Validation and Quality Control for Dried Blood Spot Based Assays: Learning Experience in Three Asian Countries

Perry Hu, MD, PhD

UCLA Division of Geriatric Medicine

Three Health and Retirement Study-Type Surveys in Asia

- **The China Health and Retirement Longitudinal Study (CHARLS)**
  - **Bioassays for its pilot study was conducted in 2010 and 2011**
- **The Indonesia Family Life Survey (IFLS)**
  - **Bioassay for Wave 4 was conducted in 2012**
- **The Longitudinal Aging Study in India (LASI)**
  - **Bioassays for its pilot study was conducted in 2012 and 2013**

Dried Blood Spot (DBS)-Based Assays by Studies

- CHARLS Pilot Study
  - C-reactive protein (CRP)
  - Hemoglobin (Hb)
- IFLS, wave 4
  - CRP
    - Hb was measured using HemoCue
- LASI Pilot Study
  - CRP
  - Hb
  - Epstein-Barr virus (EBV) antibody titer
  - Glycosylated hemoglobin (Hba1c)

Main Steps for Establishing New DBS-Based Assays

- Training of laboratory personnel

- Securing equipment for the assays

- Obtaining test reagents and supplies

- **Assay validation: including pre-test with validation samples**

- **Ongoing quality control during testing of study samples**

Training of Laboratory Personnel

- CHARLS Pilot Study

  - Collaboration with School of Public Health, Beijing University

  - Training at Dr. Thomas McDade's Laboratory at Northwestern University

  - Training on CRP and Hb assays only

  - Duration of training: 5 days

Training of Laboratory Personnel

- IFLS, Wave 4
  - Collaboration with School of Medicine, Gadjah Mada University, Yogyakarta, Indonesia
  - Training of laboratory personnel at the National AIDS Research Institute (NARI), Pune, India
  - Part of training for the World Health Organization's Study on Global Aging and Adult Health (SAGE) project, conducted by Dr. Sharon Williams from Purdue University
  - Training on CRP, Hb, Hba1c, and EBV antibody assays
  - Duration of training: 5 days

Training of Laboratory Personnel

- LASI Pilot Study

  – Collaboration with the National AIDS Research Institute (NARI), Pune, India

  – Training of laboratory personnel, as part of the WHO's Study on Global Aging and Adult Health (SAGE) project, conducted by Dr. Sharon Williams

  – Training on CRP, Hb, Hba1c, and EBV antibody assays

  – Duration of training: 5 days

# What is the Optimal Duration for Training?

- Factors to consider
  - Number of assays to be conducted
  - Prior training and experience the trainees have had
  - Level of technical support available during pre-test
    - Particularly important when there is significant delay from time of training to implementation of bioassays

Securing Equipment for the Bioassays

- Details, details, and details

- Verification, verification, and verification

Microplate Shaker
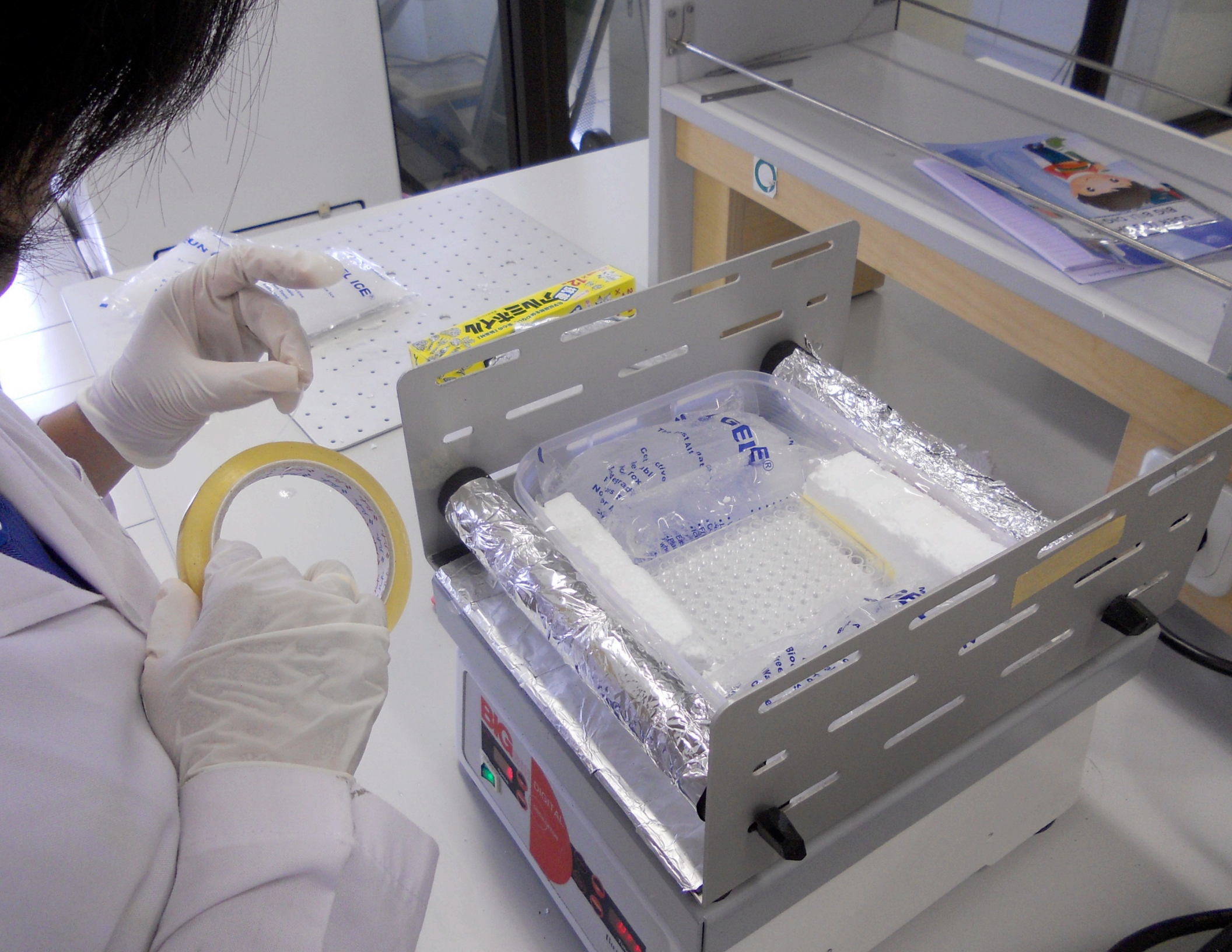
What Type of Equipment is Acceptable?

