IFLS Wave 5 Dried Blood Spot Data User Guide

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Preface

This document describes the design and implementation of dried blood spot (DBS) based assay for high sensitivity C-reactive protein (hs-CRP) and glycosylated hemoglobin (HbA1c), undertaken as part of fifth wave of the Indonesia Family Life Survey (IFLS). The laboratory analysis was done at the Clinical Pathology and Laboratory Medicine Department at the University of Gadjah Mada, under the direction of Dr. Elizabeth Henny Herningtyas. We had two technician coordinators, Sri Swastikawati for hsCRP, Sagita Adventia and later Anika Prasetyowati for HbA1c.

The dried blood spots were stored in freezers at -40C at Survey Meter. Ika Rini supervised the handling of the DBS at Survey Meter, helped by Anindita Az Zahra Lutfiatunnisa. Iip Rafaii was in charge of the programming for the DBS chemistry analyzers, assisted by Nur Arna Sucianti.

Validation samples were taken at the Department of Laboratory Medicine at the University of Washington under the direction of Dr. Alan Potter. Whole blood was drawn and dried blood spots created from these. Validation assays on both whole blood for HA1c, plasma for hsCRP and dried blood spots for both were performed at both the Department of Laboratory Medicine at the University of Washington, and for dried blood spots at the Clinical Pathology and Laboratory Medicine's Department laboratory at Gadjah Mada. USC/UCLA Center for Biodemography and Population Health contributed dried blood spots that were used as controls in the assays at Gadjah Mada.

The Indonesia Family Life Survey is a continuing longitudinal socioeconomic and health survey. It is based on a sample of households representing about 83% of the Indonesian population living in 13 of the nation's 26 provinces in 1993. The survey collects data on individual respondents, their families, their households, the communities in which they live, and the health and education facilities they use. The first wave (IFLS1) was administered in 1993 to individuals living in 7,224 households. IFLS2 sought to re-interview the same respondents four years later. A follow-up survey (IFLS2+) was conducted in 1998 with 25% of the sample to measure the immediate impact of the economic and political crisis in Indonesia. The next wave, IFLS3, was fielded on the full sample in 2000. IFLS4 was fielded in late 2007 and early 2008 on the same 1993 households and their splitoffs. IFLS5 was fielded in late 2014 and early 2015 on the same set of IFLS households and splitoffs: 16,204 households and 50,148 individuals were interviewed. Another 2,662 individuals who died since IFLS4 had exit interviews with a proxy who knew them well.

IFLS5 was a collaborative effort of RAND and Survey Meter. Funding for IFLS5 was provided by the National Institute on Aging (NIA), grant 2R01 AG026676-05, the National Institute for Child Health and Human Development (NICHD), grant 2R01 HD050764-05A1 and grants from the World Bank, Indonesia and GRM International, Australia from DFAT, the Department of Foreign Affairs and Trade, Government of Australia.

A. Chronic Inflammation and Glucose Metabolism

There is strong evidence supporting that chronic inflammation plays an important role in the process of aging and age-related diseases (Singh & Newman 2011). Persistently elevated level of C-reactive protein (CRP), a biomarker for systemic inflammation, is associated with increased risk of cardiovascular disease, functional decline, and higher mortality in older adults. Glycosylated hemoglobin (HbA1c) is a measure of glucose metabolism, and used to diagnose diabetes mellitus and monitor glucose control among diabetic patients. Abnormal glucose metabolism is also part of metabolic syndrome. Therefore, DBS-based CRP and HbA1c assays have been increasingly incorporated into community-based surveys. Compared to venous blood samples, DBS specimens are easier to collect, and do not need to be processed and frozen immediately.

B. *Sampling for DBS*

Dried blood spots were supposed to be taken for all those who had CRP assays done in wave 4, or 9,944 respondents. In the data file whether DBS were taken and the number of spots collected is recorded in Book US variable US13a. The wave 4 sampling is described in Hu et al. (2013).

There are 7,579 observations with CRP data and 7,524 observations with HbA1c in wave 5. The number of observations is less than the number sampled because some sampled respondents had died, some were not found in wave 5 and some refused to participate. Among those with samples, 109 respondents had DBS taken but should not have because they were not in the 2007 sample with assayed DBS. We retain these 109 individuals in the data, but they do not have sample weights. Users can use their judgment on whether to include them or not. Consequently there are 7,470 respondents with CRP data and sample weights

and 7,416 with HbA1c and weights. A small number, 9, had unusable DBS spots, because the circles were not filled enough to take punches for the assay.

Sampling weights

The sampling weights for wave 5 blood spots start with the wave 4 weights, which incorporate both the sampling scheme and non-participation (see Hu et al., 2013, for a description). We add to this a non-participation adjustment for those among the 9,944 respondents who had DBS assayed in 2007 and who should have contributed blood spots in 2014, but did not. The correction is an inverted probability weight constructed by first estimating a logit equation using the full 9,944 in the 2014 blood sample frame, with the dependent variable a binary indicator of whether we have DBS assays in 2014. Regressors include linear splines in 2007 age, a gender dummy variable, household composition variables, a spline in the log of household per capita expenditure in 2007, an assessment of the quality of interview in 2007, an indicator of whether their 2007 CRP was greater or equal to 3.0, and an indicator of the province in which the person resides. The logit equation is presented below.

Table 1
Logit of (DBS 14|DBS 07 assayed)

2007 variables	Coeffiicient	Std. errors
Head =1	0.380***	(0.100)
Spouse of head = 1	0.535***	(0.0991)
Child of head =1	-0.0617***	(0.0107)
Age spline		-0.0332
0 - 10	-0.0332	(0.0263)
11 - 1 5	-0.183***	(0.0344)
16 - 20	0.0941***	(0.0332)
21 - 29	0.00131	(0.0150)
30 - 45	0.0163	(0.00993)
45 - 60	-0.0542***	(0.00925)
60 +	-0.0913***	(0.00707)
Male = 1	-0.308***	(0.0604)
Household composition		
Single member HH	0.0317	(0.152)

Two members HH	0.0966	(0.0957)
# of hh members		-0.0127
0 – 9 years old	-0.0127	(0.0324)
10 -14 years old	-0.00466	(0.0410)
15 – 24 years old	-0.00258	(0.0302)
25 + years old	0.00976	(0.0250)
Age 20 x years of education	-0.0140*	(0.00724)
CRP 2007 over 3 =1	-0.187**	(0.0827)
Spline pce		-0.119**
1st - 3rd quartile	-0.119**	(0.0563)
4th quartile	-0.362***	(0.111)
Quality of interview		0.124
Excellent	0.124	(0.112)
Good	-0.0558	(0.0921)
Urban = 1	-0.381***	(0.0560)
Constant	3.579***	(0.778)
Observations	9,944	
LR $Chi^2(36)$ = 1198.59		
Prob > chi^2 = 0.000		
Pseudo R^2 = 0.1073		

Province dummy variables are included but not presented. Spline coefficients are slopes, not marginal changes in slopes.

