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
THE 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

<table>
<thead>
<tr>
<th>N = 14</th>
<th>IFLS vs. UW</th>
<th>IFLS vs. Serum</th>
<th>UW vs. Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall correlation</td>
<td>0.92</td>
<td>0.94</td>
<td>0.96</td>
</tr>
<tr>
<td>Correlation for Day 2</td>
<td>0.92</td>
<td>0.93</td>
<td>1.00</td>
</tr>
<tr>
<td>Correlation for Day 3</td>
<td>0.97</td>
<td>0.95</td>
<td>0.99</td>
</tr>
</tbody>
</table>
Average difference in CRP values (mg/L) between IFLS pre-test results, DBS-based values from University of Washington, and serum-based values

<table>
<thead>
<tr>
<th></th>
<th>IFLS vs. UW</th>
<th>IFLS vs. Serum</th>
<th>UW vs. Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N = 14</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Overall difference</strong></td>
<td>-0.48</td>
<td>0.13</td>
<td>0.60</td>
</tr>
<tr>
<td><strong>Difference in Day 2</strong></td>
<td>0.53</td>
<td>0.68</td>
<td>0.15</td>
</tr>
<tr>
<td><strong>Difference in Day 3</strong></td>
<td>-0.88</td>
<td>-0.09</td>
<td>0.79</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th></th>
<th>Average (SD) (mg/L)</th>
<th>Median (mg/L)</th>
<th>Range (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHARLS</td>
<td>1.12 (1.14)</td>
<td>0.71</td>
<td>0.12 – 4.16</td>
</tr>
<tr>
<td>McDade</td>
<td>1.42 (2.38)</td>
<td>0.59</td>
<td>0.12 – 12.16</td>
</tr>
</tbody>
</table>

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

$R^2$ for regression equation: 0.43
CRP – CHARLS DBS vs. Vermont Serum Assay

<table>
<thead>
<tr>
<th></th>
<th>Average (SD) (mg/L)</th>
<th>Median (mg/L)</th>
<th>Range (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHARLS</td>
<td>1.30 (1.44)</td>
<td>0.79</td>
<td>0.12 – 7.00</td>
</tr>
<tr>
<td>Vermont</td>
<td>2.39 (3.74)</td>
<td>1.05</td>
<td>0.33 – 20.10</td>
</tr>
</tbody>
</table>

Equation: Vermont value = 0.56 + 1.41 x CHARLS value

$R^2$ for regression equation: 0.30
## Hemoglobin – CHARLS DBS vs. HemoCue meter

<table>
<thead>
<tr>
<th></th>
<th>Average (SD) (mg/dL)</th>
<th>Median (mg/dL)</th>
<th>Range (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHARLS</td>
<td>13.3 (1.4)</td>
<td>13.1</td>
<td>11.0 - 16.5</td>
</tr>
<tr>
<td>HemoCue</td>
<td>14.0 (1.5)</td>
<td>13.8</td>
<td>11.2 – 16.9</td>
</tr>
</tbody>
</table>

Equation: Hemocue value = 5.4 + 0.7 x CHARLS value

$R^2$ for regression equation: 0.36
Ongoing Quality Control

• Ongoing monitoring of assay results
• Test results were or are being sent for review on a 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 Fingerstick 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

• Fingerstick to DBS collection:
  http://youtu.be/v2lGEABISwE

• DBS creation from venous sample:
  http://youtu.be/B5XbuStwdC4