

36 Dried blood spot samples and their validation

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- ▶ Laboratory results from the DBS assays cannot be directly compared with the results that would be obtained from assays of venous plasma samples using standard laboratory methods
 - ▶ Nevertheless, we can reliably measure A1c, HDL, CysC and CRP from DBS samples. Taking into account the blood type and the various treatment effects is very important
 - ▶ Total cholesterol is by far the weakest of the seven analytes, but the qualitative information contained in the total cholesterol values is still useful
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36.1 Introduction

The key innovation of Wave 6 was the sampling of dried blood spots (DBS) in the large international population survey of SHARE using lay interviewers. This endeavour was truly cutting-edge.

From a laboratory point of view, venous blood samples are best suited for analysing blood biomarkers. However, collecting venous samples in very large community-based settings in as many countries as in SHARE entails major logistical challenges related to collection, transportation and timely laboratory analyses, as well as significant expenses for venipuncture by certified health professionals. Moreover, a venipuncture is invasive and may harm the individual. Venipuncture may also be regarded as intrusive by respondents, thus yielding higher proportions of non-participation.

Instead of drawing venous samples, SHARE decided to collect blood using a method more adaptable to the circumstances of a very large international population survey. The DBS sampling is such a method. DBS samples are drops of capillary whole blood collected on filter paper from a simple finger prick. One of the best-known DBS applications is the screening test for phenylketonuria in new-borns for which DBS sampling is a minimally invasive method relative to collecting venous blood. In new-borns, the drop of blood is collected from the heel, but the same technique can be applied to adults' fingertips and may be carried out in a home setting by trained survey interviewers. Blood collected on filter cards can be shipped by regular mail to a laboratory and stored at -20°C (some store them at -80°C) until analyses, making DBS samples ideally suited for large population-based studies such as SHARE.

SHARE has chosen two sets of biomarkers to be assayed from the DBS samples. Priority in the selection process was given to their relevance in evidencing typical diseases at older age, such as cardiovascular disease (CVD), cancer, diabetes and cognitive decline. Additional criteria were the existence of suitable assays (i.e. reagents and procedures to detect the biomarker) and the availability of comparative population values from (inter)national health registers (e.g. the Robert-Koch-Institute in Germany, Eurohealth or WHO) and other population surveys, including HRS and its sister studies. Selection was also guided by the ability to share a DBS extract across multiple analyses, thereby limiting the amount of blood needed. The first set contains markers used in routine blood analyses. For these markers, reference values from venous blood samples are well established and include the following.

1. *Glycosylated haemoglobin* (HbA1c or A1c) is a marker for diabetes. An excess of sugar molecules in the blood irreversibly bind to haemoglobin. The so-formed A1c signals longstanding and chronically high levels of blood sugar.
- 2–4. *High density lipoprotein* (HDL) cholesterol, (total) cholesterol (TC) and triglycerides (TG) are molecules of the lipid panel and important players in lipid metabolism, such as serving as building blocks for cells and transport molecules for lipids. Imbalances in lipid metabolism lead to various diseases of the cardiovascular system.
5. *Cystatin C* (CysC) is a marker for kidney function and CVD. CysC, though a measure for the clearance of degradation products from blood, also signals risk of CVD. Those with elevated cystatin C levels have been shown to be at highest risk for CVD, even when kidney dysfunction is mild; those with the highest levels of CysC are older and have hypertension, dyslipidaemia, high BMI and higher levels of CRP.
6. *C-reactive protein* (CRP) marks the general level of inflammation in the body caused by acute infections or chronic diseases. Inflammatory processes are involved in CVD, diabetes, obesity and cognitive decline.
7. *Total haemoglobin* (tHb) is a marker of anaemia that indicates a decrease in red blood cells or haemoglobin, thereby lowering the ability to carry oxygen in the blood. Anaemia may arise from loss of blood, pathological removal of blood cells, diseases of the haematopoietic system, chronic inflammatory diseases, kidney disease, wasting diseases (e.g. cancers) and more.

Approximately 8,000 probes from SHARE respondents were assayed in the United States at the University of Washington (UW) in Seattle for these markers.