C. Methods of DBS-based CRP and HbA1c Assays

For the DBS-based CRP and HbA1c assays, IFLS collaborated with the laboratory at the Clinical Pathology and Laboratory Medicine Department at the University of Gadjah Mada (UGM), Yogyakarta, Indonesia, headed by Dr. Elizabeth Henny Herningtyas.

CRP concentrations in DBS specimens were measured using a high-sensitivity CRP (hsCRP) ELISA method validated by Department of Laboratory Medicine, University of Washington (UW, Laboratory director: Alan Potter). The UW protocol uses the hsCRP enzyme immunoassay kit manufactured by Percipio Biosciences (Catalog Number 11190). The CRP concentrations of 87 DBS samples analyzed by the DBS assay correlated well with the CRP concentrations of paired plasma samples (Pearson R = 0.99) and were linearly related. The DBS-based HbA1c assay was based on a validated protocol (Hu et al, 2015). The correlation coefficient between DBS and whole blood results was 0.960.

D. Validation and Quality Control of DBS-based Assays

1. *Preparation of validation and quality control (QC) samples*

   In preparation for training, pre-test, and QC at UGM, UW prepared 16 validation samples that have corresponding plasma CRP values (ranging from 0.4 mg/L to 30.86 mg/L) and whole blood HbA1c values (ranging from 4.6% to 16.7%). Each validation sample contained five blood spots; each allowed eight 3.2 mm punches.

   USC/UCLA Center on Biodemography and Population Health prepared additional blood spots that were used as controls for CRP and HbA1c assays.

2. *Pre-test*

   a. CRP assay

   During the 5-day training, CRP levels were measured on 16 UW validation samples on two separate days. All validation samples were measured in duplicate on both days. Figures 1a and 1b summarize the relationship between mean IFLS DBS CRP results and responding UW plasma values. The R-square was 0.99 during the first test and 0.98 during the second.

Figure 1a. The relationship between mean IFLS DBS CRP results and corresponding UW plasma values during first testing.

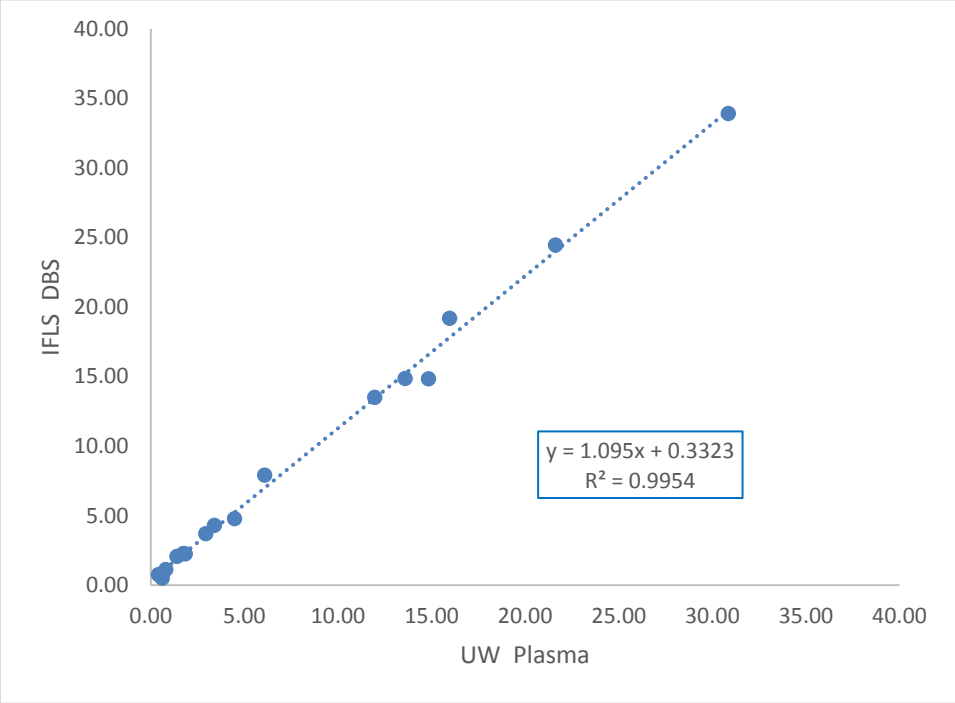
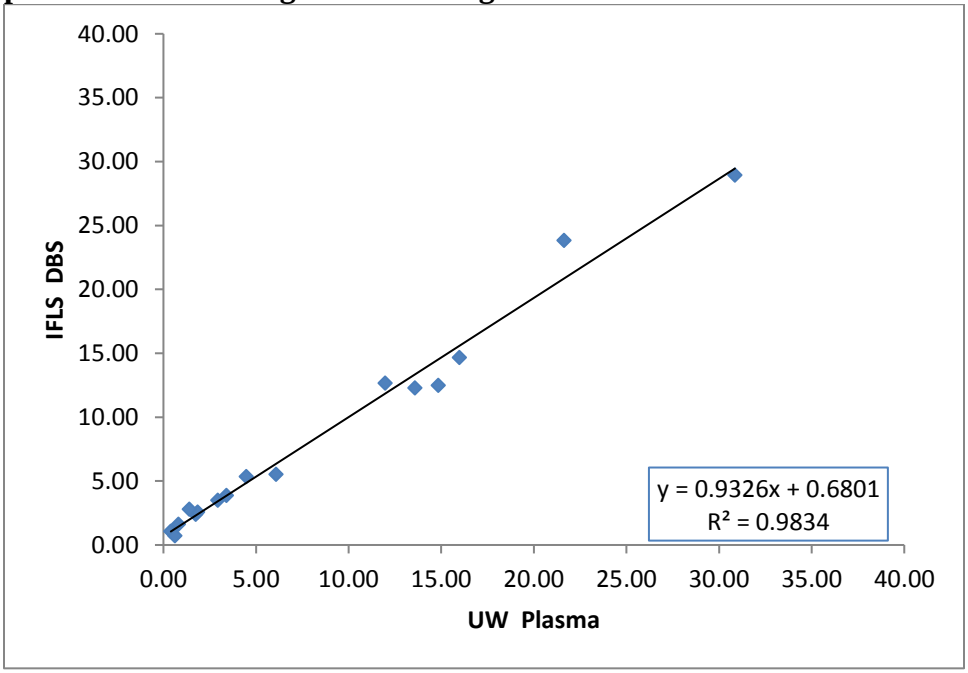


