Optimization of DNA extraction from dried blood spot samples for use in a telomere length assay

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Research Objectives

In collaboration with colleagues at the World Health Organization (WHO), we are conducting a longitudinal study on global AGEing and adult health (SAGE). The SAGE project seeks to investigate patterns and determinants of aging in individuals around the world. Dried blood spots (DBS) are currently being collected from adults in six middle-income countries. These DBS will then be analyzed for a variety of biomarkers, including telomere length (TL).

Before we can measure TL in our samples, it is first necessary to assess if genomic DNA extracted from DBS cards is sufficient for use in TL assays. Cawthon's quantitative real-time PCR method for measuring TL (Cawthon, 2009) used 20ng of DNA per reaction, although others have had success using less (Chae et al., 2014). However, it is unclear if six punches are necessary and if subtle variation in storage conditions, collection protocols and processing techniques influence DNA yield and downstream assays.

We sought to determine if differences in storage conditions and processing techniques affect DNA quantity. In order to do this, we tested the influence of 3 variables on DNA yield from DBS:

- DBS card storage (-20°C vs. -80°C)
- spot size (25uL vs. 50uL)
- number of punches per extraction (3, 4, or 6 3.2mm punches)

Methods

Venous blood was collected from 5 adults as part of an IRB approved validation study. For each individual, we immediately pipetted the whole blood onto eight Whatman Protein Saver 903 DBS cards. Cards were then dried at room temperature. In order to test if drop size influences DNA quantity, we used 25uL drops for half of the cards (n=4) and 50uL drops for the remainder (n=4). We then stored two of each of these sets of cards at -20°C and two of each of these sets of cards at -80°C for two to four months.. For each freezer condition (-20°C vs. -80°C) and volume condition (25uL vs. 50uL), three, four and six 3.2mm punches were taken from the inner circle of the DBS and three, four and six 3.2mm punches were taken from the outer circle of the DBS (Figure 1). This protocol was repeated for each of the 5 individuals sampled.

Genomic DNA was then extracted from each sample using the QIAamp Mini Kit following the manufacturer's protocol. The amount of DNA present in each sample (ng/mL) was measured using a Qubit 2.0 Fluorometer. From this measurement, the total DNA yield was calculated based on the total amount of buffer the sample was eluted in. The samples were then analyzed using a univariate GLM. Results were compared to those obtained from a multilevel analysis and found to be similar (data not shown). Significance was determined by a p value ≤ 0.05.

