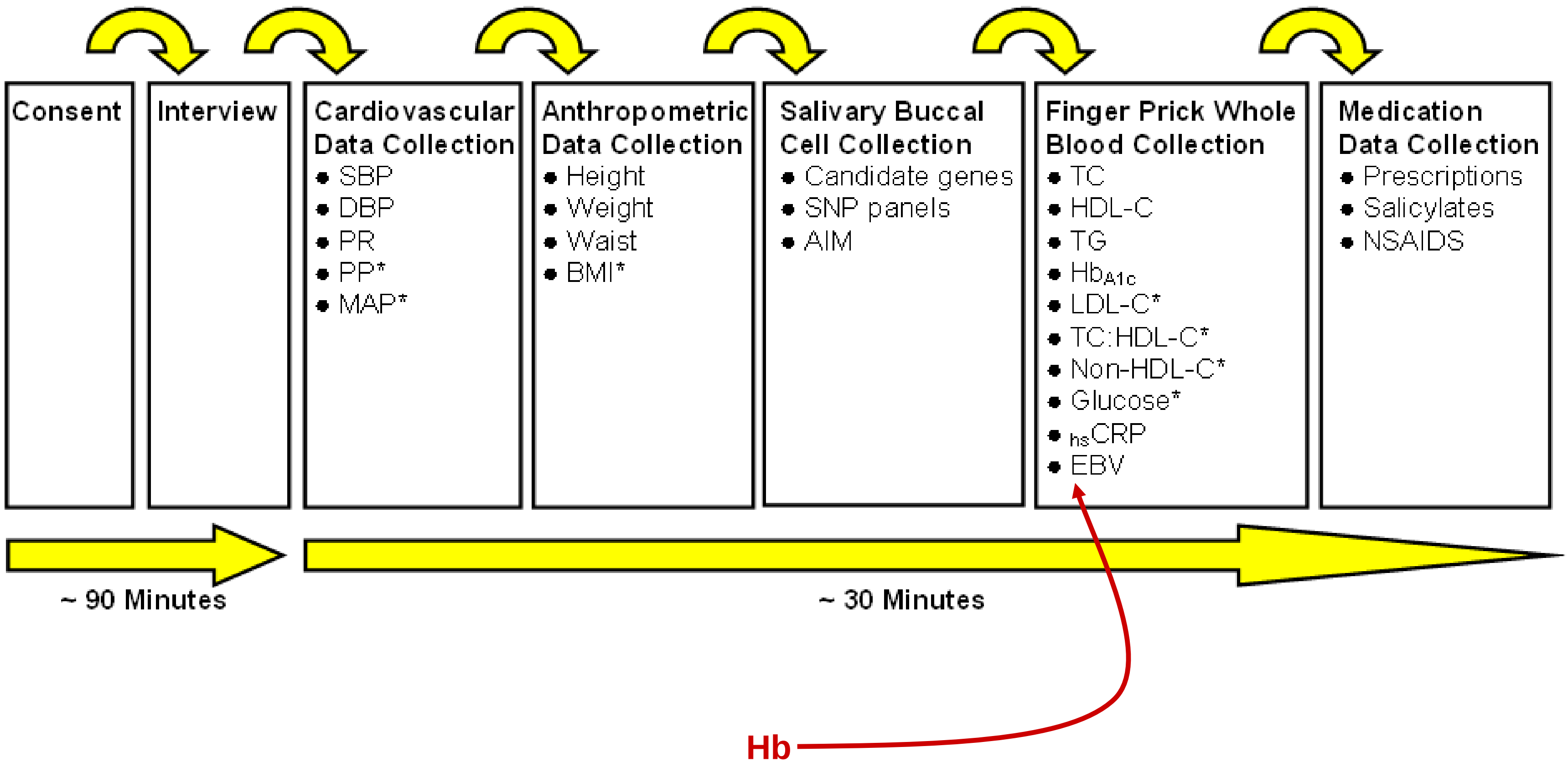
    - above, if conversion of OD → g/dL possible

Wave IV Biomarkers – Measures

- Genetic
  - candidate gene loci & SNP panels } focusing on DA, 5-HT & NA neurotransmission systems with known roles in behavior and health
  - ancestry informative markers (AIM)
  - 10^6 SNPs
- Pharmacologic
  - prescription medications
  - select over-the-counter medications (salicylates & NSAIDS)
  - classification according to Multum therapeutic category
    - examples

lisinopril ACEI	metoprolol BB
nifedipine CCB	hctz DIURETIC
clonidine CASM	
hydralazine VASODILATOR	

Wave IV Biomarkers – Collection Sequence



*Secondary

Wave IV Biomarkers – Collection Methods

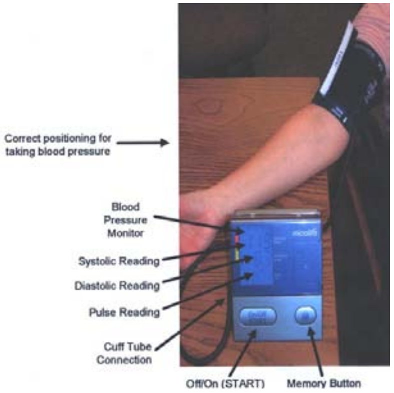
- Cardiovascular Data Collection Equipment

Measures	Equipment	Picture	Specifications
SBP	MicroLife		30-280/40-200 BP/PR range
DBP	3MC1-PC_IB		1-unit graduations
PR	Oscillometric		198 measure recall
PP*	BP		3 mmHg accuracy
MAP*	Monitor		5 beat pulse accuracy
			BHS-approved
			4 "AA" battery-powered
			w/ AC adapter + USB cable
			2 cuffs (24-41 cm)
			160 x 140 x 98 mm
			735 g (w/ batteries)
			< $65

*Secondary

Wave IV Biomarkers – Collection Methods

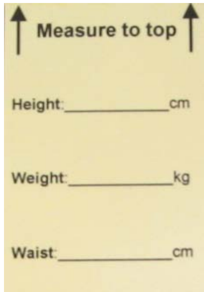
- Cardiovascular Data Collection Protocol
  - trained & certified staff
  - resting & seated respondents
  - cuff matched to arm circumference
  - SBP, DBP & PR
    - measure 3X
    - @ 30-sec intervals
    - double enter
    - automatically average over last 2



The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure (JNC 7). Hypertension 2003;42:1206.

Wave IV Biomarkers – Collection Methods

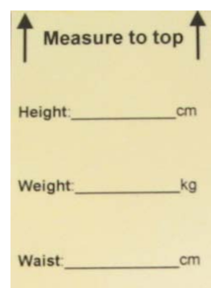
- Anthropometric Data Collection Equipment

Measure	Equipment	Pictures	Specifications
Height	**Carpenter's Square**		**portable** **light weight** **inexpensive** **true 90 angle**
	Tape Measure		**10 ft** **steel tape**
	Post-it Notes		**adherent** **pre-labeled**

Wave IV Biomarkers – Collection Methods

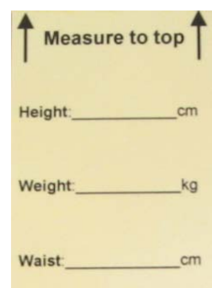
- Anthropometric Data Collection Equipment

Measure	Equipment	Pictures	Specifications
Weight	**Health-O-Meter 844KL High Capacity Digital Scale**	see below	**4-point load cell digital display in lb / kg 440 lb maximum 0.1 lb graduations long life Li^{++} battery low battery warning 1-year warranty 12.6 x 12.6 in 4.5 lb < $70**