- Factors to consider
  - Assay methodology
  - Funding availability
    - IFLS had to purchase a new microplate shaker and a new ELISA reader after pre-test, which again underscore the importance of site visit and pre-test

Obtaining Test Reagents and Supplies

- CHARLS Pilot Study

  – All reagents and supplies were available through local vendors in China

  – Higher price compared to vendors in the U.S.

    • May not be cheaper to perform DBS-based assays in developing countries despite lower labor cost

  – Quality problem with second vial of coating antibody for CRP assay

- LASI Pilot Study

  – All reagents and supplies were available through local vendors in India

  – No quality problems encountered

Obtaining Test Reagents and Supplies

- IFLS, Wave 4
  - Some test reagents were not available locally and had temperature requirement for transportation and storage
  - Assistance from WHO to secure these reagents, and ship them into Indonesia
  - Difficulties with purchase, shipment, and re-ordering, leading to multiple delays
    - A more serious problem when sample size is large and study samples have to be tested over a relatively long period of time

Pre-test for DBS-based Assays

- Goals
    - Evaluate the technical skills of trained laboratory personnel, including transfer of knowledge to other technicians in these laboratories
    - Verify that correct equipment is used for the planned bioassays
    - Verify that correct test reagents and supplies are being used
    - Evaluate general condition of the laboratory: e.g. adequate work space, proper temperature control.
    - Evaluate the reliability and validity of the assay results, by using both study and validation samples

Validation Samples for Pre-test

- CHARLS Pilot Study
  - DBS and venous specimens were collected from 50 volunteers recruited through USC/UCLA Biodemography Center
  - Collected two DBS cards via finger stick, with two blood spots per card
  - Hemoglobin levels were measured, using a point-of-care HemoCue meter
  - Serum samples were sent to University of Vermont for hsCRP assay
  - One set of DBS cards were sent to Northwestern University for DBS-based hsCRP assay
  - Second set of DBS cards were sent to Beijing for DBS-based hsCRP and Hb assay

Validation Samples for Pre-test

- IFLS, Wave 4

  - DBS and venous specimens were collected from 67 volunteers recruited through USC/UCLA Biodemography Center

  - Collected two DBS cards via finger stick, with two blood spots per card

  - Serum samples were sent to University of Washington for hsCRP assay

  - One set of DBS cards were sent to University of Washington for DBS-based hsCRP assay

  - Second set of DBS cards were sent to Indonesia for DBS-based hsCRP assay

Validation Samples for Pre-test

- LASI Pilot Study
  - Venous specimens collected from 50 volunteers recruited through USC/UCLA Biodemography Center
  - **DBS cards were created from venous blood**
  - Five blood spots per card
  - Serum samples were sent to UCLA Clinical Laboratory for Hb, hsCRP, EBV antibody titer, and Hba1c assays
  - One set of DBS cards were sent to UW for DBS-based Hb, hsCRP, and EBV antibody titer assays
  - Two sets of DBS cards were sent to NARI for DBS-based Hb, hsCRP, EBV antibody, and Hba1c assays

Why USC/UCLA Biodemography Center?

- Experience and efficiency in volunteer recruitment

- Infrastructure for and experience in sample processing, storage, and shipment

- Connection with the laboratories that may serve as gold standard for various assays

- IRB APPROVAL!

- Potential for subsidized rate!!

Validation Samples & Pre-test

- Some of the lessons learned
  - Blood spot collection through direct finger stick versus making DBS cards from venous blood
    - Validation work done by USC/UCLA Biodemography Center
  - How to send frozen DBS cards to foreign countries?
    - Shipping conditions (e.g. temperature, humidity) may affect results from these validation samples
  - How to determine acceptable between-laboratory variability in assay results?

