

**Establishing Dried Blood Spot Based  
Assays in Indonesia and China:  
Experience from the Indonesia Family  
Life Survey (IFLS) and the China  
Health and Retirement Longitudinal  
Study (CHARLS)**

**Perry Hu, MD, PhD**

**UCLA Division of Geriatric Medicine**

# Main Steps for Establishing New Bioassays

- Training of laboratory personnel
- Securing equipment for the bioassays
- Obtaining test reagents and supplies
- Pre-test with validation samples
- Testing of study samples and ongoing quality control

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DEVIL IS IN  
THE DETAILS

# Dried Blood Spot (DBS) Based Assays

- IFLS, wave 4
  - C-Reactive Protein (CRP)
    - Hemoglobin (Hb) was measured using HemoCue
- CHARLS Pilot Study
  - CRP
  - Hb

# Training of Laboratory Personnel

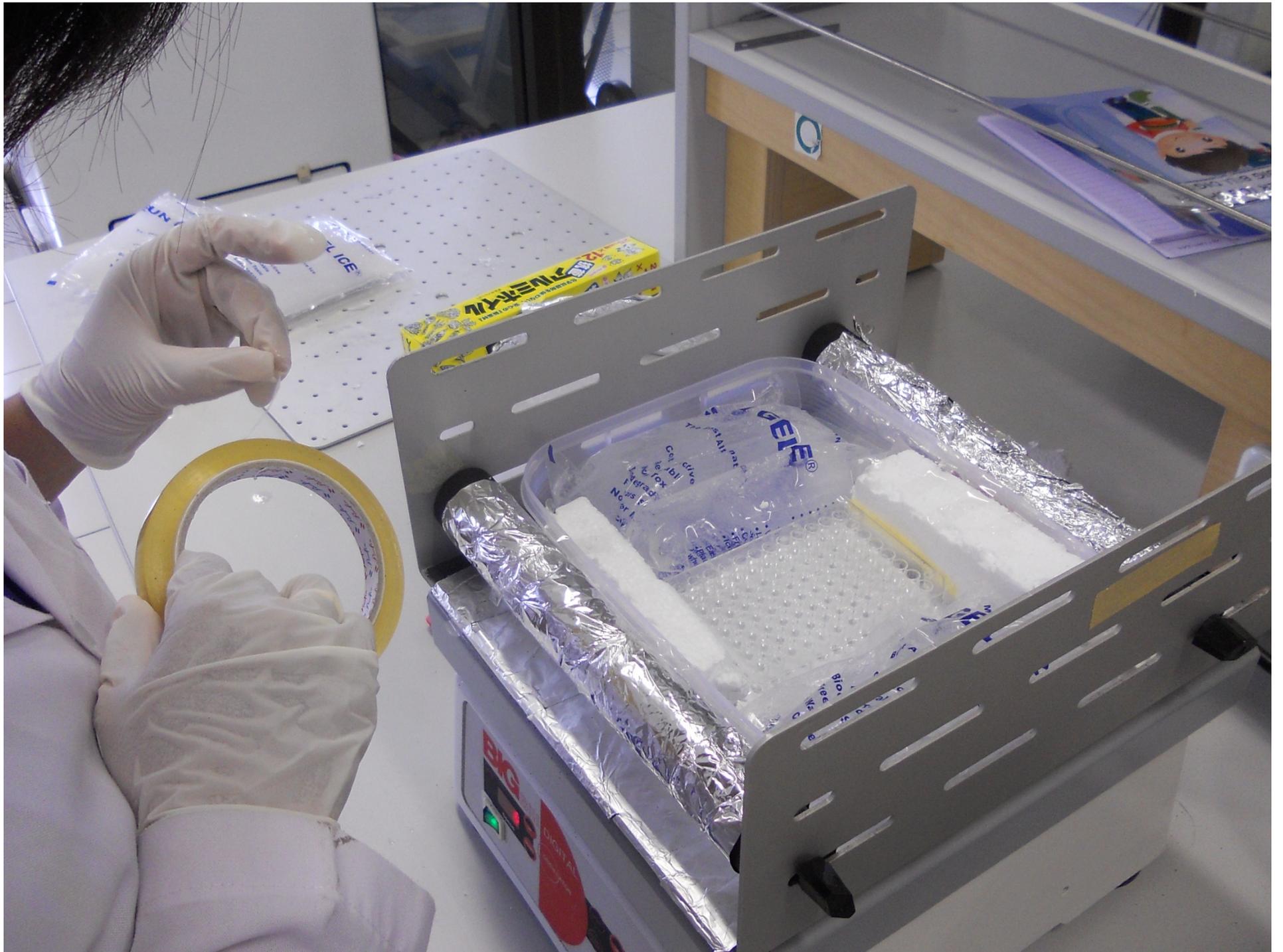
- IFLS, wave 4
  - Collaboration with School of Medicine, Gadjah Mada University
  - Training of laboratory personnel at the National AIDS Research Institute (NARI), Pune, India
  - Conducted by Dr. Sharon Williams from Purdue University
  - Part of training for the WHO's Study on Global Aging and Adult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