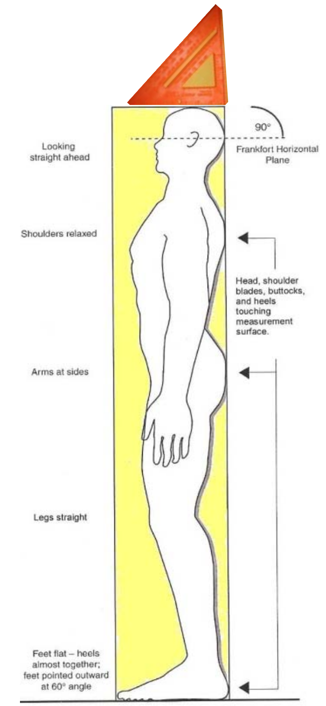
Wave IV Biomarkers – Collection Methods

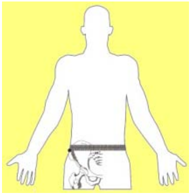
- Anthropometric Data Collection Equipment

Measure	Equipment	Pictures	Specifications
Waist	Seca 200 Circumference Tape Measure	 	200 cm maximum 2-sided cm scaling 1 mm graduations fiberglass tape plastic case automatic roll-up end-peg positioner 90 x 25 x 65 mm 50 g < $13

Wave IV Biomarkers – Collection Methods

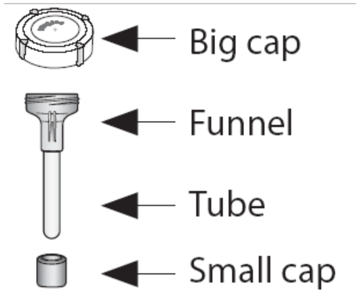
- Anthropometric Data Collection Protocol
  - trained & certified staff
  - dressed & unshoed respondents
  - standing on uncarpeted floor
  - measure
    - height to nearest 0.5 cm
    - weight to nearest 0.1 kg
    - waist to nearest 0.5 cm
      - @ superior border of iliac crest
      - @ end expiration
      - horizontal to floor
    - hair/shoe height to nearest 0.5 cm, as needed





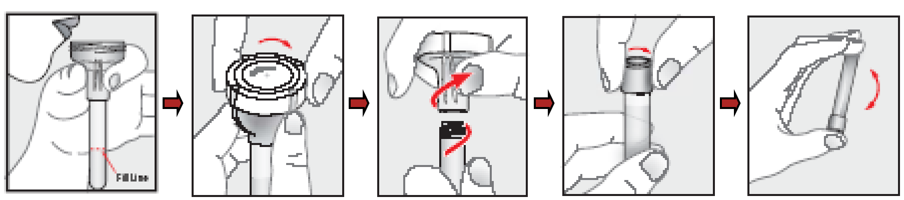
Wave IV Biomarkers – Collection Methods

- Salivary Buccal Cell DNA Collection Equipment

Measures	Equipment	Schematic	Specifications
Genes SNP panels AIM	DNAGenotek Oragene•DNA Collection Kit		collection funnel preservative-containing cap tube for storage small cap for shipping

Wave IV Biomarkers – Collection Methods

- Salivary Buccal Cell DNA Collection Protocol
  - trained & certified staff
  - respondents spit into funnel
  - remove funnel
  - turn big cap clockwise to add preservative
  - turn big cap counter-clockwise to remove it
  - replace big cap with small cap
  - invert 5X to mix saliva and preservative
  - package & FedEx to lab



Wave IV Biomarkers – Central Labs

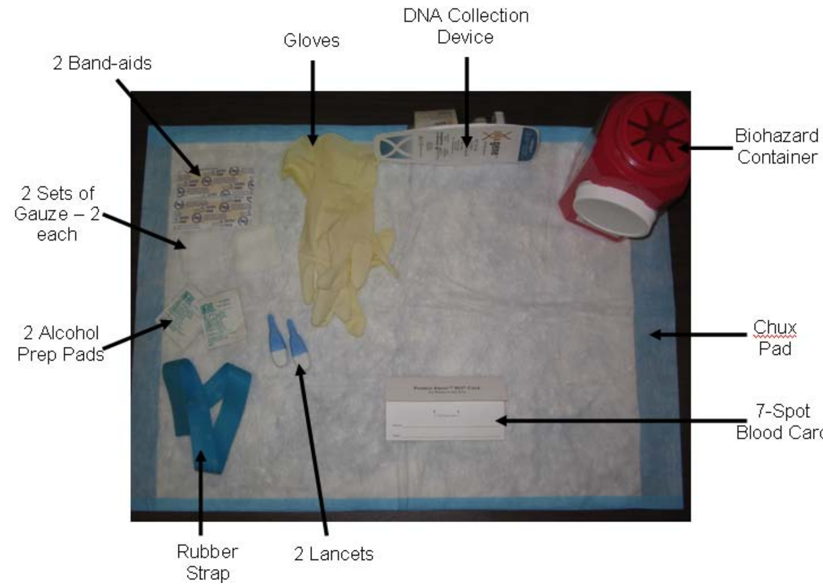
- Salivary Buccal Cell DNA
  - **Andy Smolen, Ph.D. (PI)**
    Director, Genotyping Laboratory
    Institute for Behavioral Genetics
    University of Colorado (Boulder, CO)

Wave IV Biomarkers – Collection Methods

- Dried Whole Blood Spot Collection Equipment

Measures	Equipment
TC	Gloves
HDL-C	Band-aids
TG	Gauze
Hb_{A1c}	Alcohol prep pads
LDL-C*	Tourniquet
TC:HDL-C*	Lancets
Non-HDL-C*	7-spot cards
Glucose*	Chux pad
$_{hs}$CRP	Biohazard container
EBV	
Hb	