Lesson #1: Advantage of making DBS cards from venous blood over blood spot collection through direct finger stick

DBS validation samples used for CHARLS

DBS validation samples used for LASI

Whatman 903™ [LOT] W102 6892711

Whatman 903® [LOT] W083 6837809

Tuck Cover Here
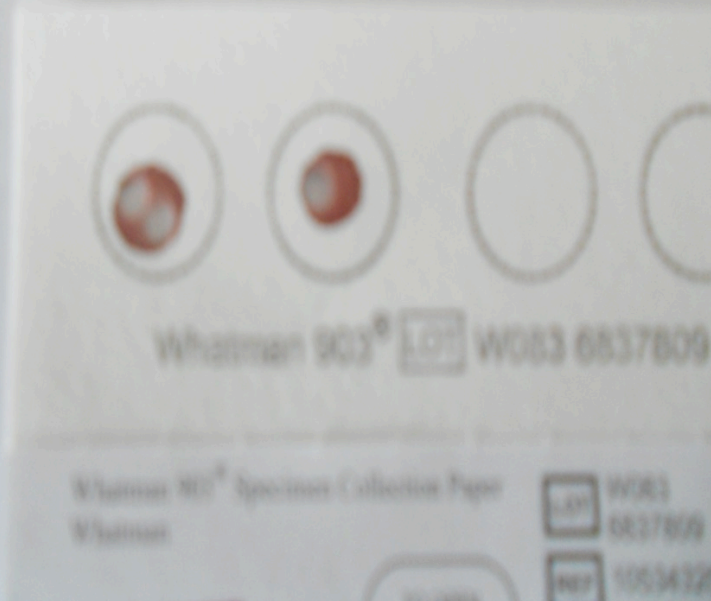
Lesson #2: How to Send DBS Validation Samples to Asian Countries?

Passage into China

Our way to India…but it costs about $1,000 to ship 50 DBS cards by World Courier

WORLD COURIER
An AmerisourceBergen Company

Change in Temperature during Validation Sample Shipment to India
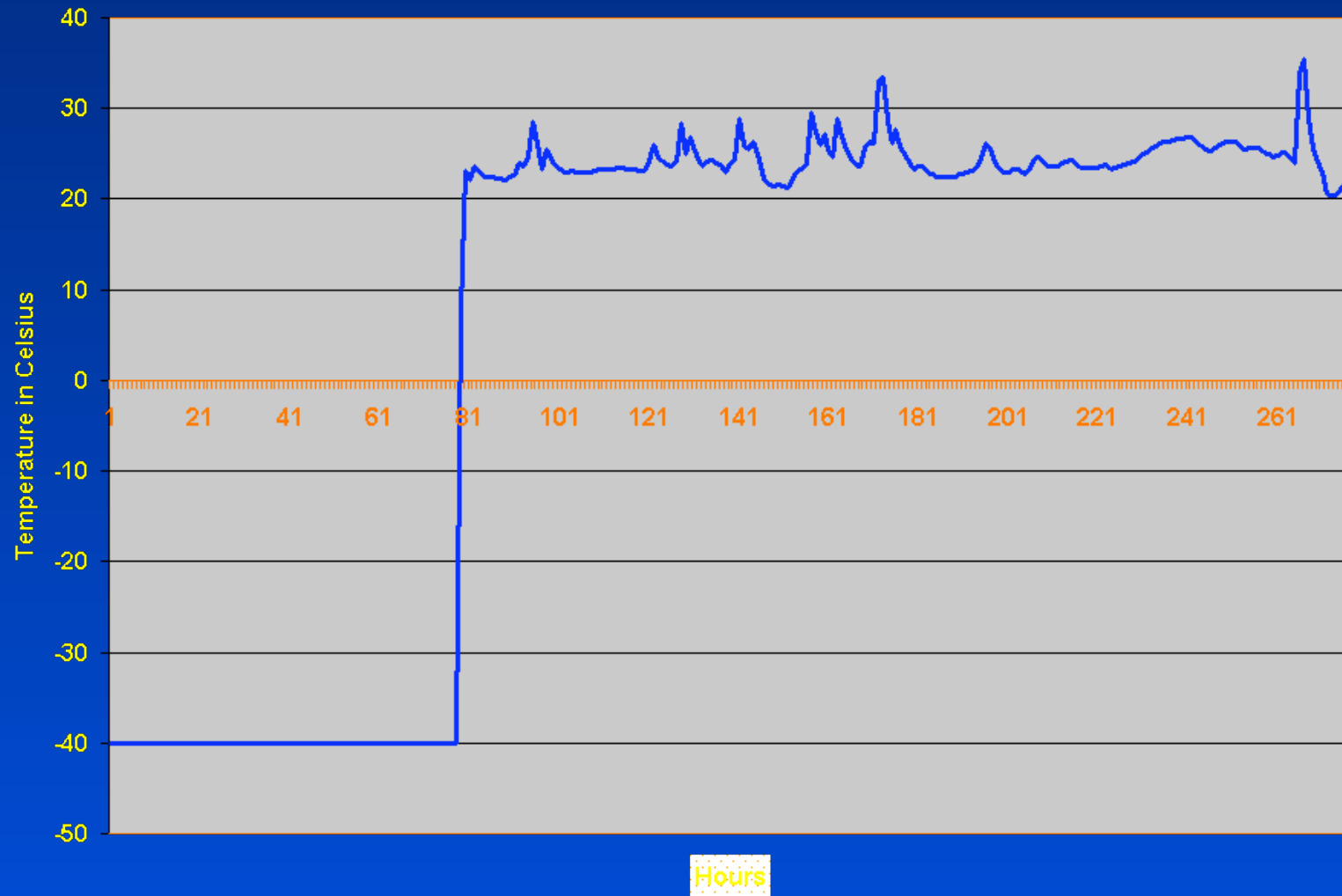
How to Determine Whether Assay Results are Valid?

