Assessing Quality and Functionality of DNA from Fresh and Archival Dried Blood Spots and Recommendations for Quality Control Guidelines

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Background: Dried blood spots (DBS) are a convenient and inexpensive method for biobanking. Although many countries have established population-based DBS biobanks from neonatal screening programs, the quality and usefulness of DNA from DBS have not been extensively assessed.

Methods: We compared 4 common DNA extraction methods (Qiagen, EZNA, Chelex 100, and alkaline lysis) in a pilot study using fresh DBS with known lymphocyte count. We assessed suitability for multiple displacement amplification (MDA) and subsequent single-nucleotide polymorphism (SNP) analyses. We selected the EZNA method for DNA extraction from archival samples up to 27 years old, stored at room temperature or −20 °C, and SNP analyses were performed after MDA.

Results: Extraction using alkaline lysis failed in most tests, and Chelex 100 was unsuccessful in real-time PCR, whereas the EZNA and Qiagen methods were successful by all evaluated quality indices. DNA extraction by EZNA, MDA, and SNP analyses were successful for the archival samples stored at −20 °C.

Conclusion: Routine protocols for evaluation of the quality and functional integrity of DNA based on DNA yield, DNA size, and quantification of amplifiable DNA allow use of sufficient template for MDA and successful SNP analyses from both primary DBS extract and MDA product. A single 3-mm disc can yield sufficient DNA for several thousand SNP analyses. DNA from DBS is thus suitable for genetic epidemiology studies. © 2007 American Association for Clinical Chemistry
whole genome amplification methods (13, 14) have had limited success, isothermal multiple displacement amplification (MDA) reagent sets using the Phi 29 DNA polymerase (15, 16) are now commercially available and routinely used by biobanks and geneticists.

Here, we evaluated the effect of storage time and temperature on the quality of DNA in DBS and the minimum template amount necessary to ensure biallelic representation after MDA. We describe a protocol that can optimize DNA extraction, MDA, and subsequent SNP analyses and should allow several thousand successful SNP analyses from small DBS punches stored for >20 years; we also describe quality control guidelines.

**Materials and Methods**

**PILOT STUDY**

**DNA extraction.** We used the QIAamp DNA MiniKit (Qiagen) blood and body fluid spin protocol on 4 anonymized fresh EDTA whole blood samples with known lymphocyte cell counts to serve as a reference for subsequent SNP analyses. Samples were obtained from the Clinical Chemistry Laboratory, University Hospital, Malmö, Sweden. We applied 100-µL aliquots of the blood samples to marked circles, 14 mm in diameter, on filter paper (Schleicher & Schüll 2992) and punched 6-mm diameter discs from each dried filter, with careful cleaning of the punch between samples to prevent contamination. One disc from each sample was then subjected to DNA extraction, MDA, and subsequent SNP analyses and should allow several thousand successful SNP analyses from small DBS punches stored for >20 years; we also describe quality control guidelines.

- The QIAamp DNA minikit (Qiagen) was used as described in the DBS protocol.
- The EZNA Forensic DNA reagent set (Omega Bio-Tek) was used as described in the protocol for isolation of DNA from dried blood, with added mixing on a shaker during incubations 1 and 2.
- DNA extraction with alkaline lysis was performed as described in the blood cells protocol, GenomiPhi DNA Amplification Kit instructions (17). A 6-mm disc was incubated on ice in 100 µL alkaline lysis solution (400 mmol/L KOH, 100 mmol/L dithiothreitol, 10 mmol/L EDTA) for 10 min. The solution was diluted 2-fold with a neutralizing solution (400 mmol/L HCl, 600 mmol/L Tris-HCl, pH 0.6) in a new microcentrifuge tube and mixed.
- We used the Chelex 100 method as described in the instructions for DNA extraction from Guthrie cards (18). A 6-mm DBS disc was incubated in 1 mL sterile water at room temperature for 30 min on a shaker. After a 3-min, 14 000 rpm centrifugation, the supernatant was discarded and the pellet incubated in 150 µL of 50 mL/L Chelex 100 resin (Bio-Rad Laboratories, cat. no. 142-2822) in water at 56 °C for 20 min on a shaker, with additional mixing every 5 min. After a 3-min centrifugation at 14 000 rpm, the supernatant was transferred to a new microcentrifuge tube. High temperatures and vortex-mixing were avoided to minimize fragmentation of the extracted DNA.