Workstation



*Secondary

Wave IV Biomarkers – Collection Methods

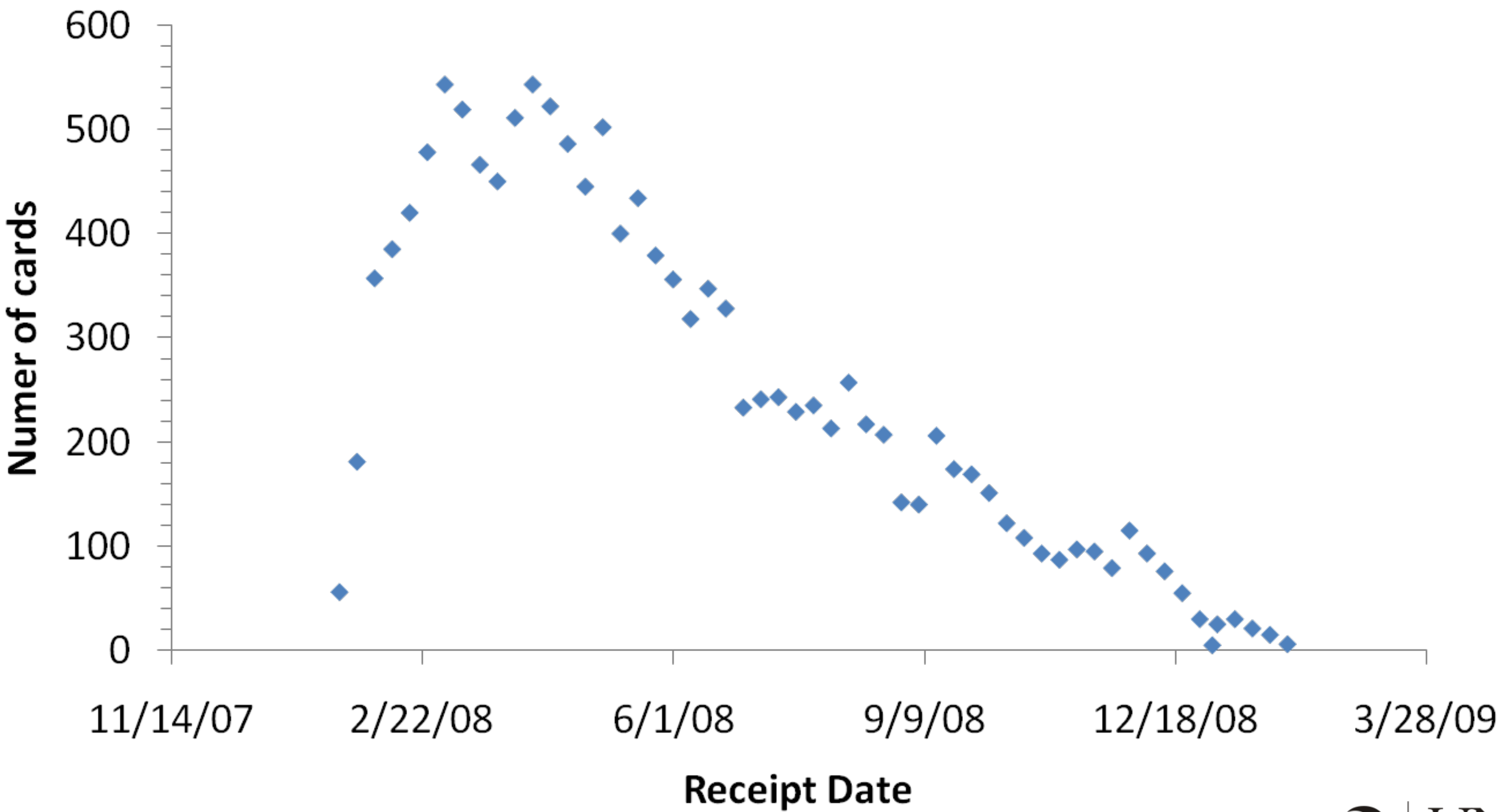
- Dried Whole Blood Spot Collection Protocol
  - trained & certified staff
  - non-fasting respondents
  - clean middle or ring finger w/ alcohol prep pad
  - apply tourniquet to arm
  - prick finger & firmly wipe away 1st drop
  - drop up to 7 blood spots onto card
  - repeat prick X1, if necessary
  - air dry over desiccant
  - package & FedEx to lab

Wave IV Biomarkers – Central Labs

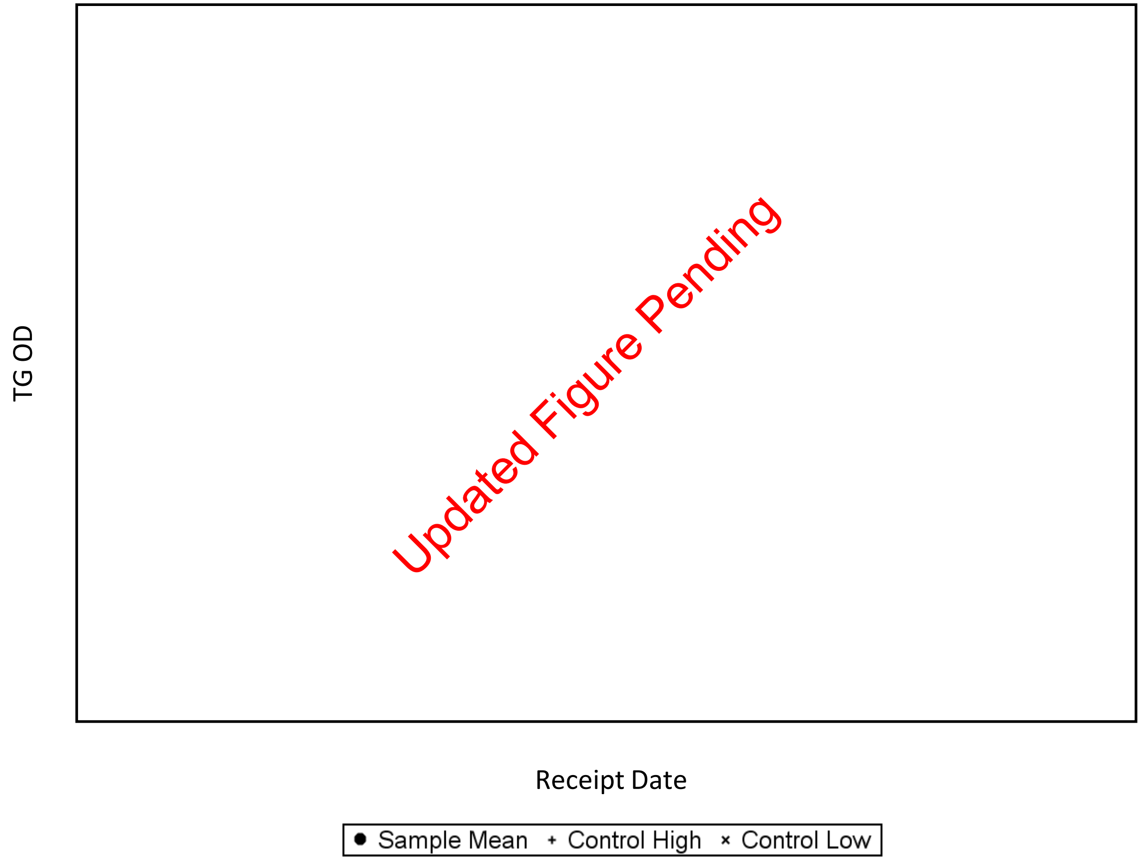
- Dried Whole Blood Spots
  - **Mark H. Wener, M.D. (PI)**

    Director, UW Medical Center Laboratories
    Department of Laboratory Medicine
    University of Washington (Seattle, WA)
  - **Robert Ray, Ph.D.**
    Director, FlexSite Diagnostics, Inc. (Palm City, FL)