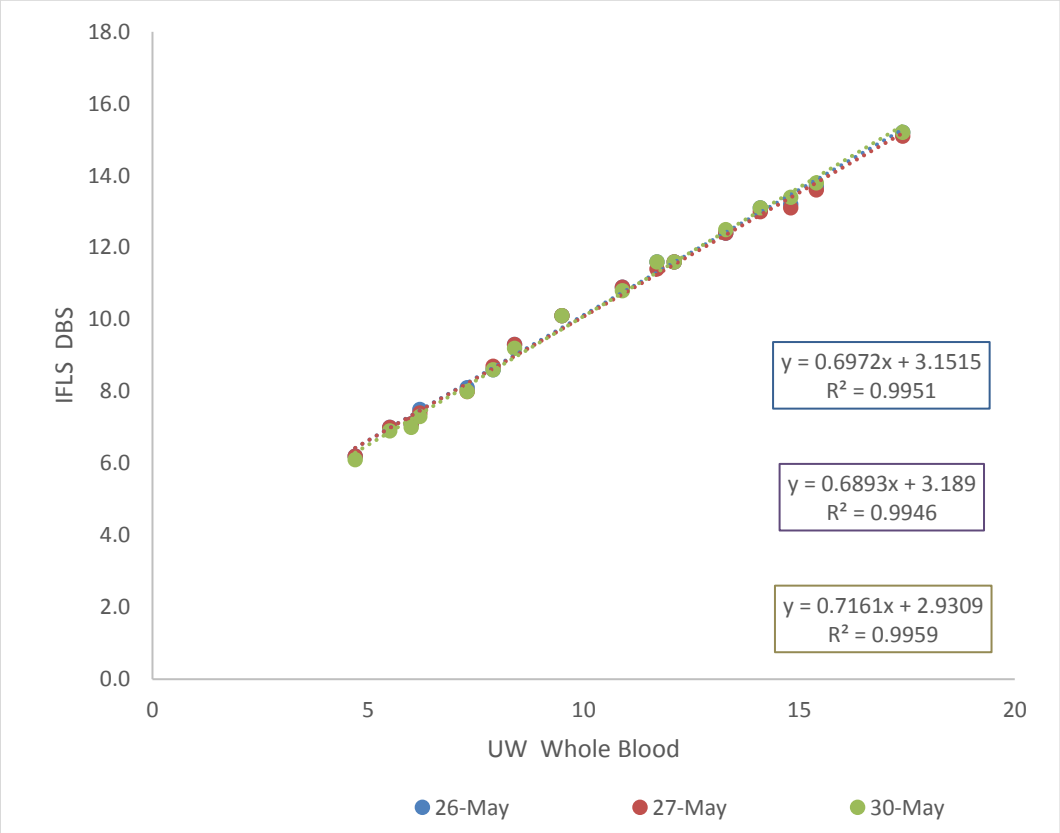
Figure 1b. The relationship between mean IFLS DBS CRP results and corresponding UW plasma values during second testing.



b. HbA1c assay

HbA1c levels were measured on 16 UW DBS validation samples for a total of three times during the pretest. The results show that IFLS DBS values were very consistent over time and highly correlated to UW whole blood values (Figure 2; all R-squares were above 0.99).

Figure 2. The relationship between IFLS DBS HbA1c results and UW whole blood HbA1c results



3. Workflow design

UGM measured CRP and HbA1c levels on 144 IFLS samples a day, five days a week. For the CRP assay, each microplate contained nine standards and two controls (low or high

CRP concentration); all were measured in duplicate. The daily workflow for HbA1c measurement was: low level liquid control (CTRL), high level liquid control (CTRH), low level DBS control (DBS CTRL), high level DBS control (DBS CTRH), 36 IFLS samples, CTRL, CTRH, 36 IFLS samples, CTRL, CTRH, 36 IFLS samples, CTRL, CTRH, 36 IFLS samples.

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4. Ongoing assay quality control with validation samples

The IFLS research team, led by Dr. Peifeng (Perry) Hu, established regular communication with the laboratory and reviewed the assay results from IFLS study samples on a weekly basis. Acceptability of the assay results was determined by comparing the analyte concentrations of the control samples with their established values. An assay was rejected if the mean value of one control sample was greater than three standard deviations above or below the established mean value, or if the mean values of two control samples were each greater than two standard deviations above or below the respective established mean value.

During the testing of IFLS study samples, UGM laboratory also measured CRP and HbA1c levels on UW validation samples on a weekly or biweekly basis. There were a total of seven repeated measurements for CRP levels and nine for HbA1c levels. The R-square for the relationship between DBS and UW plasma concentrations varied from 0.896 to 0.958 (Fig. 3). For HbA1c, the range of R-square was from 0.985 to 0.994 (Fig. 4). There is no tendency for these R^2s to fall over time.

Figure 3. The correlations between IFLS DBS and UW plasma CRP concentrations from validation samples