The second set of biomarkers is more innovative and was assayed at the *Statens Serum Institut* (SSI) in Copenhagen. This set is mainly comprised of a large set of cytokines, which are small blood-based proteins prominently

involved in inflammatory processes. Their selection and importance is described by Borbye-Lorenzen and Börsch-Supan in Chapter 39 of this volume.

- 8–12. The proteins *IL-8*, *IL-16*, *IL-18*, *IL-12/23p40* and *MCP-1* indicate an inflammation occurring in the body; all are pro-inflammatory markers but act in different tissues. Elevated blood levels of either one or several of these cytokines will better inform us about the type of inflammation and its degree; they may confirm the report of, for example, CVD or atherosclerosis by a respondent or point to yet undetected inflammation, cancer and/or (onset of) cognitive impairment.
- 13–14. *Vascular epithelial growth factor (VEGF)* and *epidermal growth factor (EGF)* are proteins involved in normal and pathologic cell and tissue growth. They are needed in healing (e.g. in blood vessels) but may also indicate proliferating tissue in the case of cancer.
15. *Brain-derived neurotrophic factor (BDNF)* is another growth factor. BDNF acts on neurons of the central and peripheral nervous system, supports the survival of neurons and encourages growth of neuronal tissue. BDNF is involved in learning and memory. Its level increases with exercise and social embeddedness. In turn, the BDNF blood level is lowered in cognitive decline.
16. *Apolipoprotein E (ApoE)* belongs to a class of proteins involved in the metabolism of fats in the body and is a component of the lipid panel. ApoE mediates the cholesterol metabolism, transports cholesterol to neurons and is the principal cholesterol carrier in the brain. ApoE is important in Alzheimer's and cardiovascular diseases.
17. *Clusterin* has the general vital function in the organism to clear misfolded proteins or cell debris; through this function, clusterin is involved in ageing and many diseases related to oxidative stress, including neurodegenerative and inflammatory diseases and cancers.
18. *Vitamin D (VitD)* is essential for several biological processes. Being deficient is associated with mortality and diseases, among them CVD but also functional loss from lower muscle function and muscle mass (sarcopenia), affecting postural stability. Additionally, osteoporosis can be caused by low VitD because it is essential for the absorption of calcium from the diet.

Laboratory results from the DBS assays cannot be directly compared with the results one would obtain from assays of venous plasma samples using standard laboratory methods (Crimmins et al. 2013; Karvanen 2003), which is particularly relevant for the first set of markers for which well-established thresholds exist. Although 'gold standard' values also have measurement

variations, DBS values of, for example, total cholesterol – known to be particularly difficult to measure from DBS samples – have both a larger mean and a larger variance, systematically influenced by many laboratory and fieldwork-related factors. Figure 36.1 shows how these differences are generated.

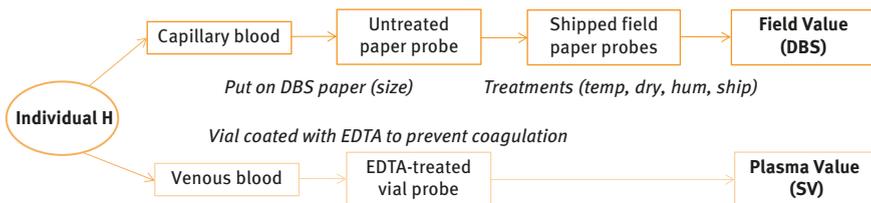


Figure 36.1: DBS fieldwork setting versus gold standard clinical setting.

Source: Own illustration.

In the centre of Figure 36.1 is a donor with characteristics H (e.g. health, age, sex) who donates both venous blood and capillary blood. The upper part of Figure 36.1 refers to the process of how capillary blood samples donated by the SHARE respondents were collected in the field and then shipped to be analysed in the two laboratories (UW and SSI): the capillary blood from the finger prick is dropped on paper, dried and later shipped to the lab under potentially unfavourable conditions T (e.g. temperature, humidity, drying time, shipment time, spot size). The sample is finally analysed to yield a value called DBS. The lower part refers to the collection of venous blood in a typical clinical setting. The venous blood from the donor is collected in a vial coated with EDTA (ethylenediaminetetraacetic acid, a chemical that prevents the coagulation of blood) and is then analysed in the lab, yielding a value SV , considered the gold standard of medical measurement.