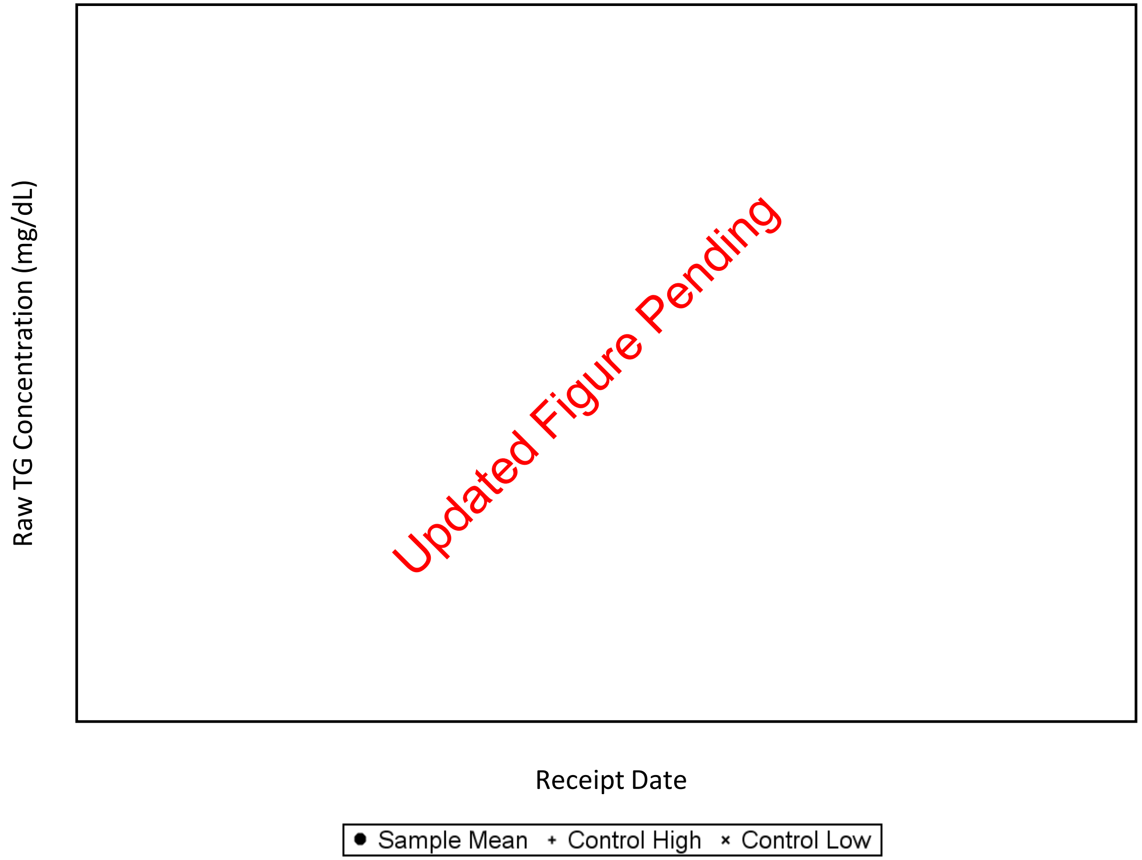
Cards Received Per Week



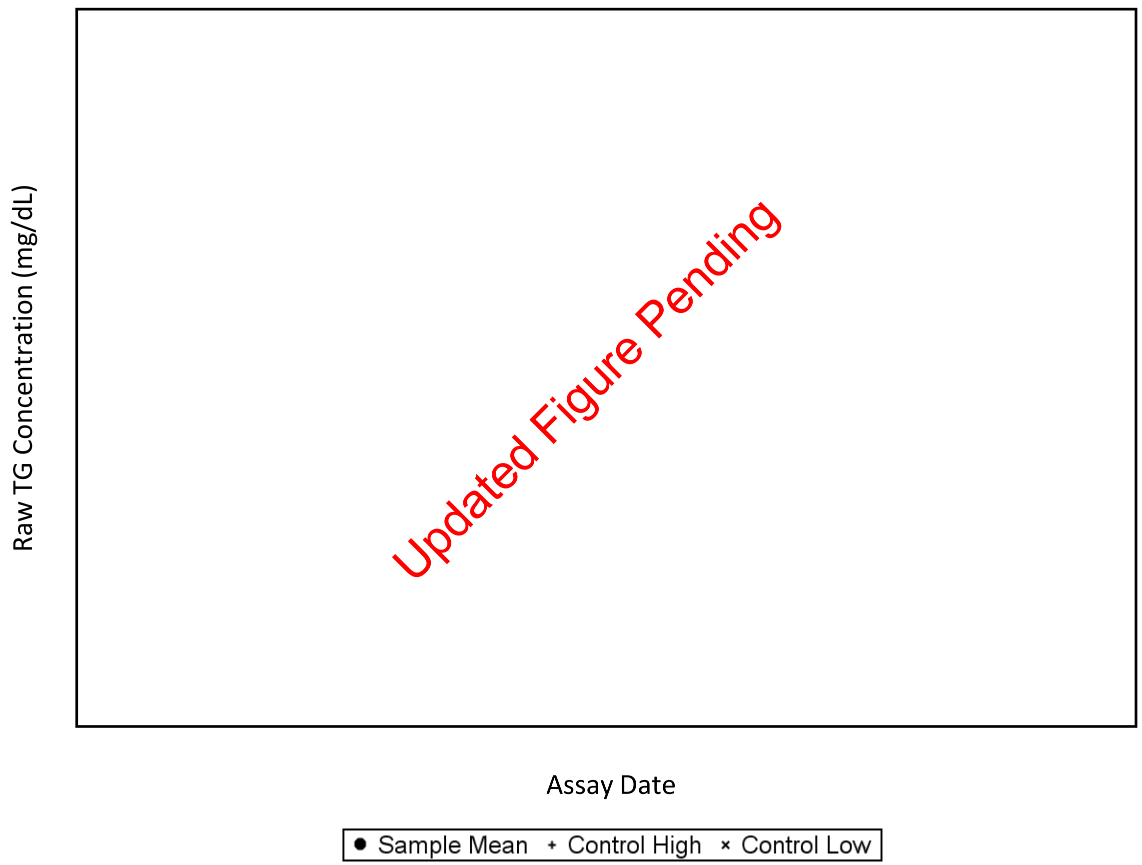
Weekly Mean TG Optical Density



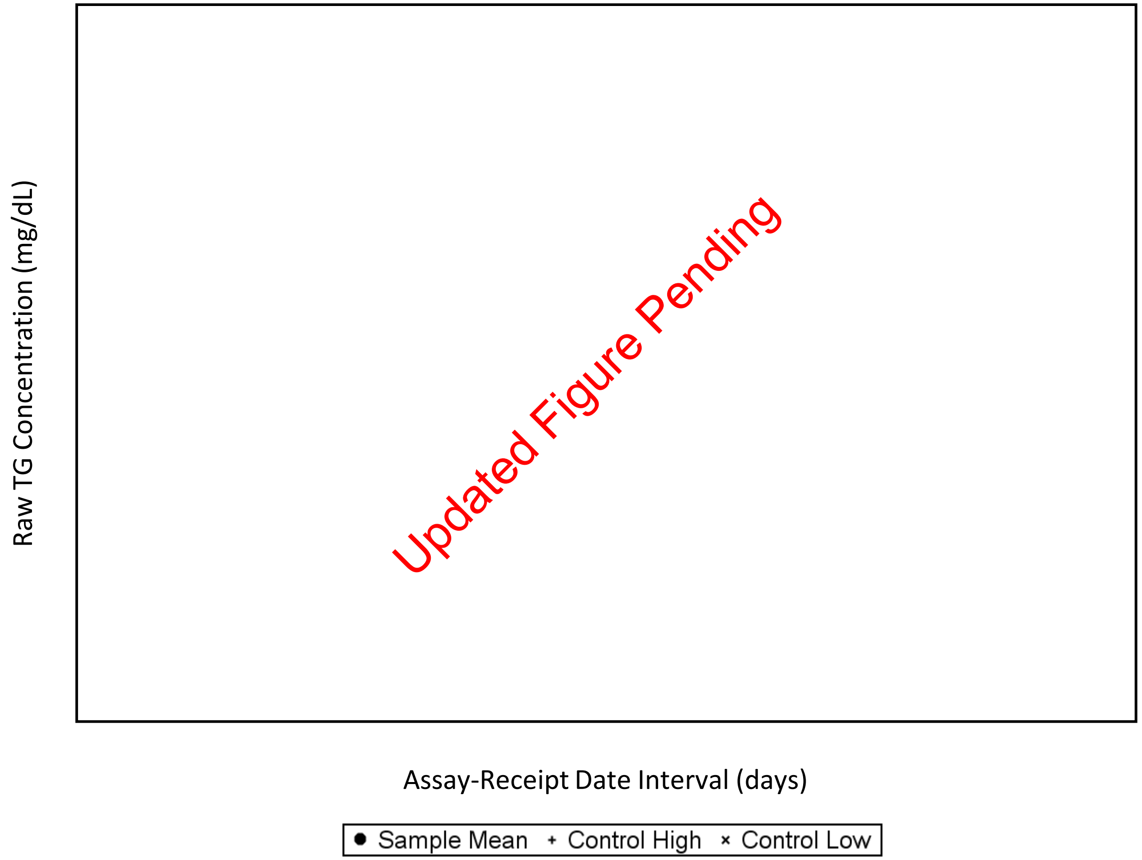
Weekly Mean Raw TG Concentration



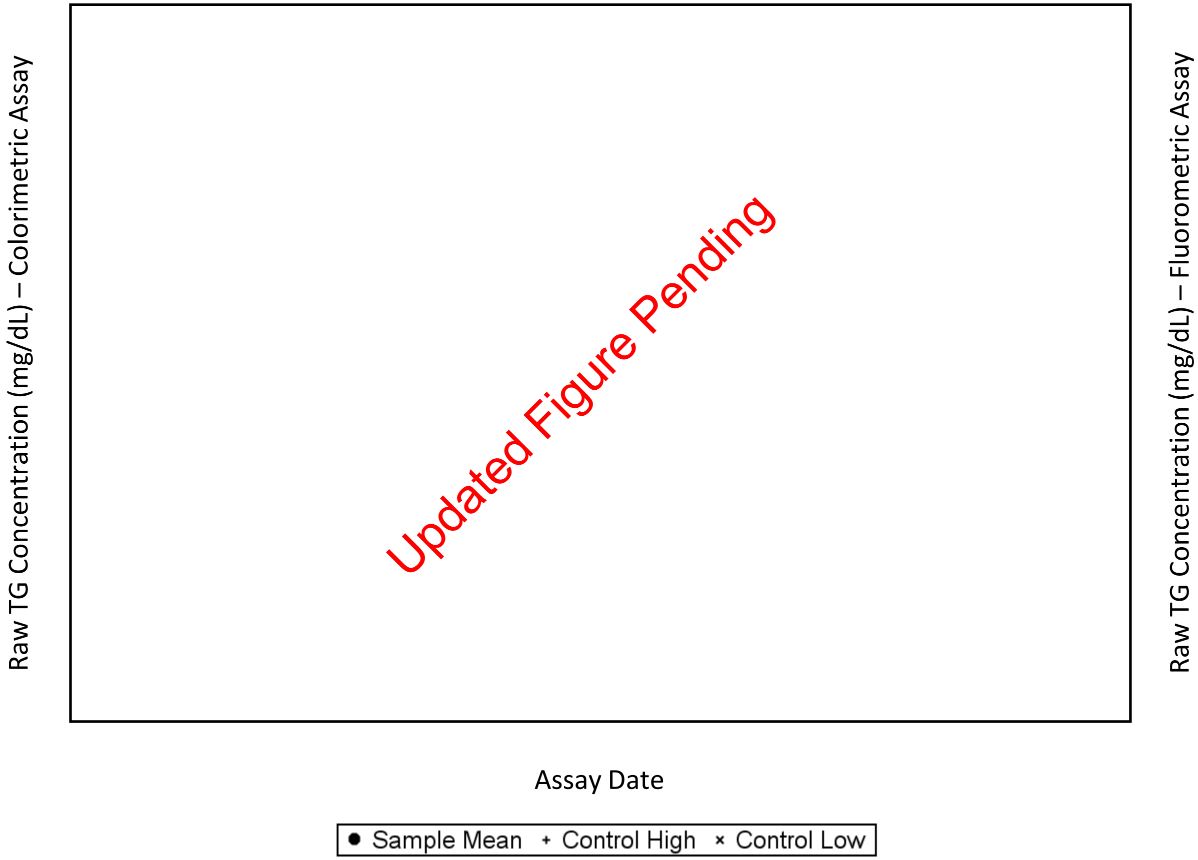
Weekly Mean Raw TG Concentration



Weekly Mean Raw TG Concentration



Weekly Mean Raw TG Concentration



Wave IV Biomarkers – QA/QC

- Issues
  - Conversion of Concentrations
    - OD → Raw → Plasma Equivalent
    - Colorimetric → Fluorometric
  - Trends in Concentrations Over
    - Receipt Dates
    - Assay Dates
    - Assay-Receipt Date Intervals
- Other Issues
  - Assay validity (colorimetric vs. criterion standard)
  - Intra-individual variation (visit 1 vs. 2)
  - Intra-sample variation (split sample 1 vs. 2)

Wave IV Biomarkers – QA/QC

- Routine components
    - automated range checks
    - duplicate entry requirements
    - post-encounter verification interviews
- External validation studies
    - to assess digit preference
    - to assess accuracy
        - cardiovascular: BP monitor calibration
        - anthropometric: weight scale calibration
        - metabolic: lipid calibration
- Inter-assay variation study
    - to inform Colorimetric → Fluorometric conversion
- Intra-individual variation study
    - to assess short-term reliability

Digit Preference

DIGIT PREFERENCE IN ANTHROPOMETRIC VARIABLES
ADD HEALTH WAVE IV, MAIN STUDY (2008-2009)

Variable	k	n	ChiSq	p	DPS
WEIGHT (kg)	10	15204	608.4	0.000	6.7
