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Use of dried capillary blood sampling for islet autoantibody screening in relatives: a feasibility study

Short title: Islet autoantibodies in dried blood spots

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Abstract

Background: Islet autoantibody testing provides the basis for assessment of risk of progression to type 1 diabetes. We set out to determine the feasibility and acceptability of dried capillary blood spot-based screening to identify islet autoantibody positive relatives potentially eligible for inclusion in prevention trials.

Methods: Dried blood spot (DBS) and venous samples were collected from 229 relatives participating in the TrialNet Pathway to Prevention Study. Both samples were tested for GAD, IA-2, and ZnT8 antibodies, and venous samples additionally tested for insulin autoantibodies and ICA. We defined multiple autoantibody positive as ≥2 serum autoantibodies, and DBS screen positive if ≥1 antibodies detected. Participant questionnaires compared the sample collection methods.

Results: Of 44 relatives who were multiple autoantibody positive in venous samples, 42 (95.5 %) were DBS screen positive and DBS accurately detected 145 of 147 antibody negative relatives (98.6%). Capillary blood sampling was perceived as more painful than venous blood draw, but 60% of participants would prefer initial screening using home finger stick with clinic visits only if autoantibodies found.

Conclusion: Capillary blood sampling could facilitate screening for type 1 diabetes prevention studies.
**Introduction**

Islet autoantibody testing provides the basis for assessment of risk of progression to type 1 diabetes, but screening generally requires venous blood sampling which can be traumatic for children [1]. Collecting capillary blood samples offers a potential alternative [2-4], and could also give additional flexibility for staff and, if samples can ultimately be collected at home, could mean that families recruited for screening would not need to come to a clinic, hospital or laboratory for venipuncture and therefore enhance recruitment. We set out to determine the feasibility and acceptability of sample collection using dried capillary blood spots (DBS), and to evaluate its performance in identifying multiple autoantibody positive relatives at increased risk of type 1 diabetes who would be potentially eligible for inclusion in TrialNet prevention trials. We envisaged DBS-based testing being used for first line screening with confirmation in a venous sample if an individual screened autoantibody positive.

**Research Design and Methods**

We recruited relatives of people with type 1 diabetes participating in the TrialNet Pathway to Prevention (PTP) Study at 15 TrialNet Clinical Centers in North America and Europe [5]. Recruitment was stratified by age to ensure that adequate numbers of young children were enrolled, and participants attending for semi-annual monitoring visits were preferentially selected to ensure inclusion of individuals positive for two or more islet autoantibodies [6]. Participants were asked to provide both DBS and venous samples at a screening or follow-up visit. All samples were collected by research nurses...
using standard procedures. Staff were trained to collect capillary blood samples using BD Microtainer® contact-activated lancets (Becton Dickenson, Franklyn Lakes, NJ) and were asked to fill 5 circles (diameter 1 cm) on filter paper (Whatman 903 Protein Saver card, GE Healthcare Bio-Sciences, Pittsburgh, PA), which was air dried before sealing in a plastic envelope and mailing to the laboratory. Venous samples were handled in accordance with PTP operating procedures.

Serum samples were tested using the established TrialNet strategy: screening for autoantibodies to GAD (GADA), islet antigen 2 (IA-2A), and insulin (IAA) with supplementary testing for zinc transporter 8 antibodies (ZnT8A) and islet cell antibodies (ICA) if any autoantibodies were positive on initial screen [6]. DBS samples were tested after overnight soaking and elution at 40°C in 60 µl of 20 mM Tris-HCl (pH7.4) buffer containing 150 mM NaCl, 0.1% BSA, 0.15% Tween-20, and 0.1% NaN3 and assays performed on 20 µl of retrieved eluate. GADA, IA-2A, ZnT8A and IAA were determined by radioimmunoassay and ICA by indirect immunofluorescence as previously described [7,8]. The same GADA, IA-2A and ZnT8A assays and thresholds were used for serum and eluted DBS samples.