The field-measured value of DBS is not equal to the gold-standard value of SV for three reasons. First, the DBS samples collected in the field have different sizes and are exposed during shipment to varying temperatures, humidity and other factors, called ‘fieldwork effects’. Second, the capillary blood of the donor is dried in the field and then re-liquefied in the lab, potentially creating chemical changes called the ‘dry-liquefy effect’. Finally, capillary blood given for DBS is a more heterogeneous mixture of arterial and venous blood, interstitial fluid and red blood cells, whereas venous blood from venipuncture is treated with EDTA to prevent coagulation, and most markers are analysed from only plasma. We call this difference the ‘blood-type effect’.

36.2 Fieldwork effects

We tested the influence of different fieldwork circumstances using data from the UW assays of approximately 8,000 probes that were linked to the SHARE data. Details are given in Chapter 37 of this volume. We regressed DBS on fieldwork conditions and donor health. We included interviewer fixed effects to isolate the fieldwork effects that hold across different interviewers or regions. Figure 36.2 shows that the influence of field conditions or sample quality is significant and differs among assays. Small spot size is the main challenge in the field, especially for TC and A1c, but not for TG, CysC or CRP. TC is also sensitive to high outside temperatures and long shipment times. HDL is sensitive to the combination of long shipment times and insufficient humidity protection. TG and CysC are also sensitive to short drying times. CRP and tHb seem relatively robust to field conditions and sample quality.

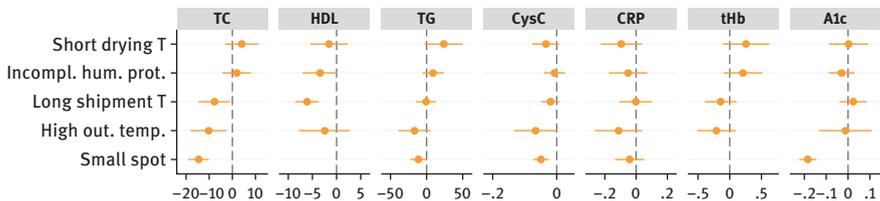


Figure 36.2: Influence of SHARE fieldwork conditions (N ~ 8,000).

36.3 Dry-liquefy effect

In previous studies, the dry-liquefy effect has been studied extensively at UW (e.g. Crimmins et al. 2013); the effect is small. UW has collected venous blood samples in EDTA tubes. Those samples were then analysed using conventional clinical chemistry analysers. In parallel, blood from each EDTA tube was dropped onto filter paper to create artificial DBS samples and then analysed similar to field-collected DBS. The relation is linear and tight Table 36.1.

Table 36.1: Lab-created DBS samples from venous blood versus gold standard.

	A1c	TC	HDL	TG	CysC	tHb	CRP
Tightness of fit (R^2)	0.99	0.86	0.87	0.95	0.91	0.99	0.87

36.4 Blood-type effect

EDTA is known as a preservative for cholesterol and various haematological assays, but the implications of the blood-type effect for the assays have yet to be studied. The implications can only be measured in combination with the dry-liquefy effect (Figure 36.1). We dropped both capillary blood and EDTA-treated venous blood from the same donor onto filter paper under lab conditions and compared the obtained values. We also compared the lab-created DBS values (i.e. without treating them in other fieldwork and shipping conditions) with the gold standard SV. Table 36.2 shows tight correlations for all markers except for TC and tHb, after controlling for spot size, age, sex and BMI, which substantially improves the fit to the gold standard SV.

Table 36.2: Lab-created DBS samples from capillary blood versus gold standard.

	A1c	TC	HDL	TG	CysC	tHb	CRP
Tightness of fit (R²)	0.96	0.60	0.85	0.98	0.92	0.36	0.997

36.5 Polish nurse experiment

The main challenge is now to set up a translation formula that takes into account donor and fieldwork conditions and converts the DBS values into gold standard SV values. The ideal way to conduct such a validation study is to obtain both DBS and venous blood samples in the field. We conducted such an experiment in a small sub-study in Poland in which nurses took both DBSS and venous blood samples that were then assayed for A1c, TC, HDL, TG and CRP. This process is described in detail in Chapter 38 in this volume. Although the distributions of the DBS laboratory results are different from the distributions of the results obtained from plasma values (wet blood values in case of A1c), conversion formulae that include fieldwork conditions produced a very good fit between plasma and DBSS values for all markers (Table 36.3).