Whatman Protein Saver 903 DBS Card

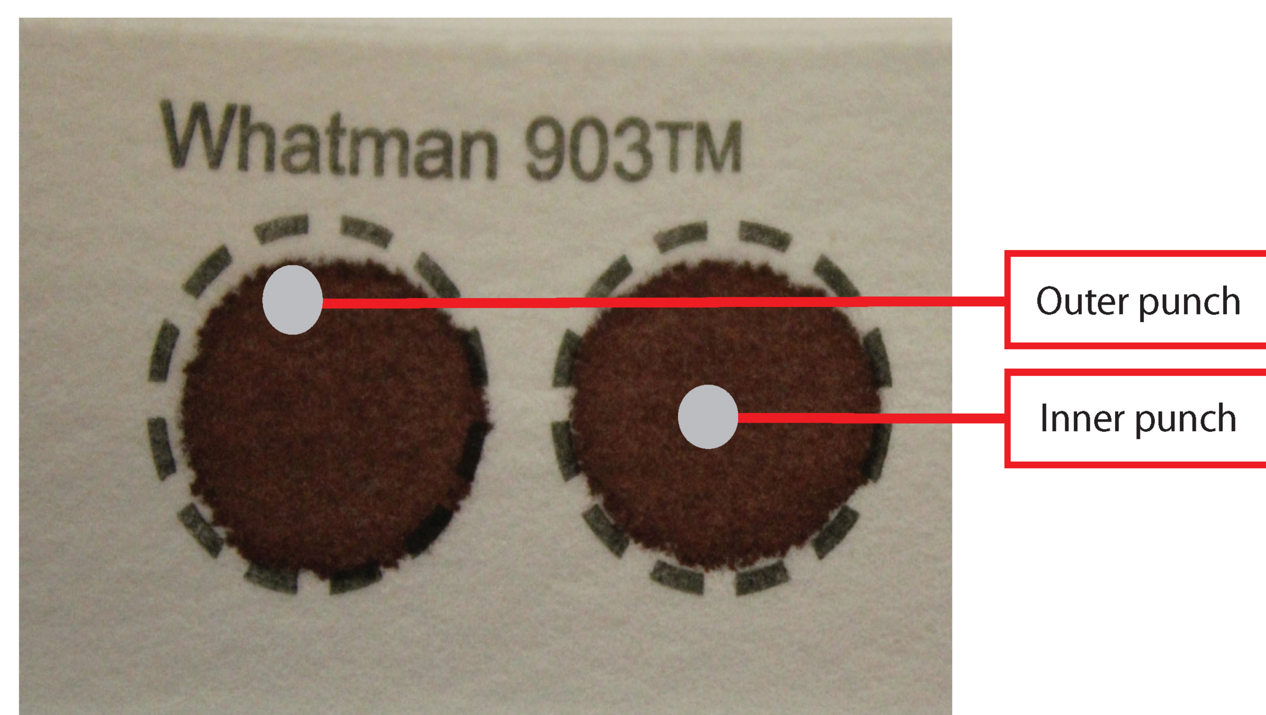


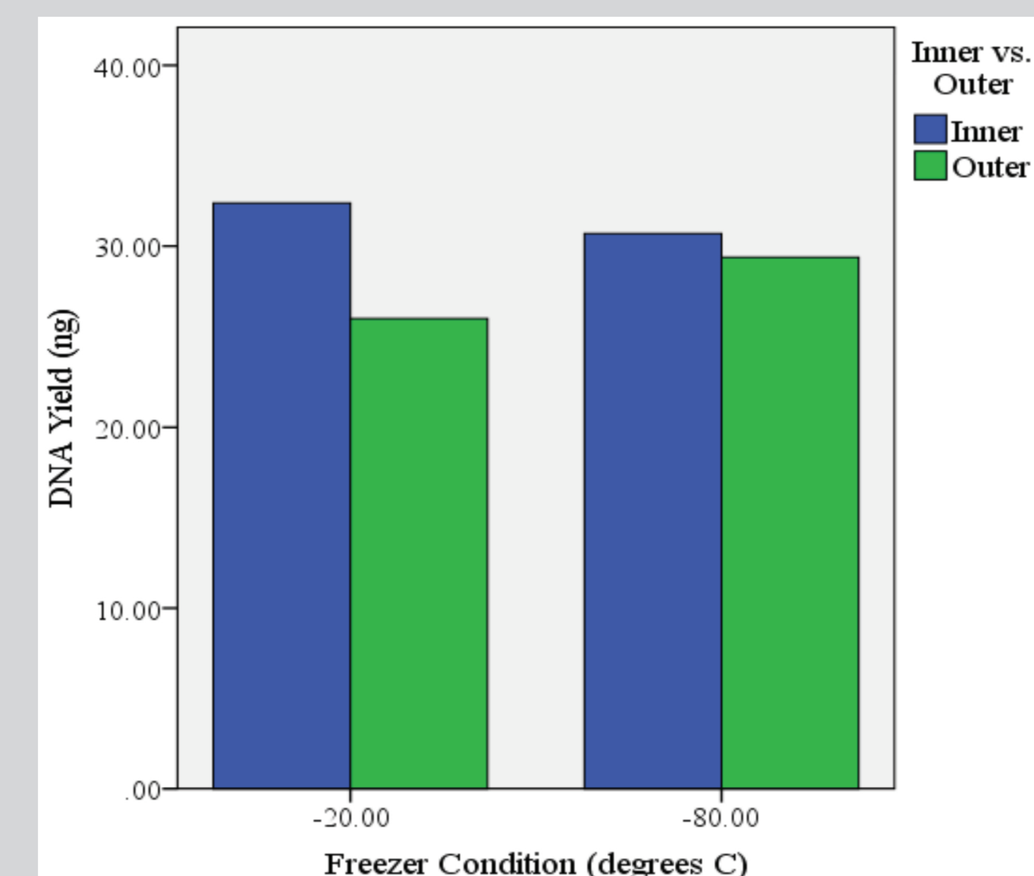
Figure 1: Inner vs. outer punch on a DBS card (50uL)

Results

Variables examined with average DNA yield.