HEIGHT (cm)	10	15373	63.4	0.000	2.1
WAIST (cm)	10	15357	26.3	0.002	1.4

Sorted from high to low DPS. DPS = Hense (1991) digit preference score = 100*(Chisq/(n*(k-1)))**0.5. Range = 0-100. Chisq = goodness of fit test stat, where ni = observed cell freq & sum(ni/k) = expected cell freq in cell i. k = number of possible terminal digits, 0-9 by 1 for weight. k = number of possible penultimate digits, 0-9 by 1 for height and waist. p = p value.

Digit Preference

Distribution of terminal digits,
Add Health Wave IV, Main Study (2008-2009)

Weight	n	%
x.0	2369	(15.6)
x.1	1338	(8.8)
x.2	1426	(9.4)
x.3	1358	(8.9)
x.4	1331	(8.8)
x.5	1717	(11.3)
x.6	1482	(9.8)
x.7	1366	(9.0)
x.8	1484	(9.8)
x.9	1333	(8.8)

Digit Preference

DIGIT PREFERENCE IN CARDIOVASCULAR VARIABLES
ADD HEALTH WAVE IV, MAIN STUDY (2008-2009)

Variable	k	n	ChiSq	p	DPS
SBP2 (mm Hg)	10	15298	19.3	0.023	1.2
SBP1 (mm Hg)	10	15347	12.5	0.187	1.0
SBP3 (mm Hg)	10	15222	11.7	0.230	0.9
DBP1 (mm Hg)	10	15347	67.9	0.000	2.2
DBP3 (mm Hg)	10	15222	61.8	0.000	2.1
DBP2 (mm Hg)	10	15298	45.0	0.000	1.8
PR1 (beat/min)	10	15262	18.7	0.028	1.2
PR3 (beat/min)	10	15137	10.4	0.319	0.9
PR2 (beat/min)	10	15210	10.0	0.349	0.9

Sorted from high to low DPS. DPS = Hense (1991) digit preference score = 100*(Chisq/(n*(k-1)))**0.5. Range = 0-100. Chisq = goodness of fit test stat, where ni = observed cell freq & sum(ni/k) = expected cell freq in cell i. k = number of possible terminal digits, 0-9 by 1 for weight. p = p value.