- **Assay parameters**
  - **OD values for blanks**
  - **Shape and R-square of standard curves**
  - **OD values and concentrations for controls/calibrators**
  - **%CV of controls and actual study samples – all are done in duplicates**
- **Between-laboratory variability**
  - **Correlation coefficient**
  - **Absolute and relative differences**
- **What kind of differences are considered as acceptable?**
  - **Lack of specific criteria**

Quality Control during Study Sample Testing

- Laboratories were asked to send in test results for review on weekly basis, with rapid feedback on the samples that need re-testing
  - Compliance issue

- Issues to consider
  - Standard curves
  - OD Values for blanks, standards, and controls/calibrators on each plate
  - CVs of duplicate samples and controls/calibrators
  - Retesting of specimens with values significantly outside normal range
    - e.g. for Hb assay, may consider diagnostic criteria for polycythemia vera

Quality Control during Study Sample Testing

- Periodic testing of validation samples to monitor the possible laboratory assay result drift over time
  - Need for large number of dried blood spots

CHARLS Validation Sample Results

- Validation samples were assayed in the beginning and half way through the testing period

- N = 33 – 42, due to suboptimal condition of dried blood spots
  - Less dried blood spots than originally planned for pre-test
  - Inadequate dried blood spots for frequent quality monitoring

CRP – CHARLS DBS vs. McDade DBS

	Average (SD) (mg/L)	Median (mg/L)	Range (mg/L)
CHARLS	1.12 (1.14)	0.71	0.12 – 4.16
McDade	1.42 (2.38)	0.59	0.12 – 12.16

Equation: McDade value = - 0.11 + 1.37 x CHARLS value

R^2 for regression equation: 0.43

CRP – CHARLS DBS vs. Vermont Serum Assay

	Average (SD) (mg/L)	Median (mg/L)	Range (mg/L)
CHARLS	1.30 (1.44)	0.79	0.12 – 7.00
Vermont	2.39 (3.74)	1.05	0.33 – 20.10

Equation: Vermont value = 0.56 + 1.41 x CHARLS value

R^2 for regression equation: 0.30

Hemoglobin – CHARLS DBS vs. HemoCue meter

	Average (SD) (mg/dL)	Median (mg/dL)	Range (mg/dL)
CHARLS	13.3 (1.4)	13.1	11.0 - 16.5
HemoCue	14.0 (1.5)	13.8	11.2 – 16.9

Equation: Hemocue value = 5.4 + 0.7 x CHARLS value

R^2 for regression equation: 0.36

IFLS Pre-test of CRP Assay

- Pre-test schedule
  - Day 1: 20 IFLS samples
  - Day 2: 32 IFLS samples (4 were repeats from Day 1), plus 5 validation samples
  - Day 3: 27 IFLS samples (6 were repeats from Day 2), plus 10 validation samples
- All DBS samples were tested in duplicates
- Duplicate samples that had CV > 10% was 4.3%
- Correlation coefficient of 10 IFLS samples with repeated measurements: 0.998

Correlation coefficients between IFLS pre-test results, DBS-based values from University of Washington, and serum-based values

N = 14	IFLS vs. UW	IFLS vs. Serum	UW vs. Serum
Overall correlation	0.92	0.94	0.96
Correlation for Day 2	0.92	0.93	1.00
Correlation for Day 3	0.97	0.95	0.99

Average difference in CRP values (mg/L) between IFLS pre-test results, DBS-based values from University of Washington, and serum-based values

N = 14	IFLS vs. UW	IFLS vs. Serum	UW vs. Serum
Overall difference	- 0.48	0.13	0.60
Difference in Day 2	0.53	0.68	0.15
Difference in Day 3	- 0.88	- 0.09	0.79

Ongoing Quality Control for IFLS

- Periodic monitoring of assay parameters and study sample results

- Eight validation samples were tested for approximately every 1,200 study samples due to limited number of blood spots in validation samples
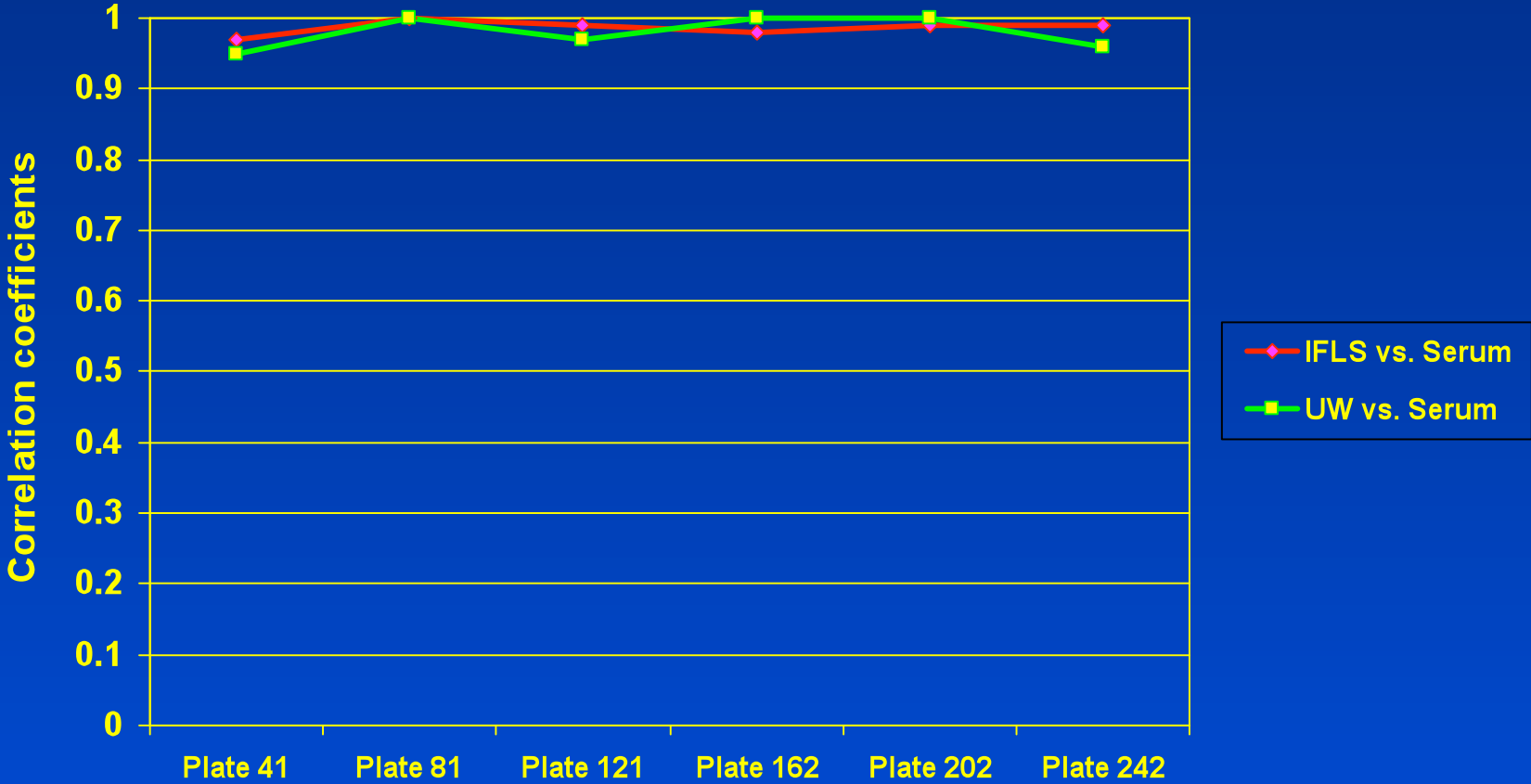
LASI Pre-test