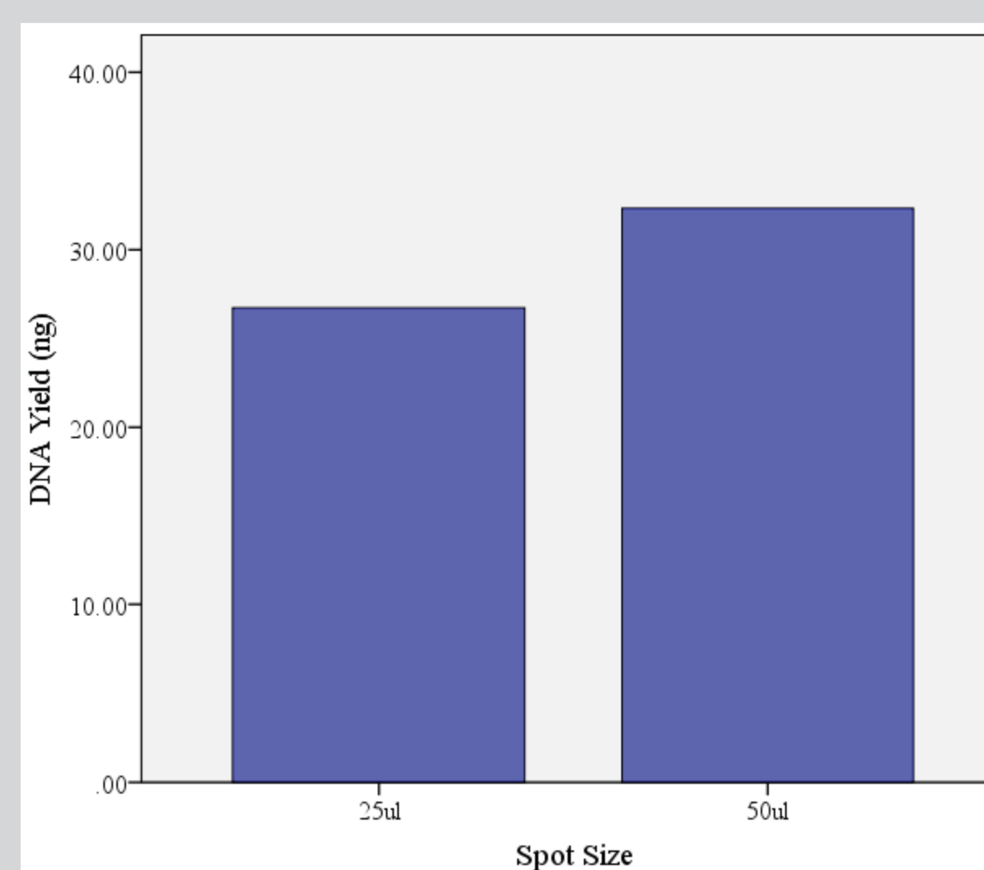
Freezer Temperature (°C)	Spot Size (uL)	Number of Punches	Punch Location	Average DNA Yield (ng; n=5)
-20	25	3	Inner	22.89
-20	25	3	Outer	12.49
-20	25	4	Inner	27.52
-20	25	4	Outer	20.25
-20	25	6	Inner	38.12
-20	25	6	Outer	27.84
-20	50	3	Inner	29.62
-20	50	3	Outer	22.15
-20	50	4	Inner	28.72
-20	50	4	Outer	29.7
-20	50	6	Inner	43.56
-20	50	6	Outer	43.71
-80	25	3	Inner	12.72
-80	25	3	Outer	18.83
-80	25	4	Inner	28.08
-80	25	4	Outer	23.75
-80	25	6	Inner	42.93
-80	25	6	Outer	34.17
-80	50	3	Inner	21.93
-80	50	3	Outer	17.36
-80	50	4	Inner	30.63
-80	50	4	Outer	32.13
-80	50	6	Inner	43.23
-80	50	6	Outer	45.27

Does freezer storage temperature affect the total DNA yield?