Digit Preference

Terminal Digit Preference of Blood Pressure, Add Health Wave IV

	SBP Measure			DBP Measure		
Terminal Digit	1	2	3	1	2	3
	n (%)[b]	n (%)[b]	n (%)[b]	n (%)[b]	n (%)[b]	n (%)[b]
0	1606 (10.5)	1630 (10.7)	1626 (10.7)	1591 (10.4)	1637 (10.7)	1599 (10.5)
1	1545 (10.1)	1517 (9.9)	1519 (10.0)	1445 (9.4)	1442 (9.4)	1411 (9.3)
2	1560 (10.2)	1500 (9.8)	1492 (9.8)	1667 (10.9)	1685 (11.0)	1700 (11.2)
3	1590 (10.4)	1554 (10.2)	1462 (9.6)	1753 (11.4)	1628 (10.6)	1682 (11.1)
4	1538 (10.0)	1598 (10.5)	1560 (10.3)	1558 (10.2)	1468 (9.6)	1490 (9.8)
5	1491 (9.7)	1453 (9.5)	1502 (9.9)	1476 (9.6)	1467 (9.6)	1476 (9.7)
6	1509 (9.8)	1533 (10.0)	1525 (10.0)	1419 (9.3)	1529 (10.0)	1508 (9.9)
7	1500 (9.8)	1445 (9.5)	1527 (10.0)	1506 (9.8)	1478 (9.7)	1445 (9.5)
8	1552 (10.1)	1533 (10.0)	1507 (9.9)	1476 (9.6)	1503 (9.8)	1442 (9.5)
9	1456 (9.5)	1535 (10.0)	1502 (9.9)	1456 (9.5)	1461 (9.6)	1469 (9.7)
Pearson χ^2	12.49	19.26	11.71	67.90	44.99	61.76
P value	0.187	0.023	0.230	<0.001	<0.001	<.0001
DPS[a]	0.95	1.2	0.9	2.2	1.8	2.1

[a]DPS = digit preference score (see methods). [b]Unweighted sample size and percent.

Digit Preference

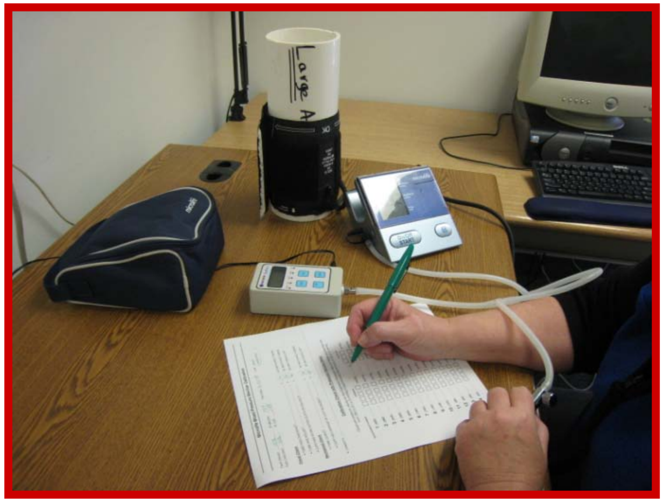
FI 122		
SBP3	n	%
95	1	(1)
99	1	(1)
100	2	(2)
101	1	(1)
105	1	(1)
108	3	(3)
110	9	(8)
112	4	(4)
113	1	(1)
114	2	(2)
115	3	(3)
116	5	(5)
118	15	(14)
119	2	(2)
120	36	(32)
122	5	(5)
124	6	(5)
128	6	(5)
130	5	(5)
132	1	(1)
134	2	(2)

FI 122		
DBP3	n	%
60	5	(5)
61	1	(1)
62	4	(4)
64	2	(2)
65	4	(4)
67	2	(2)
68	2	(2)
69	6	(5)
70	32	(29)
72	9	(8)
74	5	(5)
75	11	(10)
76	2	(2)
78	4	(4)
79	1	(1)
80	16	(14)
82	2	(2)
84	2	(2)
85	1	(1)

Digit Preference

- Summary
  - affects relatively few FIs & participants
  - yet some FI / participant data
    - consistently affected
    - severely affected
  - highly suspect FI data has been deleted & flagged

BP Monitor Calibration



BP Monitor Calibration

- Standard protocol (Dec 2008 – Jul 2009)
  - involved two technicians
  - inspected 292 monitor/cuff pairs returned from the field
    - damage
    - missing parts
    - electronic malfunction
  - attached large adult cuff to 37 cm rigid cylinder
  - connected in tandem with
    - BP monitor (Microlife 3MC1-PC_IB)
    - Pressure meter (Netech DigiMano, Model 2000)
  - recorded pressures over 280-40 mm Hg in 20-unit dec
  - attached adult cuff to 28.5 cm rigid cylinder
  - repeated pressure recordings

BP Monitor Calibration

Microlife Blood Pressure Monitor Calibration

Tech ID: ______ FI Cuff: _______ FI Monitor: _______ Test Date: _______ Cuff: ADULT or LARGE ADULT

Visual Check

◆ TUBE HAS CRACKING?	Y	N	NO MATCHING TUBING
◆ TUBE HAS HOLES?	Y	N	NO MATCHING TUBING
◆ CUFF HAS WORN OUTER CLOTH OR VELCRO?	Y	N	NO MATCHING CUFF
◆ TUBE LEAKS?	Y	N	NO MATCHING TUBING
◆ CUFF HAS LEAKAGE OF CUFF BLADDER?	Y	N	NO MATCHING CUFF