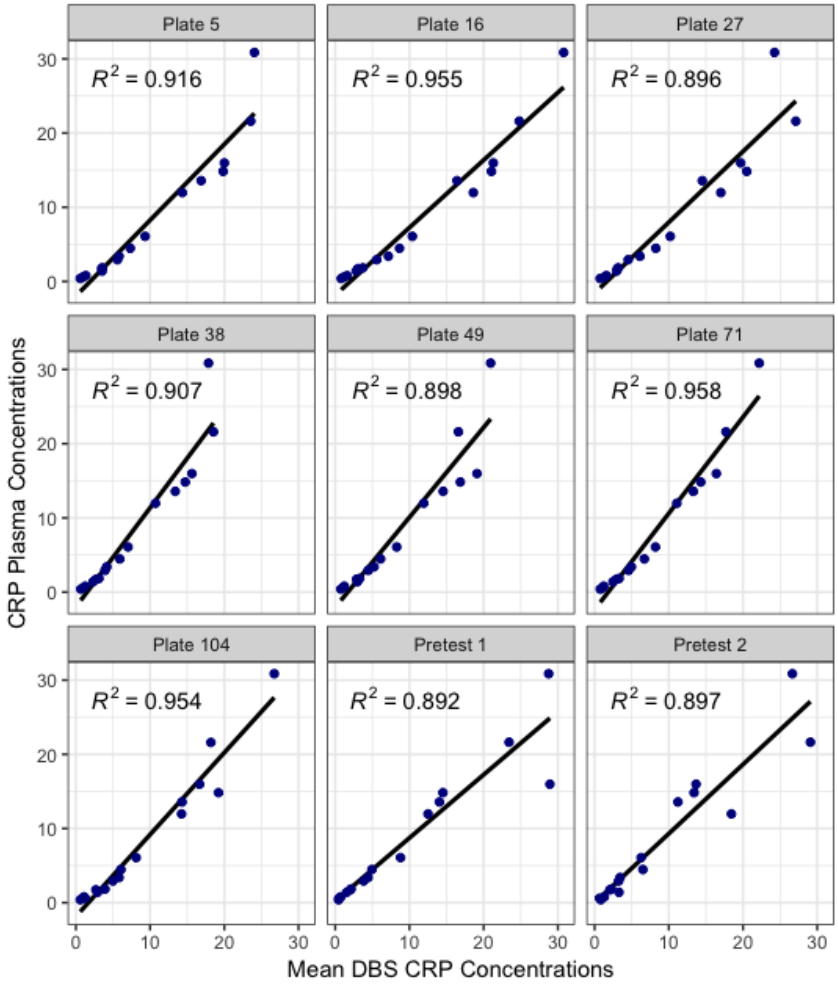
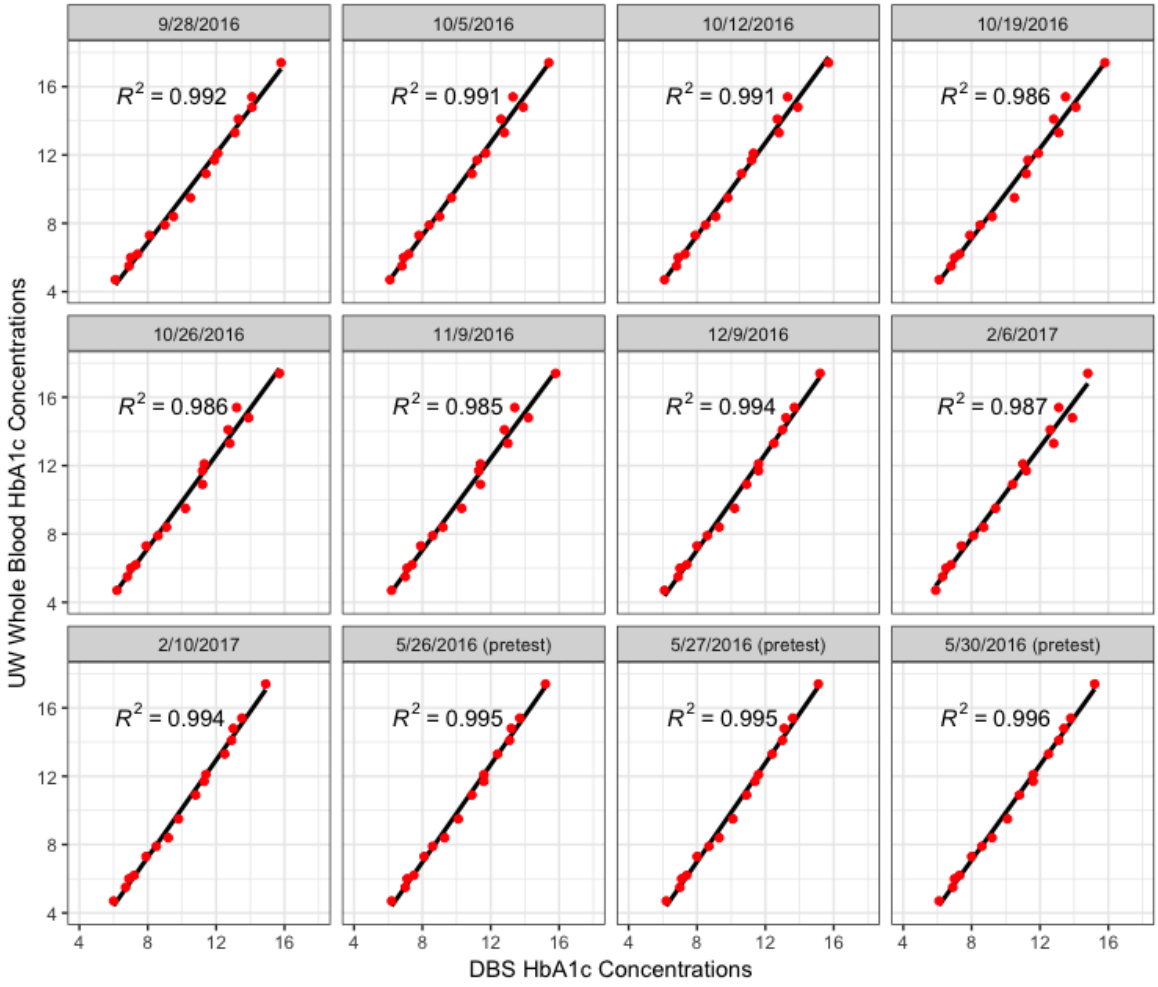


Figure 4. The correlations between IFLS DBS and UW whole blood HbA1c concentrations from validation samples



E. Conversion of DBS results to plasma-equivalent values for CRP and whole blood equivalent values for HbA1c

E.1 CRP

Based on corresponding DBS and plasma values from repeated measurements of UW validation samples, we have generated a regression equation to allow conversion from IFLS DBS values to plasma.

Ln(CRP plasma equivalent) = 1.192*Ln(CRP DBS value) – 0.684

Even though the equation may be used to convert DBS values to CRP plasma equivalent, the absolute equivalent values generated may not be entirely representative of those that would

be obtained from venous blood, because the number of UW validation samples is limited, particularly at lower CRP levels. To get appropriate CRP measures, we have performed log transformation of CRP values, because the distribution is right-skewed. We then converted the predicted log plasma equivalent values back to the original scale by taking an anti-log.

