Implementation of A New Dried Blood Spot Based C-reactive Protein Assay in Indonesia

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Background

- C-reactive protein (CRP), a biomarker of inflammation, has been included in many community-based surveys
- During wave 4 of the Indonesia Family Life Survey (IFLS), CRP levels were measured on blood spot (DBS) specimens, using a protocol developed by Thomas McDade
- This original DBS-based CRP assay protocol required individual ordering of component reagents and coating microplates with antibody
- Unfortunately, some of these component reagents are no longer available

Background

- University of Washington (UW) has developed a new DBS-based CRP assay, using the high-sensitivity CRP ELISA kit manufactured by Percipio Biosciences
- For the new UW protocol, correlation coefficient between DBS results and paired plasma samples was 0.99 (N = 87)
- IFLS-5 collaborated with the UW and laboratory at the University of Gadjah Mada (UGM), the same institution that measured CRR levels for IFLS-4

Training and Pre-test

- Five-day training and a pre-test were conducted in May of 2016
- UW created 16 validation samples with CRP levels ranging from 0.4 mg/L to 30.9 mg/L
- USC/UCLA Biodemography Center helped to create additional assay control samples
- After training, technicians at UGM measured validation samples from UW
- The correlation coefficient between UGM DBS CRP results and corresponding UW plasma values was 0.95

Modifications to UW protocol

- To accommodate the chemistry analyzer setting at UGM and minimize punch to punch variation, we decided to use two DBS punches for the assay
- As a result of using two punches, volume of elution buffer was doubled as well
- Used stop solution and measured optic densities at 450 nm
- Because the current CRP kit does not work as well at high CRP concentrations, we decided that samples with initial DBS CRP values above 15 mg/L would be diluted and re-tested

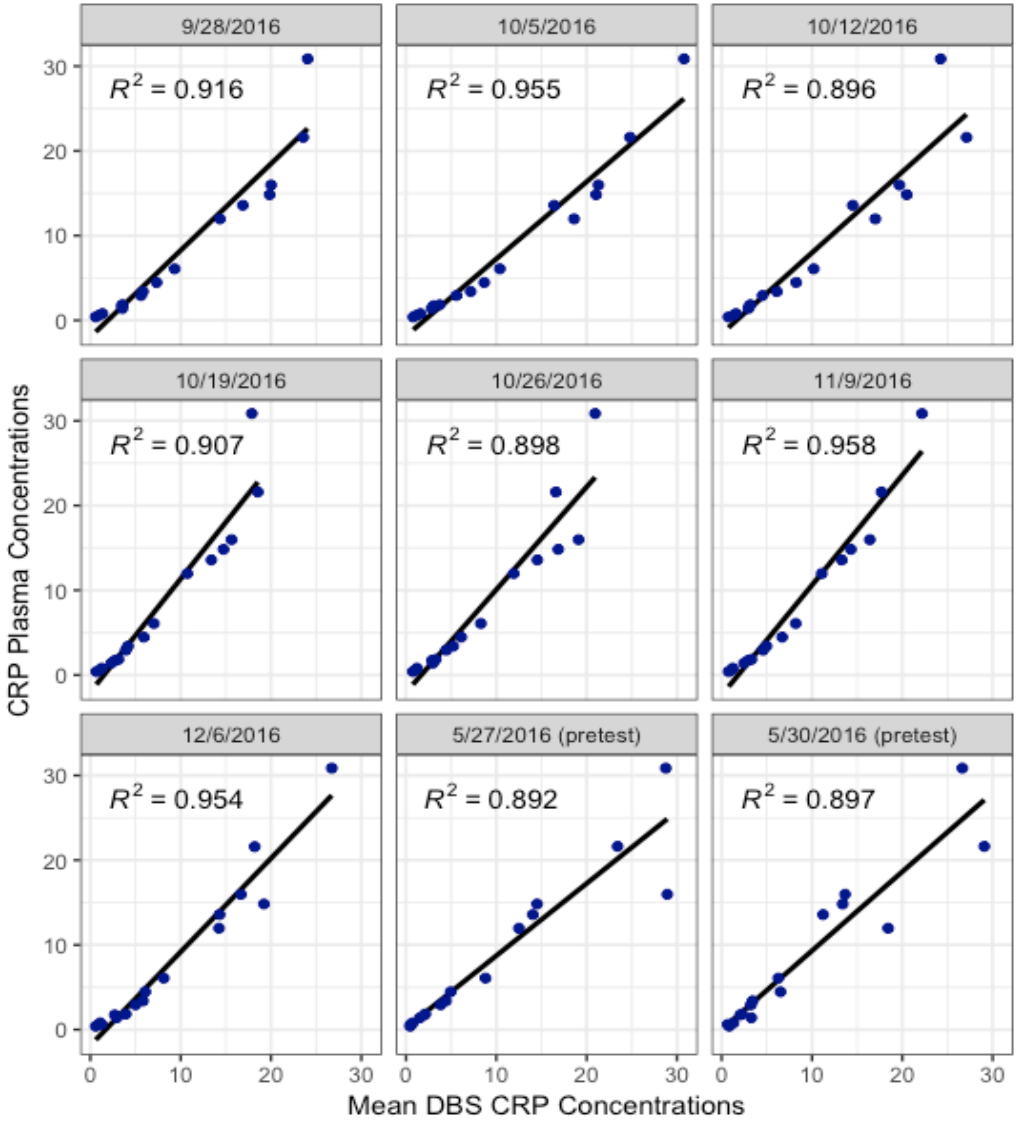
Workflow Design

- Each microplate contained one blank, nine standards and two controls; all were measured in duplicate
- Each microplate measured seventy-two study samples
- UGM laboratory measured 144 IFLS-5 study samples a day (two microplates), five days a week

Assay Quality Control

- CRP results were reviewed on weekly basis
- Acceptability of the results was determined by comparing the results of control samples on each microplate with their established values
- UGM laboratory also measured sixteen UW validation samples initially weekly and then biweekly

The correlations between UGM DBS CRP results and corresponding UW plasma values from repeated measurement of validation samples



Comparisons between Old and New DBS-based CRP Protocols

	Old Protocol	New UW Protocol
Need to coat microplates	Yes	No
Assay time	Five hours	Less than two hours
Number of study samples per microplate	37	72
Supply cost per sample	$1 to $2	$2.5

Additional Results from STAR Project

- UGM laboratory also measured CRP levels on 210 DBS samples from STAR (Study of the Tsunami Aftermath and Recovery) point-of-care device validation project
- The R-square between UGM DBS results and corresponding serum values from another laboratory in Indonesia was 0.93

Conclusions

- The new DBS-based CRP assay protocol developed by UW is reliable and much more efficient than the previous one
- The increase in per-unit assay cost is small
- The protocol may be successfully implemented in other countries

Conclusions

- All IFLS-5 data and documentations are now publicly available
- https://www.rand.org/labor/FLS/IFLS/ifls5.html

Thank you!