◆ *COMMENTS:* ____

Calibration Check with Pressure-Vacuum Meter

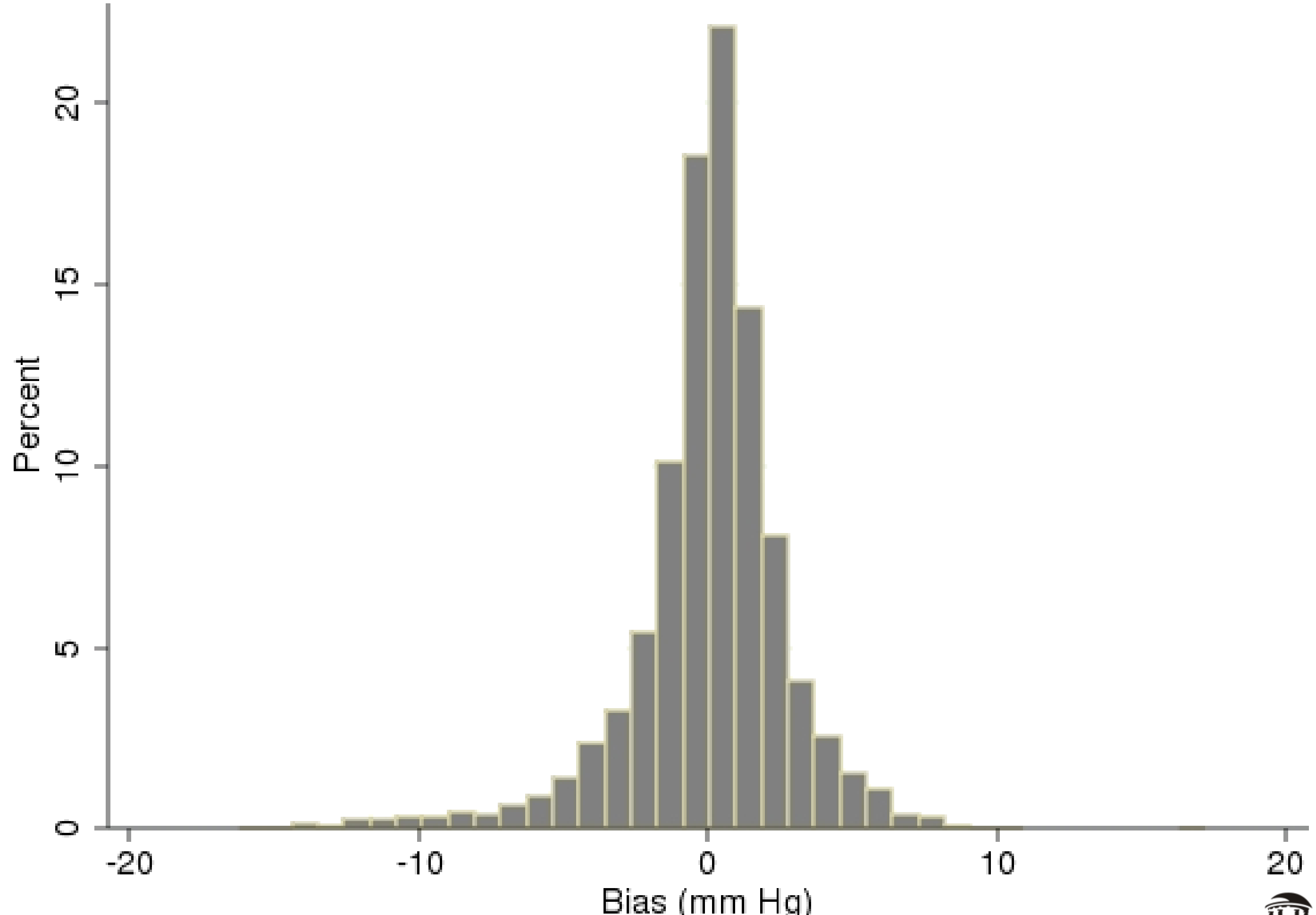
Observed pressure values on the Digimano Pressure-Vacuum Meter and the Microlife from 280 to 40 (± 2) mmHg in approximate decrements of 20 (± 2) mmHg.

MEASUREMENT NUMBER	DIGIMANO	MICROLIFE
1 (280)	☐☐☐.☐ mmHg	☐☐☐ mmHg
2 (260)	☐☐☐.☐ mmHg	☐☐☐ mmHg
3 (240)	☐☐☐.☐ mmHg	☐☐☐ mmHg
4 (220)	☐☐☐.☐ mmHg	☐☐☐ mmHg
5 (200)	☐☐☐.☐ mmHg	☐☐☐ mmHg
6 (180)	☐☐☐.☐ mmHg	☐☐☐ mmHg
7 (160)	☐☐☐.☐ mmHg	☐☐☐ mmHg
8 (140)	☐☐☐.☐ mmHg	☐☐☐ mmHg
9 (120)	☐☐☐.☐ mmHg	☐☐☐ mmHg
10 (100)	☐☐☐.☐ mmHg	☐☐☐ mmHg
11 (80)	☐☐☐.☐ mmHg	☐☐☐ mmHg
12 (60)	☐☐☐.☐ mmHg	☐☐☐ mmHg
13 (40)	☐☐☐.☐ mmHg	☐☐☐ mmHg

BP Monitor Calibration

- Analysis
  - bias (mm Hg) = meter - monitor difference
  - relative bias (%) = 100 x bias / meter pressure

BP Monitor Calibration



Accuracy of Monitor Pressure, by Meter Pressure (mm Hg), Add Health Wave IV (2008-2009)

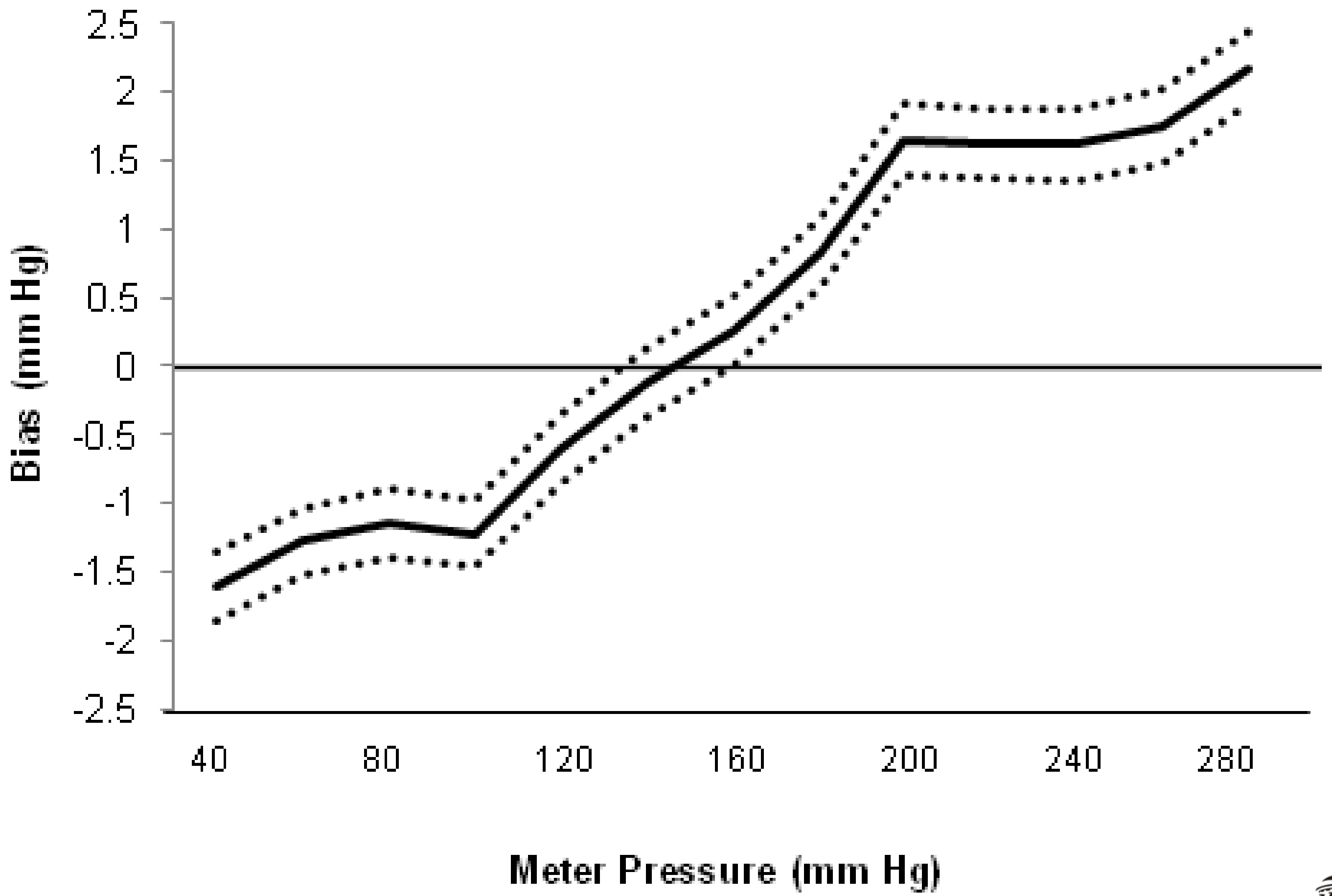
	Bias (mm Hg)[a]			Relative Bias (%)[b]		
Meter Pressure (mm Hg)	Median	Mean	SD	Median	Mean	SD
280	1.70	1.79	1.78	-0.60%	0.64%	0.64%
260	1.30	1.34	1.89	0.50%	0.51%	0.73%
240	1.10	1.20	1.89	0.46%	0.50%	0.79%
220	1.10	1.26	2.15	0.50%	0.57%	0.98%
200	1.10	1.38	2.16	0.55%	0.69%	1.08%
180	0.70	0.57	2.66	0.39%	0.31%	1.48%
160	0.30	0.06	2.69	0.19%	0.03%	1.68%
140	0.00	-0.28	2.65	0.00%	-0.20%	1.89%
120	-0.40	-0.71	2.59	-0.33%	-0.60%	2.16%
100	-0.60	-1.30	3.27	-0.59%	-1.30%	3.27%
80	-0.75	-1.23	2.74	-0.95%	-1.53%	3.42%
60	-0.70	-1.30	2.73	-1.16%	-2.17%	4.53%
40	-0.90	-1.55	2.53	-2.24%	-3.86%	6.28%

[a]Bias = meter pressure - monitor pressure. [b]Relative bias = 100 × (bias ÷ meter pressure). SD = standard deviation.