Participant questionnaires were used to compare the sample collection methods (online supplementary material). The quality of DBS samples was reported by the laboratory as ‘optimal’ (sufficient to allow all three autoantibodies to be measured in duplicate with confirmation in autoantibody positive samples if required; 3 or more circles filled); ‘borderline’ (DBS circles had blank sections but were insufficient to allow confirmatory
testing) and ‘poor’ (individual DBS circles were unevenly filled and blotchy, leading to potentially unreliable results).

Multiple autoantibody positive (high risk) status was defined as detection of two or more of the five antibodies tested in the venous sample, and DBS screening was considered positive if one or more of the three antibodies tested were detected. We calculated the sensitivity and specificity of DBS screening for detection of high risk, multiple autoantibody positive individuals and determined 95% exact confidence intervals. Differences in sample quality by age and reported level of discomfort were analyzed by chi-square testing. Antibody levels in DBS and venous blood were compared using linear regression.

**Results**

DBS and venous samples were collected from 229 individuals; 130 at screening visits, 97 at semi-annual monitoring visits and 2 at annual screening visits. The median age of participants was 20 years (interquartile range 12-38); 28 were aged 8 years or less, 83 were aged 9-18 years and 118 aged more than 19 years. 131 were female. Of the 229 serum samples, 72 (31%) were positive for GADA, 22 (10%) for IA-2A, 9 (3.9%) for IAA, 27 (12%) for ZnT8A and 37 (16.2%) for ICA. Questionnaires were completed by 212 participants. There was no difference in age between questionnaire responders and non-responders (p=0.55).
Levels of GADA, IA-2A and ZnT8A in DBS elute correlated well with serum levels (Figure 1). The sensitivity and specificity of DBS compared with serum assays for GADA, IA2A and ZnT8A is given in Supplementary Table 1.

The number of autoantibodies detected in paired DBS and venous blood samples, and the sensitivity and specificity of DBS screening for detection of multiple autoantibody positive relatives are shown in Table 1. Of 44 relatives found to be multiple autoantibody positive using the TrialNet strategy in venous samples, 42 (95.5%) were positive on DBS screening. DBS accurately detected 145 of 147 antibody negative relatives (98.6%). The 2 individuals positive on DBS screening but antibody negative in venous samples were weakly positive for ZnT8A or GADA and had insufficient DBS sample to allow confirmation. Of the 15 participants who were single antibody positive in venous but not DBS samples, 5 were positive for IAA, 1 for ICA, 8 for GADA, and 1 for IA-2A.

It was possible to report results for GADA, IA-2A and ZnT8A in all the 229 DBS samples. DBS sample quality was optimal with at least 3 circles filled in 55% of participants, but this varied from 20 to 100% between centers. The frequency of suboptimal samples was highest in adults. The median age of relatives with optimal samples was 16 years (interquartile range 11-34) compared with 30 years (14-39) in those with suboptimal samples (p=0.002). DBS sample quality was optimal in 64% of participants aged 8 years or less, 65% of those aged 9-18 yr and 46% of those more than 19 years of age (p = 0.013).
Capillary blood sample collection was perceived as more painful than venous blood draw by 51% of questionnaire respondents while 28% reported the blood draw hurt more; 34% of respondents thought they would be more worried about a blood draw and 16% about a DBS test. For future testing, 38% would choose a blood draw while 41% would prefer a DBS test as carried out in the study. However, 63% of participants/families felt they would prefer the option of initial screening using a home finger prick with clinic visits only if antibodies were detected, including 59% of relatives at screening visits and 68% of those attending for monitoring visits.

There was a linear-by-linear association between the reported level of discomfort associated with DBS collection and the quality of the sample (p=0.013); of 88 individuals whose samples were categorized as ‘poor’ quality, 18 (20%) reported that DBS sample collection was associated with ‘a lot’ of discomfort, compared with only 10 of 114 individuals whose sample was 'optimal'. This association was observed among 108 adults aged 19 years or above (p=0.005), but not in children (n=104, p=0.249).