E.2 HbA1c

Conversion of the HbA1c values reported by the Bio-Rad D10 HPLC involved additional steps. Results of a validation study conducted in the same lab with the same assay using DBS collected in Aceh, Indonesia, indicate that relative to gold-standard venous-blood values, values reported by the HPLC are significantly upward biased, (Thomas et al, 2018). The HPLC separates hemoglobin moieties detected in the DBS for each subject and generates a chromatogram which displays the concentration of hemoglobin components as empirical density functions arrayed against the retention time taken to elution. Inspection of the chromatograms indicates the HPLC excludes part of the hemoglobin A which is misidentified as a hemoglobin variant. The validation study established it is possible to calculate unbiased values of Hba1c using data from the machine-generated chromatograms. Specifically, the percent HbA1c was calculated as the ratio of the area under the A1c curve to the total area of all HbA curves up to and including Ao (but excluding fetal Hb) for each sample (Thomas et al., 2018). The resulting ratios are adjusted to take into account the HPLC-specific calibration curve and converted to whole blood equivalent values using the equation:

HbA1c whole blood equivalent = 1.44*recalculated HbA1c DBS value – 0.62

These revised whole blood equivalent values, a1c_rev, are included in the public use dataset.

The HbA1c assay protocol calls for re-assaying values of HbA1c that fall below 4.0%.

This protocol was followed for the HbA1c values reported by the HPLC but because those values are upward biased, we missed a small fraction of the recalculated HbA1c cases that were below 4%. After inspection of the distribution of these values, we identified those cases for which the recalculated HbA1c value is less than 3.5% and suppressed the specific value, classifying them as missing indicated by assigning them a value of 3 in the variable a1c_revx. We believe that it is reasonable to assume these values are around 4%. The variable a1c_revx takes the value 1 if there is a valid value of HbA1c for a target respondent, 2 if there is a valid HbA1c value (also CRP value) for a respondent who was not a target (and therefore has no blood sample weight), 3 if they have a recalculated HbA1c value less than 3.5%, and 9 if it was not possible to extract a valid HbA1c value from the DBS.

E.3 Interpretation of CRP and HbA1c levels and cut-offs

Because we are more confident about the relative order of CRP or HbA1c levels within the IFLS sample rather than the absolute equivalent values, we would recommend using DBS values for most analyses, unless users want to use clinically established plasma based or whole blood based cut-off points for categorical analysis (such as 3.0 mg/L for CRP or 6.5% for HbA1c), or make cross-country or cross-wave comparisons that involve assay results from venous blood. In those cases using the whole-blood equivalents for HbA1c or the plasma-equivalents for CRP is appropriate. We recommend that users be cautious in making prevalence estimates for both HbA1c and CRP since our estimates are likely to have more random error than venous blood samples collected in clinic-type settings.

Figures 5 and 6 show Bland-Altman plots for the validation sample, plus plots (and a regression line) between the University of Washington plasma (for CRP) or whole blood (for HbA1c) values, and DBS concentrations from the laboratory at Gadjah Mada. For CRP while

most observations are within the 95% confidence interval lines, for high levels of CRP some are outside, with the UW plasma readings being higher than the Indonesian DBS ones. However, both are high. For HbA1c, while most observations are within the confidence limits, there is a slope. For low levels of HbA1c, DBS values are higher than whole blood values, while for high levels it is the reverse, whole blood levels are higher. Again, even if the DBS values are understated for high levels of HbA1c, they are still quite high relative to standards of normal.

If you want to compare wave 5 CRP data to Wave 4 data you should use the plasma equivalent value for Wave 5 and the serum equivalent value for Wave 4, assuming equality of plasma and serum CRP concentrations. There is a difference in the assay methods between the two waves that makes some difference in the assay value. In order to determine this difference we re-assayed 216 frozen DBS samples from wave 4 using the wave 5 assay. The results showing the two values are in Figure 7 below which compares the original assay with that from the new assay of the stored sample. The R^2 relating the two is 0.87 and the equation is:

Wave 5 Plasma Equivalent value= 1.228 * Wave 4 serum equivalent value + 0.312

The wave 5 plasma equivalent values generated using this equation have been put on the 2007 blood user data file: crp_public_use.

F. Description of CRP and HbA1c data from IFLS-5

Table 2 contains weighted descriptive statistics for both CRP and HbA1c, in both the DBS concentrations and for CRP in plasma equivalent and for HbA1c in whole blood equivalents. Figure 8 shows the smoothed density plot for the logged plasma equivalent of CRP and Figure 9 the same for the whole blood equivalent of HbA1c. For log CRP the small peak on the left tail arises because there were a small number of cases for which the assay showed undetectable CRP level. We know that the true values are extremely low, not missing,

so we assigned an arbitrarily low value for these (0.001 in unlogged value). For HbA1c there is a long, thin right tail to the distribution.

G. DBS data file

The data file for the DBS data, wave5_dbs_public_use.dta (the STATA file), has the following variables:

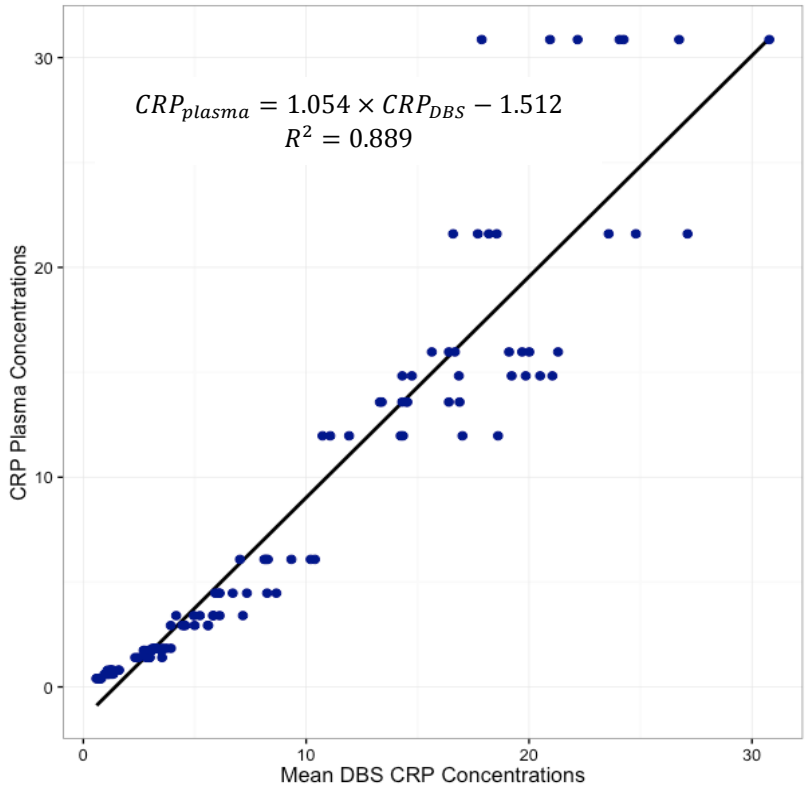
hhid14	HHID 2014
pid14	PID 2014
pidlink	Pidlink
a1c_dbs	HbA1c – DBS
crp_dbs	CRP – DBS
a1c_revx	Valid HbA1c value
a1c_rev	HbA1c - whole blood equivalent (revised)
crp_plas_equi	CRP - plasma equivalent
ln_crp_plas_equi	log CRP-plasma equivalent
notin2007	Not assayed in 2007
pwt14DBSXa	IFLS5 person X-section DBS (assayed) w/ attrition correction
pwt14DBSLa	IFLS5 DBS 2007-2014 longitudinal weight, w/attrition correction