Table 36.3: DBS versus SV under actual field conditions.

	A1c	TC	HDL	TG	CysC	tHb	CRP
Tightness of fit (R²)	0.95	0.67	0.92	0.95	n.a.	n.a.	0.99

36.6 Simulating fieldwork conditions

Because the sample was very small, these results are only indicative. Therefore, we replicated this experiment on a much larger scale in the UW lab in which we simulated field conditions. We took venous blood from approximately 50 donors, used it to create approximately 3,700 DBS samples of three different sizes on filter paper and then exposed the DBS samples to a large number of combinations of different drying times, humidity protections, outside temperatures and shipment times that are typical of the conditions experienced by field-collected DBS during collection and shipment in SHARE. We then compared the treated DBS values with the gold standard values, thereby conditioning on the treatment variables, their interactions and the available donor characteristics.

Figure 36.3 shows the results for the four markers TC, HDL, CysC and tHb. The light orange bars show the significant variation in the raw laboratory values from DBSS and their distance from the dashed equality line. The grey values use a simple bivariate regression while the darker orange values are based on estimations accounting for the simulated fieldwork conditions and donor characteristics.

36.7 Combining the components

By combining all components (treatment effects, dry-liquefy effect and blood-type effect), we obtain a conversion formula that translates the DBS values obtained under simulated fieldwork conditions into gold standard values. The prediction accuracy of this conversion formula is high for A1c, HDL, CysC and CRP (Table 36.4). Prediction accuracy is lower for TG and tHb in one component but high in the other, whereas TC is weak in both components.

Table 36.4: Prediction accuracy.

	A1c	TC	HDL	TG	CysC	tHb	CRP
Blood-type and dry-liquefy effect (R^2)	0.97	0.76	0.82	0.97	0.89	0.79	0.99
Treatment effects (R^2)	0.87	0.79	0.94	0.75	0.95	0.98	0.97