- Satisfactory pre-test results for 3 assays
  - CRP
  - Hb
  - EBV antibody titer
- Challenges with Hba1c assay

LASI Pre-test for Hba1c Assay

Comparison of DBS and Serum Hba1c Levels
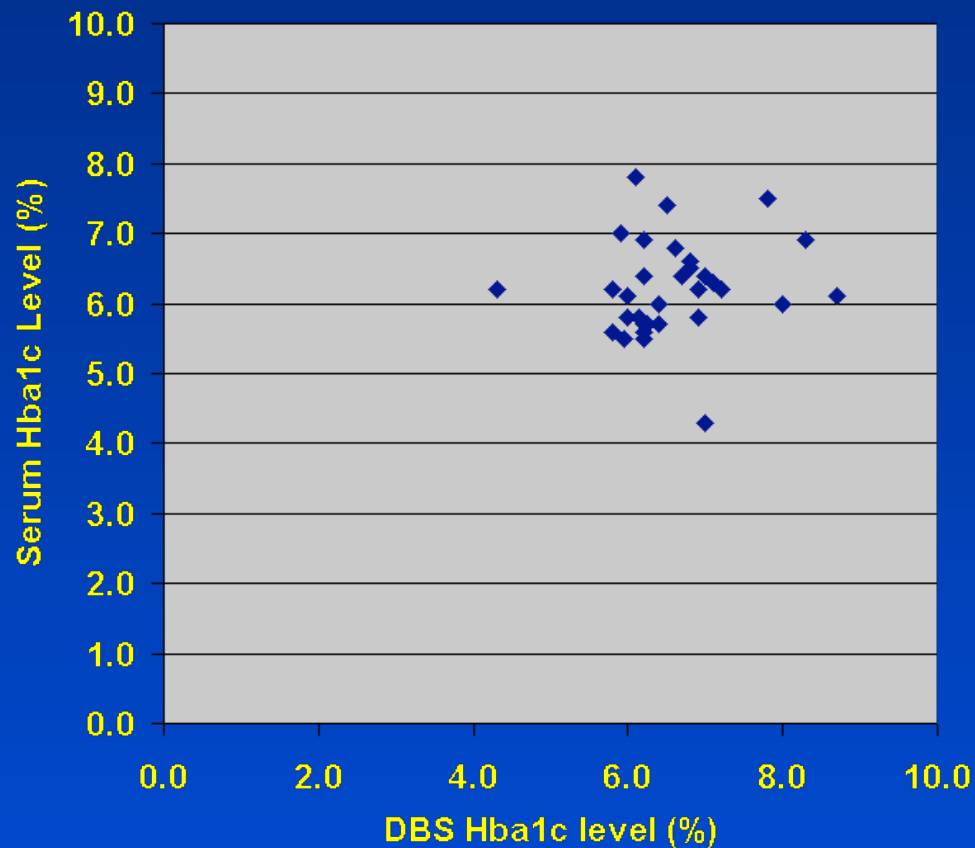
Correlation coefficient = 0.11 (N = 33)

Mean difference: 0.3%
**S.D.: 1.0%**
Range: - 1.9% to 2.7%
P-value (paired t-test): 0.07

Ongoing Quality Control for LASI

- Key difference was the amount of blood spot material available for validation purpose

- Measured 5 validation samples per microplate for the first 10 plates, followed by 8 validation samples every 150 study samples