Table 2. Descriptive Statistics of Biomarkers in Entire IFLS Wave 5 (Weighted)

	N	Mean (SD)	Median	5% pct	25%	75%	95%	Ranges	% with High Risk Levels (>= 3 mg/L)
CRP DBS concentration	7,470	2.89 (5.09)	1.33	0.15	0.55	3.36	10.35	0.01-200	
CRP Plasma Equivalent	7,470	2.11 (5.33)	0.71	0.05	0.25	2.14	8.18	0.0001~278.82	18.0%

	N	Mean (SD)	Median	5% pct	25%	75%	95%	Ranges	% with High Risk Levels (>= 6.5%)
HbA1C DBS concentration	7,416	7.69(1.01)	7.5	6.5	7.1	8.0	9.3	4.1-18.2	
HbA1c Whole Blood Equivalent	7,347	5.49(0.96)	5.4	4.4	5.0	5.8	6.6	3.5-15.7	6.9%

Figure 5 Bland-Altman CRP Plots



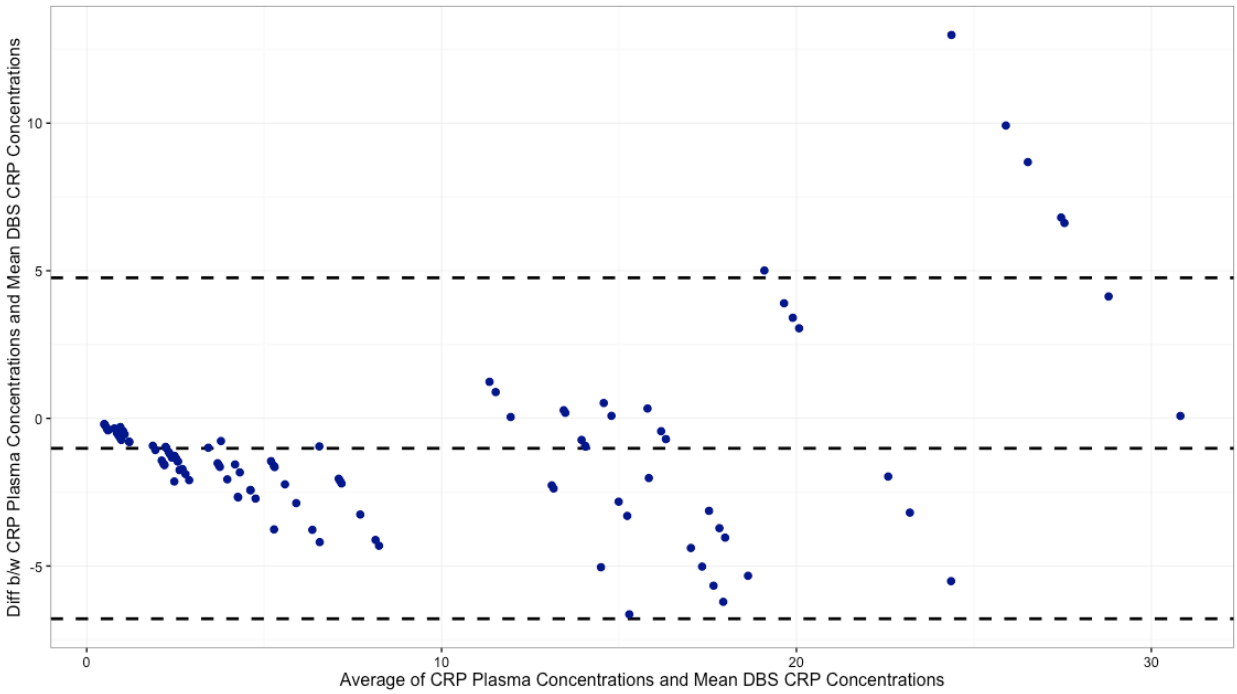
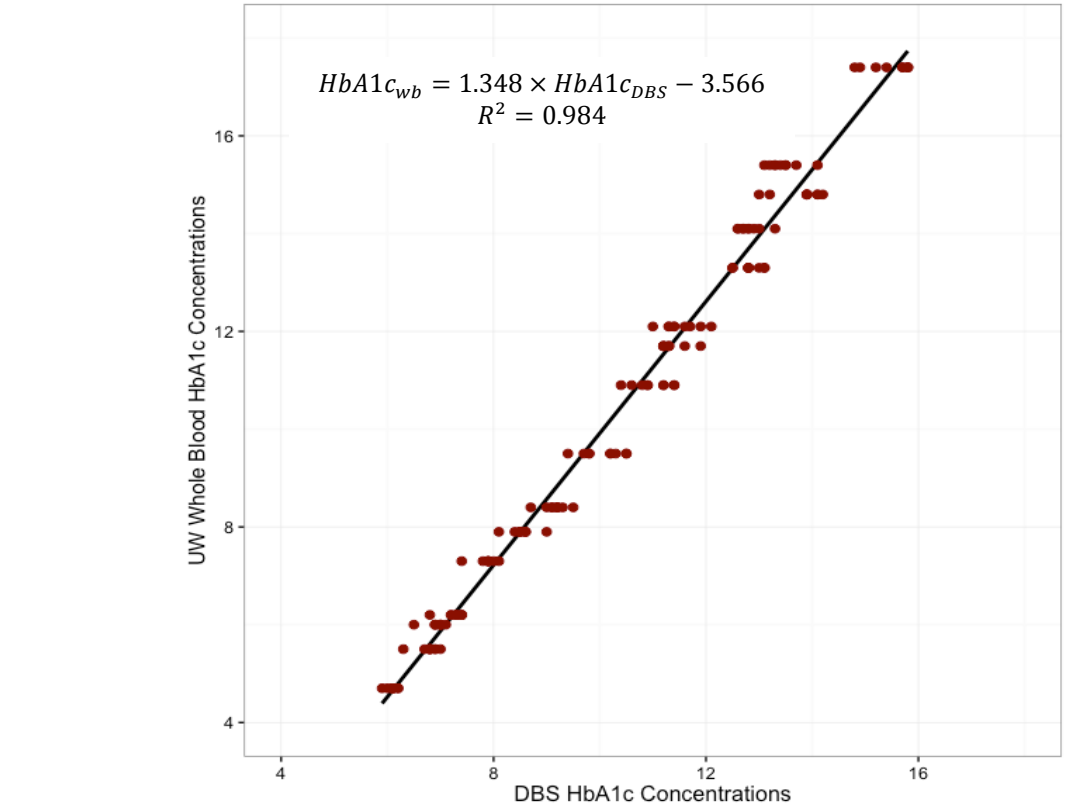