# 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

# Securing Equipment for the Bioassays

- Details, details, and details
- Verification, verification, and verification



# Obtaining Test Reagents and Supplies

- IFLS, wave 4
  - Some test reagents are not available locally and have temperature requirement for transportation and storage
  - Assistance from WHO to secure reagents and supplies, and ship them into Indonesia
  - Multiple issues with purchase and shipment
- CHARLS Pilot Study
  - All reagents and supplies were available through local vendors in China
  - Quality issue of reagents

# Pre-test

- Goals
  - Evaluate the technical skills of trained laboratory personnel, including transfer of knowledge to other technicians in the laboratory
  - 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

- IFLS, wave 4
  - DBS and venous specimens were collected from 67 volunteers recruited through USC/UCLA Biodemography Center
  - Serum samples were sent to University of Washington for CRP assay
  - One set of DBS cards were sent to University of Washington for DBS-based CRP assay
  - The second set of DBS cards were “sent” to Indonesia for DBS-based CRP assay

# Validation Samples for Pre-test

- CHARLS Pilot Study
  - DBS and venous specimens collected from 50 volunteers recruited through USC/UCLA Biodemography Center
  - Measurement of hemoglobin levels, using a point-of-service HemoCue meter
  - Serum samples were sent to University of Vermont for CRP assay
  - One set of DBS cards were sent to Northwestern University for DBS-based CRP assay
  - The second set of DBS cards were “sent” to Beijing for DBS-based CRP and Hb assay

# Only Way to Safely “Send” DBS Validation Samples to Indonesia or China

- **Three criteria used to select a courier**
  - A PhD degree
  - Professor in a major university
  - Willing to do it for free



# Pre-test of CRP Assay - IFLS

- 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
- % of 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**

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

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

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

# CHARLS Validation Sample Results

- Validation samples were assayed in the beginning and half way through the testing period
- N = 33- 42

## CRP – CHARLS DBS vs. McDade DBS

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

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

R<sup>2</sup> for regression equation: 0.43

## CRP – CHARLS DBS vs. Vermont Serum Assay

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

Equation: Vermont value = 0.56 + 1.41 x CHARLS value

R<sup>2</sup> for regression equation: 0.30

## Hemoglobin – CHARLS DBS vs. HemoCue meter

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

Equation: Hemocue value = 5.4 + 0.7 x CHARLS value

R<sup>2</sup> for regression equation: 0.36

# Ongoing Quality Control

- Ongoing monitoring of assay results
- Test results were or are being sent for review on weekly basis, with rapid feedback on the samples that need re-testing
- Issues to consider
  - Standard curves
  - Values of standard and control samples on each plate
  - CVs of duplicate samples
  - Values significantly outside normal range
- Periodic testing of validation samples to monitor the possible laboratory assay result drift over time

# Lessons Learned

- DBS is a viable alternative to venous blood for many biomarkers
- Many factors could contribute to the cross-laboratory differences in validation sample results
  - Cross-laboratory difference in predictors of assay variability
    - Reagents
    - Equipment
    - Personnel: skills, deviation from validated protocols
    - Physical environment: temperature, humidity
    - True difference between DBS values and serum/plasma values
  - Issues with validation samples themselves
    - Quality (e.g. size) of DBS samples for validation
    - Temperature condition during sample shipment
- Experience and practice do make a difference

# Lessons Learned

- Funding availability often limit the number of validation samples that can be generated and tested
- Expect the unexpected
- We may need time, more time, and even more time
- We may need funding, more funding, and even more funding

# Thank You!



# Web Videos

- Collection of blood spots from fingerstick
- Creation of blood spots from venipuncture collection
- For each video, allow for separate sound tracks in multiple languages
- Provide screen shots in format like PowerPoint for inclusion in training or in cases where video-streaming difficult

# Suggestions for Fingertick Collection Video

- Good example of an ideal case (good bleeder, ability to use heating pad)
- Additional suggestions:
  - Include examples of front and back of correctly saturated cards
  - Demonstrate use of chemical heat packs (in case heating pads not feasible)
  - Show more detail about how to hold finger for lancet puncture
  - Add information about ppt being hydrated
  - Include a problematic case, including warming the arm, milking arm, etc
  - Clarify that different types of cards may have different requirements for collection (e.g., multiple drops)
- New video will be available by July 1, 2012

# Links to Videos

- Fingertstick to DBS collection:  
<http://youtu.be/v2IGeABISwE>
- DBS creation from venous sample:  
<http://youtu.be/B5XbuStwdC4>