- Ability to track values for selective validation samples over time
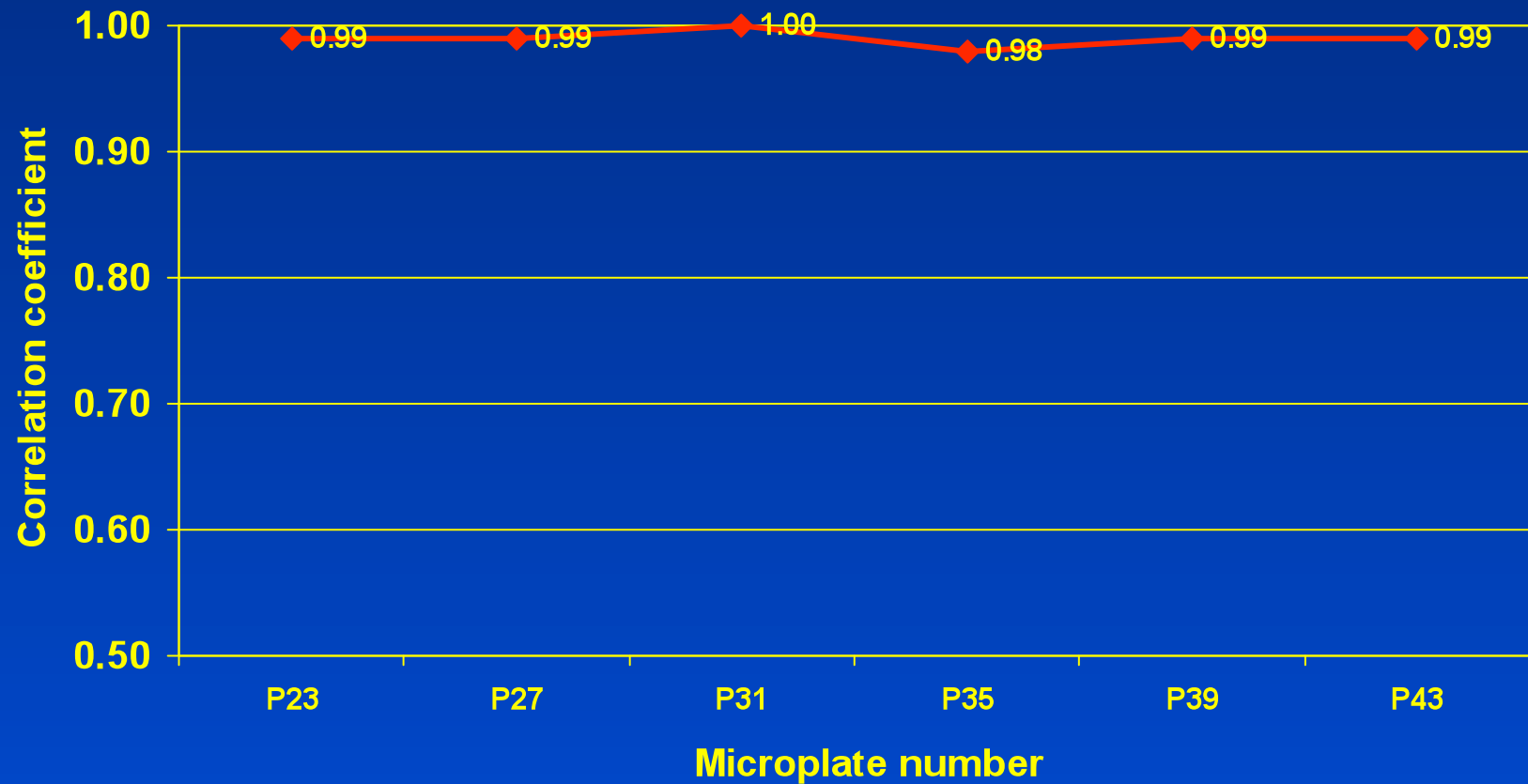
Correlation coefficients between LASI and UW results on validation samples, by microplate numbers
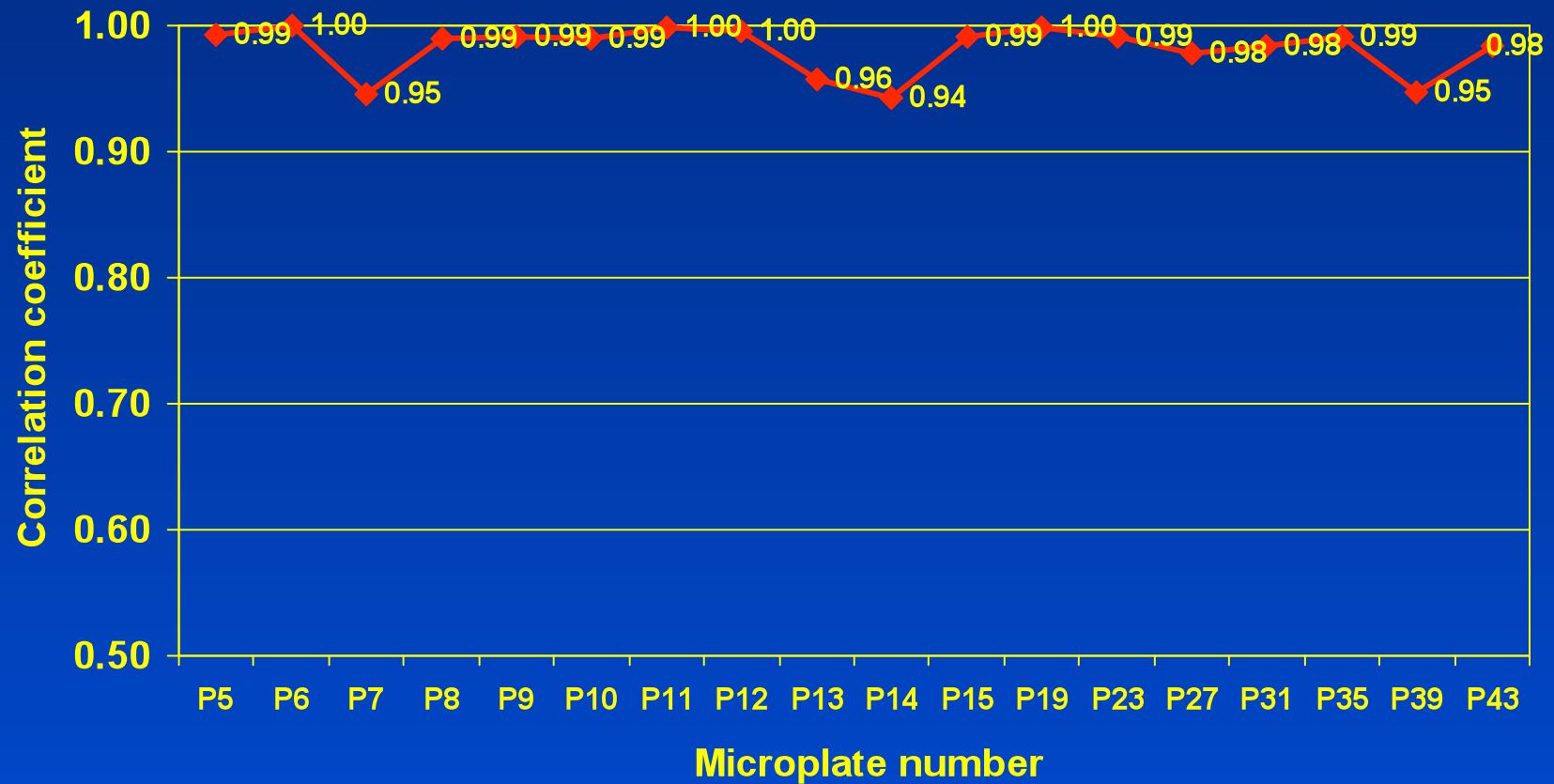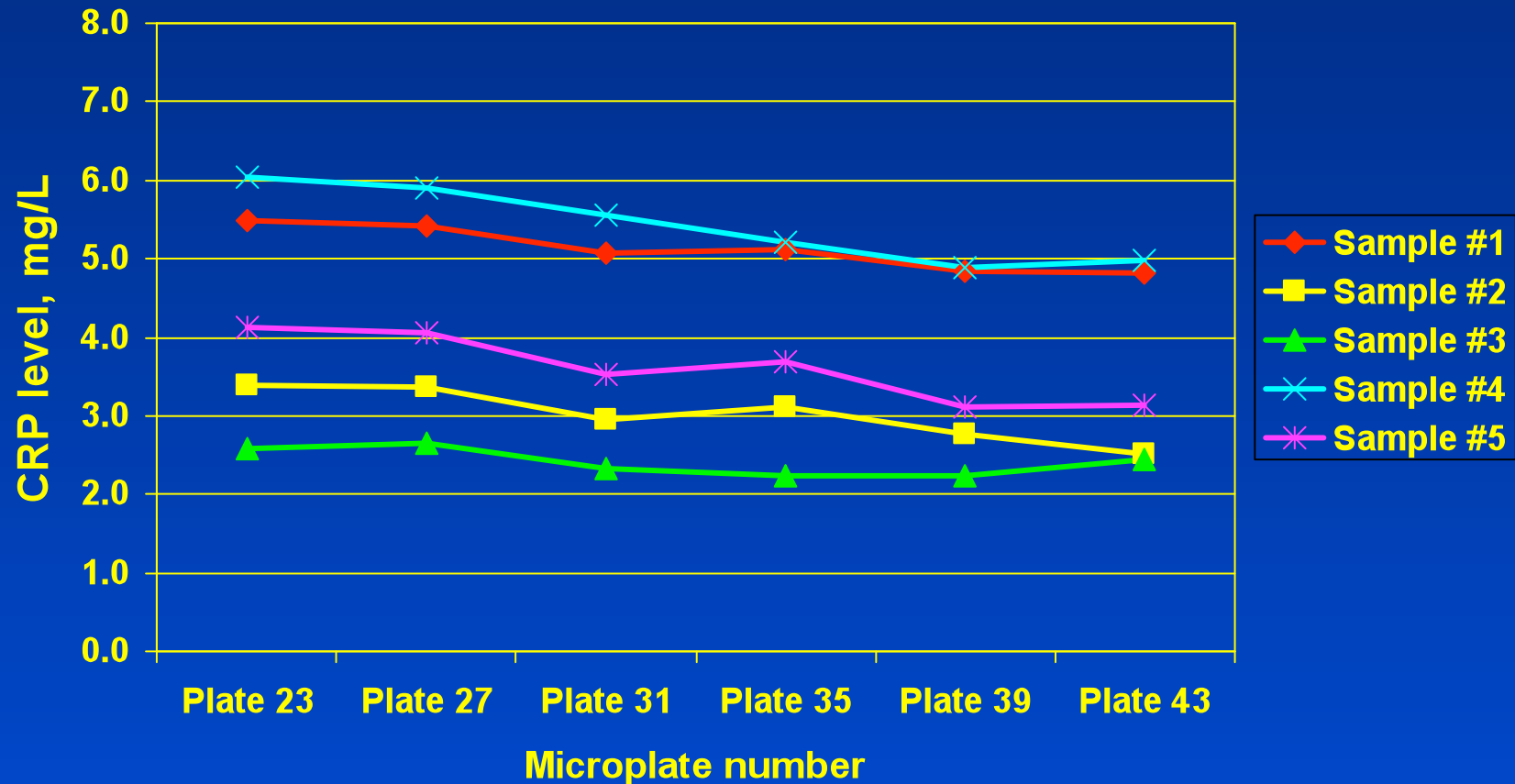
Absolute CRP values for selective validation samples across microplates
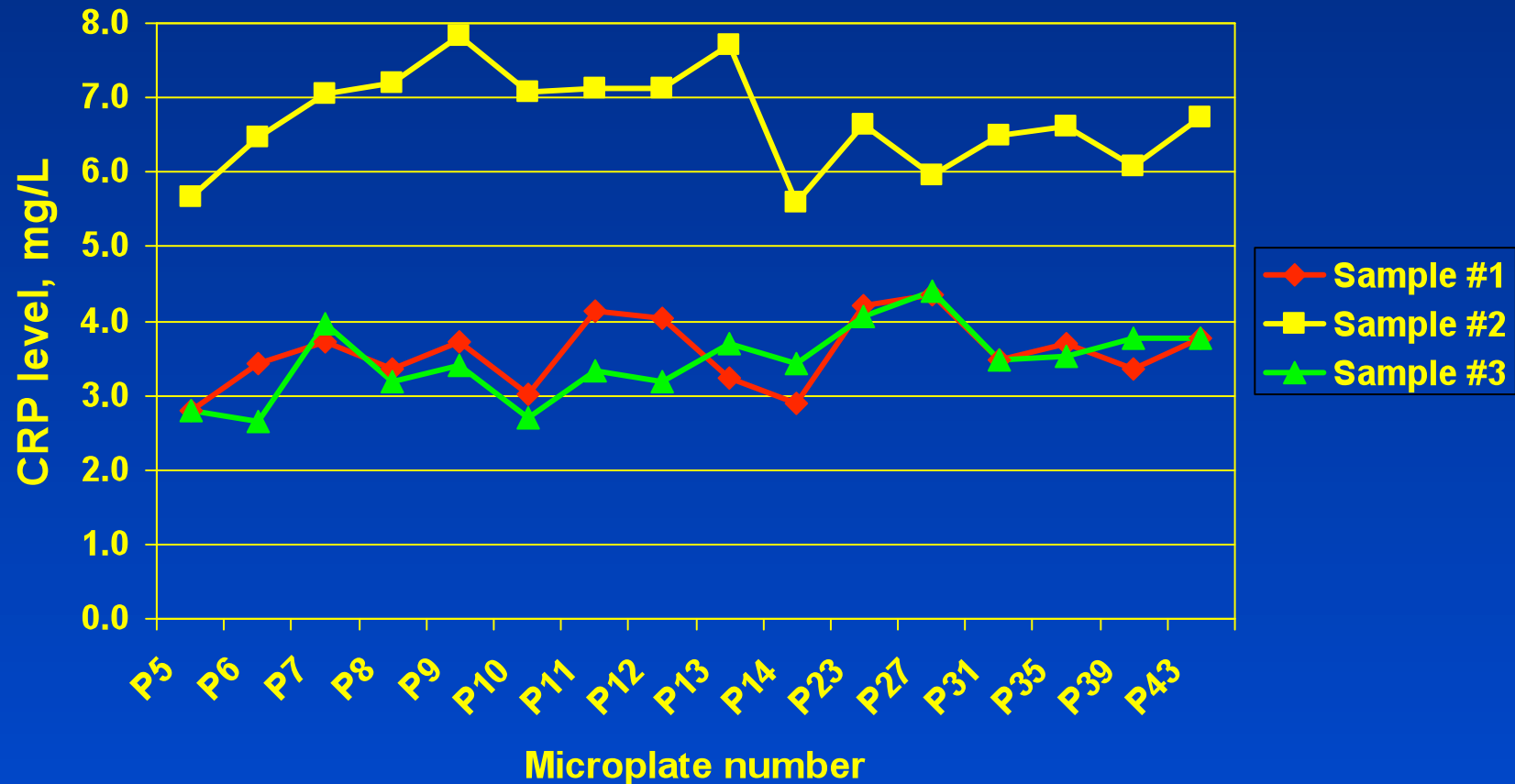
Correlation coefficients between LASI and serum values on validation samples, by microplate numbers

Absolute CRP values for selective validation samples across microplates

More Lessons Learned

- It is essential to conduct well-designed pre-test and ongoing monitoring to ensure data quality for DBS-based assays

- Good pre-test and quality control rely on availability of adequate validation samples, which requires infrastructure, expertise, and funding typically beyond what studies can do individually

- Maintain ongoing and timely communication with the laboratories that conduct DBS-based assays is crucial, but may be challenging

More Lessons Learned

Major Questions Remain

- There are no specific criteria that are being used to define acceptable assay quality and between-laboratory variability

  – How good is good enough (or how bad is not good enough)?

- In addition to good pre-test and quality control for each individual study, how can we compare DBS-based results across different surveys/countries?

  – Need for macro-level infrastructure

  – Laboratories for most surveys are operational for only a short period of time

- What are the other support/services USC/UCLA Biodemography Center may be able to provide?

Thank You!