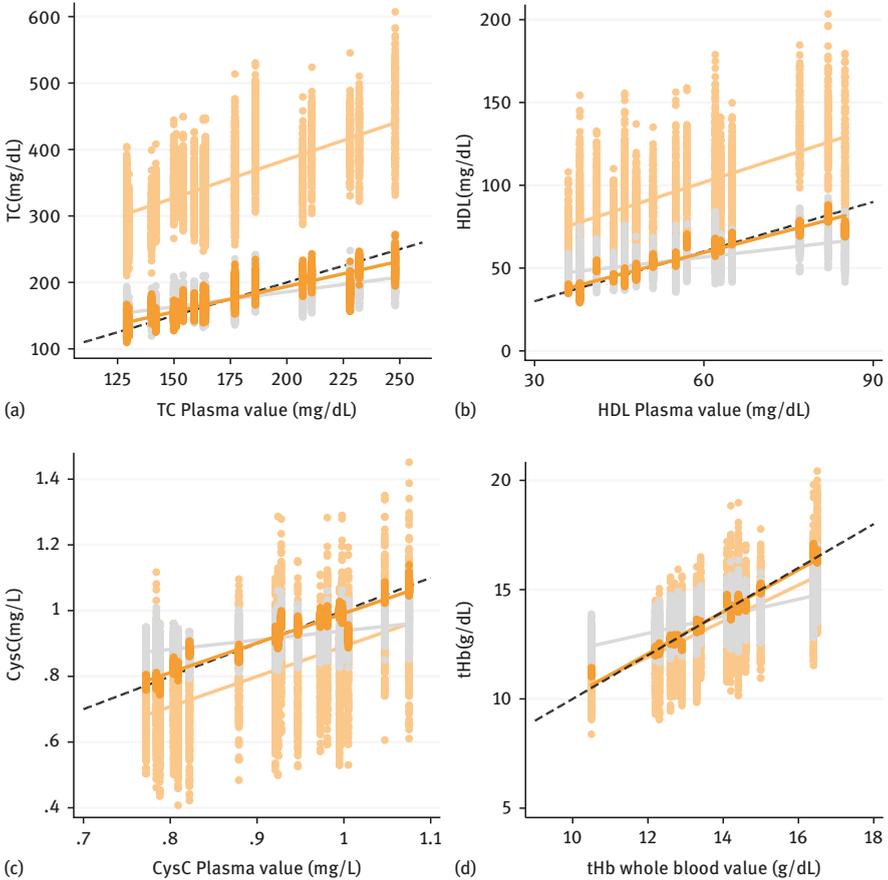


Figure 36.3: Adjusting for simulated fieldwork conditions (N ~ 3,700).
Note: For more details and legend of colours: see text.

We finally applied this conversion formula to the values obtained in the Polish nurse experiment to test its validity. Figure 36.4 shows the original DBS values (light orange) and the values obtained from the conversion formula (orange) on the vertical axis plotted against the actual gold standard values taken from plasma on the horizontal axis. The grey dots represent the predicted values if the blood-type effect is ignored. Note that the plasma values were obtained from a laboratory in Poland with different analysers from those used in our UW-based validation studies and that the circumstances of the transportation of the venous blood in Poland generated additional variation in the plasma values. Additionally, not all

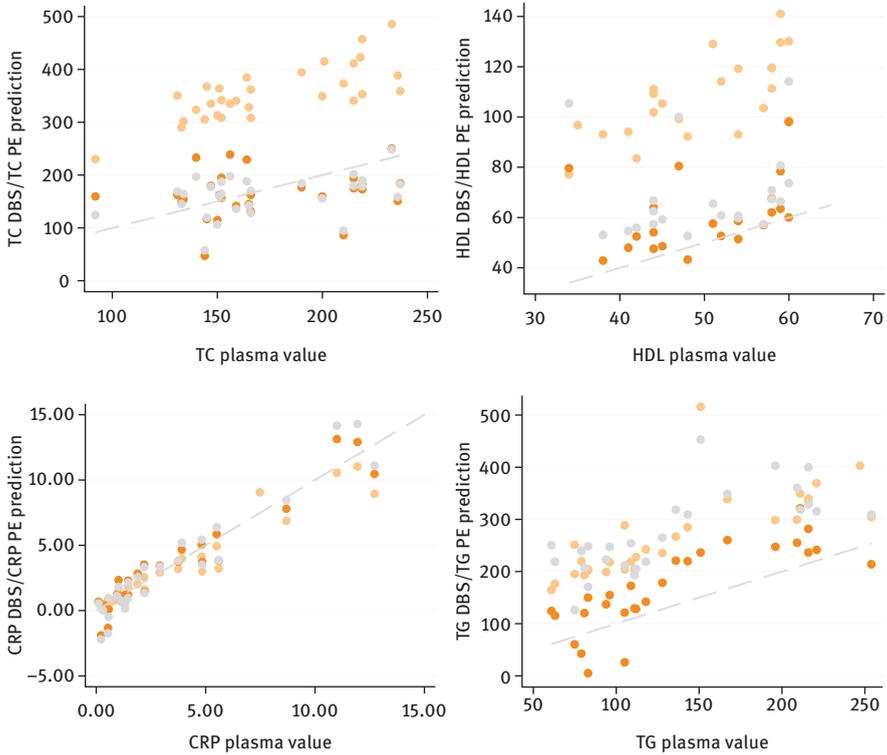


Figure 36.4: Raw and estimated gold standard values versus actual gold standard values.

Note: For more details and legend of colours: see text.

fieldwork conditions previously mentioned could be used to create the used formula because not all of them were observed in the Polish nurse experiment. Nevertheless, we observe a good convergence to the dashed equality line.

36.8 Conclusions

The overall conclusion from our structured validation experiments is that we can reliably measure A1c, HDL, CysC and CRP from DBS samples. We also learned that it is very important to take into account the blood type and the various treatment effects. The treatment effects are somewhat less accurately

predicted for TG and the blood-type effect for tHb. As expected, TC is by far the weakest of the seven analytes, but the qualitative information contained in the TC values is still useful.

References

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