BP Monitor Calibration

- Model: multi-level, random-intercept
  - $Y_{ijk} = \beta_0 + \beta_1 P_{ijk} + \beta_2 C_{ijk} + \gamma_{1k} + \gamma_{2j(k)} + \varepsilon_{ijk}$
  - where
    - *i* = i^{th} cuff pressure (level 1)
    - *j* = j^{th} cuff (level 2)
    - *k* = k^{th} monitor (level 3)
  - and
    - Y_{ijk} = bias (mm Hg)
    - β_0 = intercept
    - P_{ijk} = vector of meter pressure categories (1-13)
    - C_{ijk} = vector of covariates, e.g. technician ID
    - $\gamma_{1k} + \gamma_{2j(k)}$ = random intercepts @ levels 3 & 2
    - ε_{ijk} = random error @ level 1

BP Monitor Calibration



BP Monitor Calibration

- Summary
  - bias: < 2 mm Hg
  - relative bias: < 4%
  - both: > 0 @ BP < 140
    @ BP > 140 mm Hg

Lipid Calibration

- Standard Protocol (Apr 2008 - Jul 2008)
  - procured 3 CDC LSP plasma pools, together representing
    - low
    - medium
    - high

    } TC, HDL-C & TG concentrations
  - mixed each pool with washed RBCs
  - spotted 20 cards with each plasma-RBC mixture
    - pool 1
    - pool 2
    - pool 3

    } x 20 cards = 60 cards
  - sent 1 card/pool to the lab, 2x/wk x 10 wk
  - masked lab & technicians to origin of card
  - processed the spots per Add Health protocol

Lipid Calibration

- Analysis
  - lab | CDC correlations (Pearson; Spearman)
  - bias (mg/dL) = lab - CDC difference
  - relative bias (%) = 100 x bias / CDC
  - coefficient of variation = sd (lab) / mean (lab)

Lipid Calibration

Correlation of lipid concentrations, lab vs. CDC

Variable		Pearson*	Spearman*
Primary	TC		
	HDL-C		
	TG		
Secondary	LDL-C		
	NON-HDL-C		

Updated table pending

LDL-C = TC - HDL-C - TG ÷ 5 when TG < 400 (Friedwald). NON-HDL-C = TC - HDL-C. *P values < 0.0001 for all tests of H_0: r = 0.0.

Lipid Calibration

Difference in lipid concentrations (mg/dL), lab vs. CDC

Variable		Mean (SD) Lab	Mean (SD) CDC	Bias	Relative Bias	CV	p
Primary	TC						
	HDL-C						
	TG						
Secondary	LDL-C						
	NON-HDL-C						

Updated table pending
Updated table pending
Updated table pending

LDL-C = TC - HDL-C - TG ÷ 5 when TG < 400 (Friedwald). Bias = lab - CDC. Relative bias = (100 * bias) / CDC. NON-HDL-C = TC - HDL-C. P = p value for student's t test of H_0: Bias = 0.

Lipid Calibration

- Summary
  - pending completion of trend & conversion analyses

Inter-Assay Variation Study

- Standard Protocol
  - identified a race/ethnicity-, gender- & TG-stratified random sample of 96 Main Study participants
    - four race/ethnic groups: NHW, NHB, Hispanic & other
    - three TG concentrations: low, medium & high
    - two genders: male & female
    - 4 x 3 x 2 = 24 groups
    - 4 participants / group
  - resubmitted calorimetrically assayed cards for fluorometric assay
  - masked labs & technicians to participant identity
  - processed the lipids

Inter-Assay Variation Study

- Summary
  - pending completion of trend analysis

Intra-Individual Variation Study

- Standard Protocol (2007 - 2009)
  - computed n needed to estimate the reliability assuming
    - measures are interval-scale
    - reliability estimated as an ICC
    - underlying ICC = 0.7 & precise, 95%CI = 0.2
  - identified a race/ethnicity- & gender-stratified random sample
    - ~ 50 participants in Pre-Test & ~ 50 in Main Study
  - excluded siblings and pregnant women
  - examined participants 2x, 1-2 wk apart
    - visit 1: full interview + biomarkers
    - visit 2: abbreviated interview + biomarkers
  - masked labs & technicians to respondent identity
  - processed the biomarkers

Intra-Individual Variation Study

Characteristics of the IIV population*

Characteristic		Mean (sd) or n (%) Visit 1	Visit 2
Age (y)		28.5 (1.9)	
Female		50 (50%)	
Race/Ethnicity	Non-Hispanic White	64 (64%)	
	Non-Hispanic Black	16 (16%)	
	Hispanic	12 (12%)	
	Other	8 (8%)	
Pre-Test Participant		42 (42%)	
Time of Day		x:xx pm	x:xx pm
Fasting duration (hr)		x.x (x.x)	x.x (x.x)
Fasting ≥ 8 hr		xx (xx%)	
V1-V2 Interval (d)		8.6 (3.0)	
Same Field Interviewer Both Visits		84 (84%)	

*Based on a final sample of 100 IIV participants.

Intra-Individual Variation Study

- Analysis
  - Nested, random-effects model partitioning biomarker variance into its components:
  - $Y_{ijk} = \mu + P_i + V_j(P_i) + \varepsilon_{ijk}$
  - where:
    - Y_{ijk} = value @ j[th] visit of i[th] participant
    - μ = intercept
    - P_i = i[th] participant
    - $V_j(P_i)$ = j[th] visit nested within i[th] participant
    - ε_{ijk} = random error
  - assuming:
    - $P_i, V_j(P_i)$ and ε_{ijk} are independent and ~ $N(0, \sigma^2)$
  - under this assumption:
    - $\sigma_T^2 = \sigma_{BP}^2 + \sigma_{BV}^2 + \sigma_{WV}^2$ and ICC = $\sigma_{BP}^2 / \sigma_T^2$

Intra-Individual Variation Study

- Summary of reliability
  - anthropometric measures
    - excellent, near unity
  - cardiovascular measures
    - SBP / DBP acceptable
    - PR marginal
  - other measures
    - pending completion of trend & conversion analyses

To Learn More

- Resources
  - General
    - http://www.cpc.unc.edu/projects/addhealth
  - User Guides
    - http://www.cpc.unc.edu/projects/addhealth/data/guides
      - cardiovascular & anthropometric measures
      - BP monitor calibration form & protocol
      - FI verification script
  - Wave IV Data
    - http://www.icpsr.umich.edu/icpsrweb/DSDR/access/add-health.jsp

Wave IV Biomarkers – Discussion