Conclusions

Our findings demonstrate that collecting capillary blood on filter paper provides a feasible and acceptable alternative to venous blood draw for obtaining samples for islet autoantibody testing. The DBS-based screening strategy achieved high sensitivity for identifying multiple antibody positive relatives at high risk of developing type 1 diabetes but, as expected from earlier studies [2], was less sensitive for detection of single
antibody positive individuals. We also found that obtaining DBS samples can be difficult, even for healthcare professionals, and 45% of samples were not of sufficient quality to confirm positive results. Importantly, the questionnaires showed that the potential to avoid clinic visits was very important to families. Even though participants found the finger prick test more painful than the venous blood draw, and they might be asked to come to clinic for a confirmatory blood draw, families expressed a preference for collecting capillary blood samples at home.

By including participants with a range of antibody levels, we were able to assess both the sensitivity and specificity of our screening strategy. We showed that DBS screening is very sensitive for detection of high risk relatives and, although, some individuals invited back to give a venous blood sample would not be confirmed as multiple autoantibody positive, the majority of these would be single autoantibody positive and thus potentially eligible for follow-up in the PTP and future prevention studies. DBS-based screening therefore offers a suitable alternative for initial testing in situations when venipuncture is difficult, for example at camps and community events.

A further strength is evaluation of the acceptability of the two blood sampling techniques, although these data have some limitations in informing more widespread use of capillary blood-based screening. First, samples were collected by health care professionals rather than participants or family members as would be necessary for home screening. Second, parts of the questionnaire were necessarily theoretical; for example, ‘do you think that you could do this at home?’  Also, our study design meant that we were only able to obtain the preferences of individuals who had experienced both venipuncture and capillary sampling. It is possible that people recruited for
screening without prior experience of either method may have different views. Our study therefore represents only one step in process of developing a strategy for self-collection of screening samples.

The use of DBS in islet autoantibody screening has some drawbacks. Previous studies have found low sensitivity for detection of IAA [2]. We therefore elected not to test for these, but rather to substitute ZnT8A in the initial screen. As IAA generally provide the first indication of autoimmunity in infancy [9], there may be problems with using DBS for screening young children. We also need to overcome the technical problems associated with collection of DBS samples and reduce the variability and proportion of suboptimal samples obtained. This could perhaps be accomplished by better training and/or collecting capillary whole blood samples from which serum can be obtained. We were interested to find age-related differences in the quality of DBS sample collected but do not have an adequate explanation. The observation that poor sample quality is associated with higher reported levels of discomfort in adults but not in children may be relevant, and it will be interesting to see whether these differences are observed in future studies. In this study we used the same thresholds to define autoantibody positivity in serum and DBS samples. It is possible that the performance of DBS-based screening – particularly the sensitivity for identifying single antibody positive individuals - could be enhanced by optimizing these thresholds. The number of samples in this study did not allow us to do this, but it should be considered if DBS testing is to be applied on a larger scale.
We conclude that, with samples collected by research staff as in this study, capillary blood-based screening using DBS could provide a useful recruitment tool for prevention trials. In the future, this approach could also be suitable for general population screening for risk of type 1 diabetes and other autoimmune conditions. Taken together with acceptability and the families’ enthusiasm for the possibility of collecting samples at home, these results clearly justify further exploration to enhance the feasibility and/or acceptability of home-based sample collection techniques which have proved successful in other settings [10].
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Author Disclosure Statement

No competing financial interests exist for any authors

Author Contributions

P.J.B. wrote the manuscript. L.E.R., D.M., L.Y., A.K.S. and C.H. conducted the study, assisted in writing the manuscript and reviewed the manuscript. C.A.B. and D.C.B. provided statistical support, analyzed the data, assisted in writing the manuscript and reviewed the manuscript. C.A.B. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis
References


Figure Legends

**Figure 1**: Assay Concordance in venous serum and dried blood spots. Solid lines equate to identical autoantibody levels in venous and dried blood spot samples. The correlation coefficients \((r)\) for GADA (upper panel), IA-2A (middle panel) and ZnT8A (lower panel) were 0.866, 0.960 and 0.894 respectively (all \(p<0.001\)). Dotted lines indicate the thresholds used to define autoantibody positive status.