Figure 6 Bland-Altman HbA1c Plots



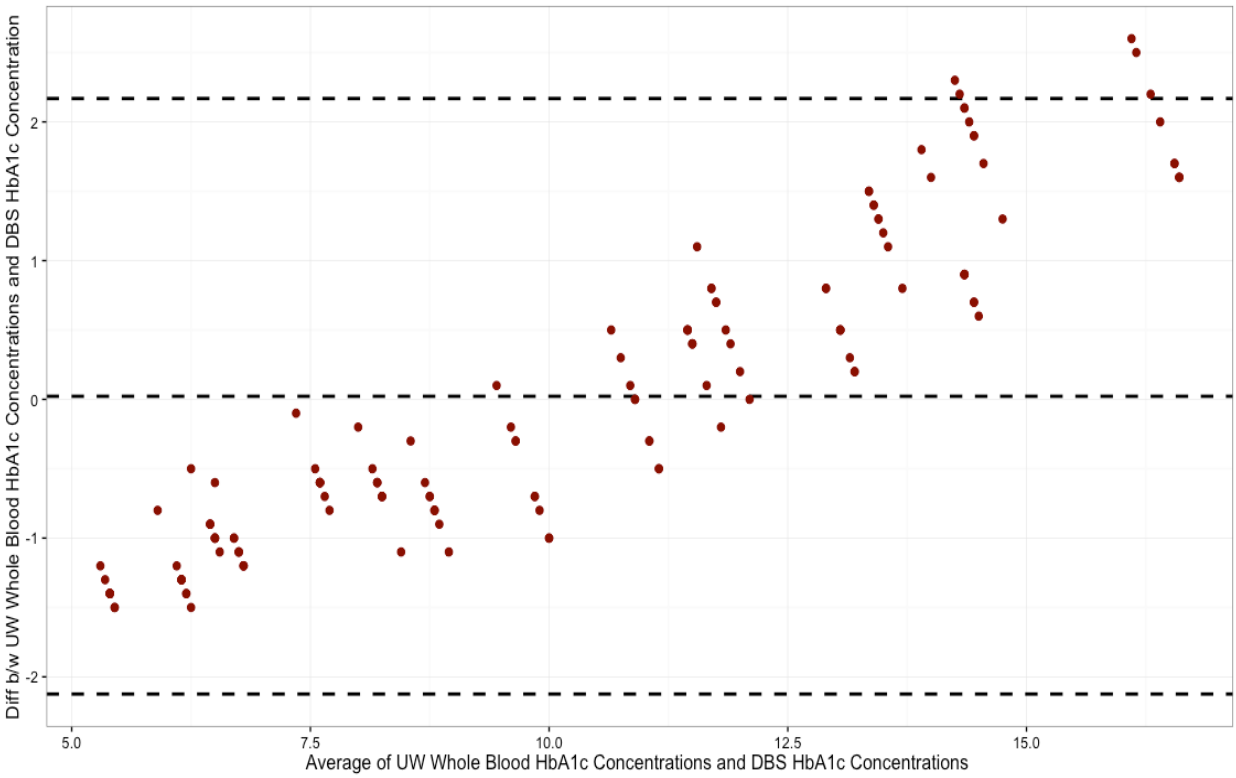


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Figure 7: Stored Wave 4 DBS assayed for CRP at Wave 5 and Original Assay Value