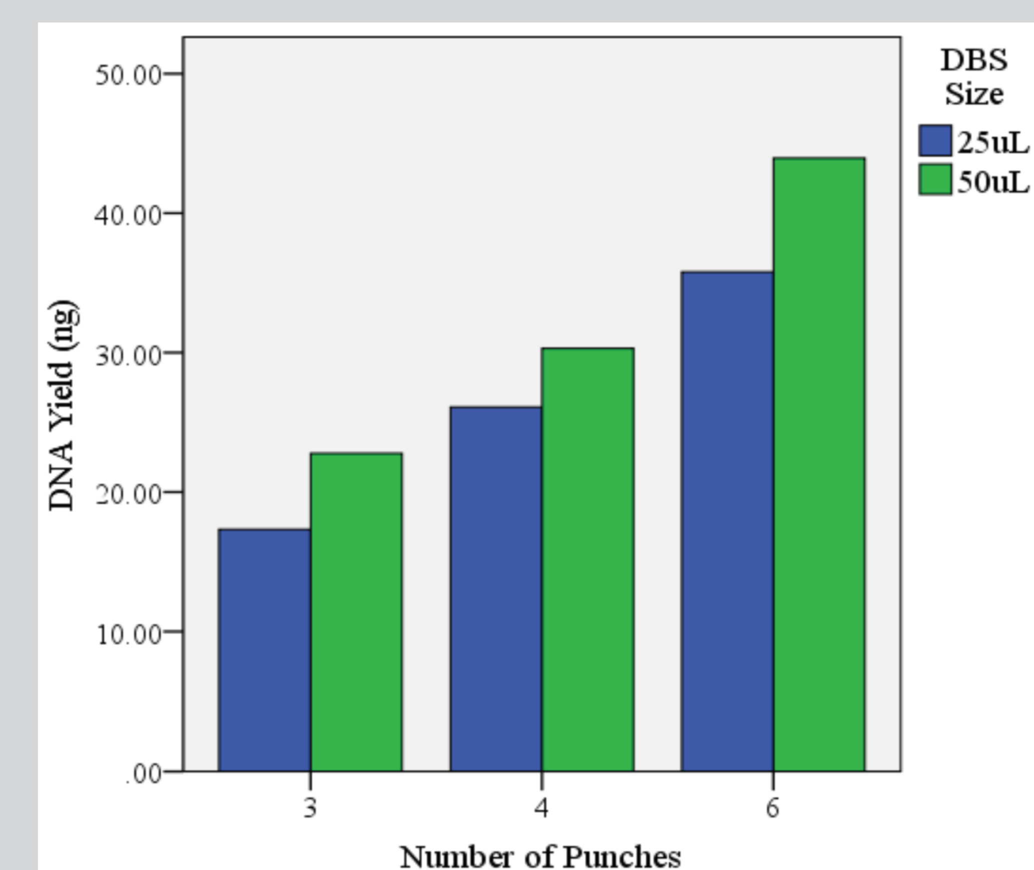
There was no significant difference in DNA yield between cards stored at -20°C and cards stored at -80°C, regardless of punch location.

Does DBS size affect the average DNA yield?



The amount of DNA extracted from 50uL DBS was significantly higher than the amount of DNA extracted from 25uL DBS (p=0.03).

Does number of punches affect DNA yield?



DNA yield increased with more punches ($p < 0.001$), regardless of DBS size.

Research Findings and Future Directions

This study sought to determine which conditions are optimal when collecting, storing and processing DBS that will be used for TL assays. Our preliminary results suggest that blood spot size and punch number significantly influenced DNA yield. More specifically:

- **Punches taken from 50uL spots provide significantly more DNA than 25uL spots.**
- **As expected, DNA quantity increased as punch number increased.**

In summary, our preliminary findings support that DBS cards can be used to extract genomic DNA. The most optimal conditions for extracting the largest quantity of genomic DNA from DBS are to utilize six 3.2mm punches from a 50uL DBS. Interestingly, our results also suggest that one could obtain the minimum amount of DNA necessary for running a TL assay using three DBS punches rather than six. However, DNA yield increases with an increase in number of punches. The next phase of our study will examine if DNA quality is affected by the variables tested in this study and more directly test how much DNA is required for a successful TL assay.

Acknowledgements

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References

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