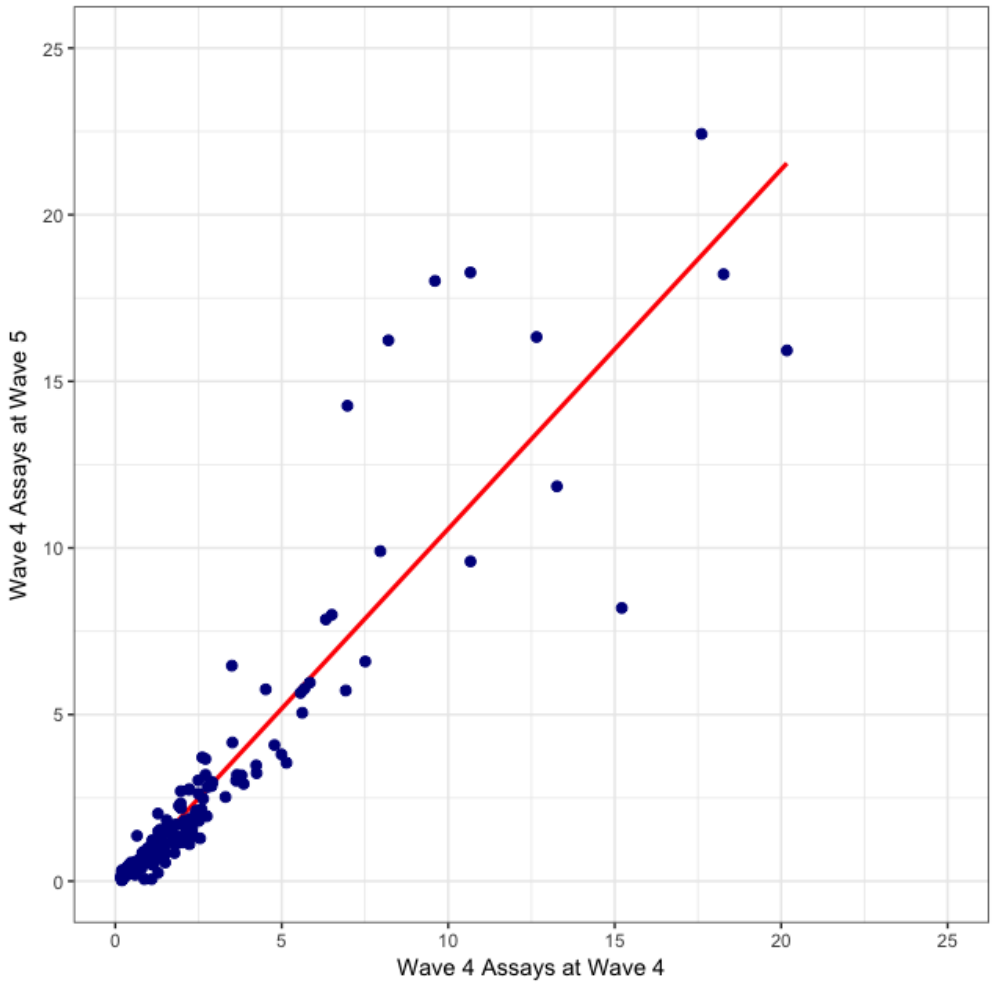


Figure 8 Mean Logged Plasma Equivalent CRP distribution (with weight)

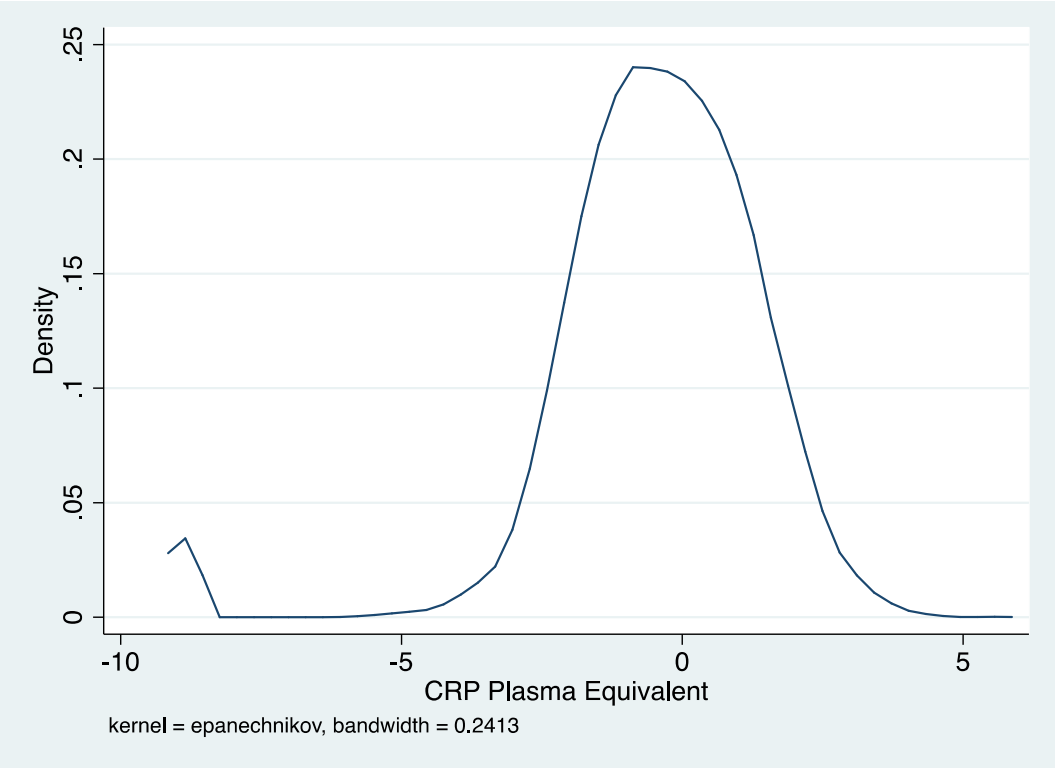
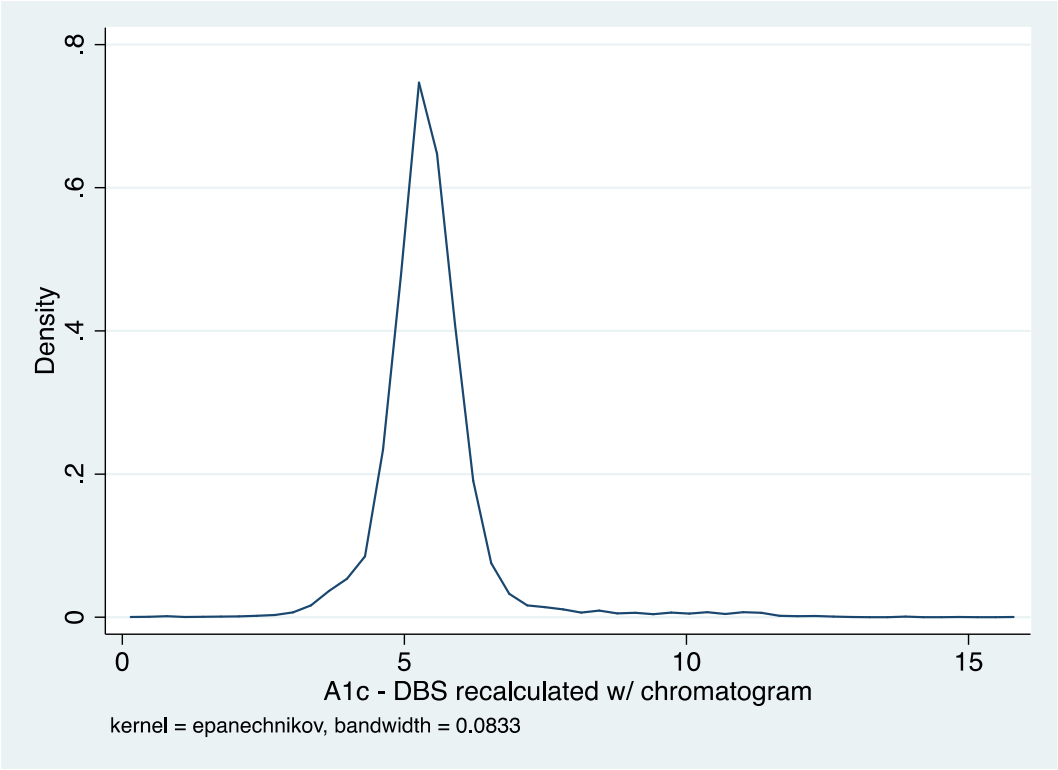


Figure 9 Mean Whole Blood Equivalent HbA1c distribution (with weight)



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