**DNA SIZE AND CONCENTRATION**

We measured the concentration of double-strand DNA in primary DBS extract and whole blood by use of the PicoGreen dsDNA Quantification Kit (Molecular Probes) on a FLUOstar Optima plate reader (BMG LABTECH GmbH). We measured total DNA concentration using the OliGreen ssDNA Quantification Kit (Molecular Probes). Yield is expressed as percentage of theoretical DNA content calculated as 7 pg DNA per cell.

The size of extracted DNA fragments was visualized after electrophoresis in a 8 mL/L agarose gel (SeaKem, Cambrex BioScience) in TBE buffer (0.09 mol/L Tris, 0.09 mol/L boric acid, 1 mmol/L EDTA) stained with 0.4 mL/L ethidium bromide (Applichem GmbH) and compared to the size of a mixture of 2 markers (SMM0191 and GenEvent 50 bp DNA Ladder, MBI Fermentas).

We analyzed a dilution series from 10 µL to 1 × 10⁻⁴ µL primary DBS extract in a 20-µL real-time PCR assay followed by end-point allelic discrimination using reagents designed for a robust F2⁵ [coagulation factor II (thrombin)] g.20210G>A (dbSNP (19) rs1799963) TaqMan MGB assay on a 7900 HT Sequence Detection System (instrument and all TaqMan SNP genotyping assay reagents from Applied Biosystems).

**MDA**

We subjected serial dilutions containing from 5 to 0.05 ng (based on PicoGreen quantification) primary DBS extract to MDA using the GenomiPhi Kit. Each aliquot was evaporated to dryness at 37 °C in a 96-well plate before addition of GenomiPhi reagents. After enzyme inactivation, the MDA products were diluted 10-fold with TE buffer (1 mmol/L EDTA, 10 mmol/L Tris-HCl, pH 8.0) and solubilized at 4 °C for at least 6 h. The quantity, by real-time PCR, and size of the MDA product were evaluated as above.

**TaqMan MGB SNP ANALYSES**

We performed analyses of the high frequency SNPs MTHFR [5,10-methylenetetrahydrofolate reductase (NADPH)] c.677C>T (rs1801133), MTHFR c.1298A>C (rs1801131), and APOE (apolipoprotein E) c.334T>C (rs429358) on the 7900 HT using 2-µL undiluted extracts from whole blood, DBS, or a 100-fold dilution of MDA product in 6-µL reactions. All assays are accredited by SWEDAC for routine diagnostic testing and were performed with internal controls having wild-type/wild-type, wild-type/mutant, and mutant/mutant genotypes in each assay batch.

We used the VIC-6-carboxyfluorescein (FAM) ratio of fluorescence from ~2200 samples previously analyzed

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3 Human genes: F2, coagulation factor II (thrombin); MTHFR, 5,10-methylenetetrahydrofolate reductase (NADPH); APOE, apolipoprotein E.
with the same assays for the MTHFR SNPs in our laboratory as criteria for genotype determination. MDA samples were accepted as homozygotes if the FAM:VIC ratio was within 3 SD of the reference homozygous mean ratio and as heterozygotes if the ratio was outside 4 SD of the reference homozygous mean ratio.

Because no such reference data using the same APOE reagents and protocol were available, we confirmed APOE genotypes of samples that were not automatically determined by the 7900 HT software with restriction fragment length polymorphism as described by Hixson and Vernier (20).

**Mass Spectrometry SNP Analyses**

We analyzed a panel of 254 SNP assays on a MALDI-TOF mass spectrometer (SEQUENOM MassArray) using iPLEX reagents and protocol (SEQUENOM) and primer sets (Metabion) previously validated on in-house samples and 10 ng whole blood DNA or MDA product of DBS as PCR template if available.

**Archival DBS**

We obtained anonymous punched discs, 3 mm in diameter, from 10 samples stored for 3 months at -20 °C (Schleicher & Schüll 903), 10 samples stored for ~22 years (obtained 1980–1989) at -20 °C, and 10 samples stored for ~26 years (obtained 1979–1981) at room temperature (both Schleicher & Schüll 2992) from the Danish National PKU Biobank (State Serum Institute). DNA was extracted from a single disc of each sample with the EZNA method and eluted in 50 μL buffer. The 22-year-old samples were extracted in duplicate, 1 disc according to the protocol and a 2nd disc with the proteolysis time increased to 24 h. Five of the 26-year-old samples were extracted in duplicate with increased proteolysis time, and 5 samples had 1 disc extracted according to the protocol and a 2nd disc extracted with the increased proteolysis time.

We measured the DNA concentration by use of real-time PCR and visualized the size of DNA fragments as described above.

Ten microliters of the primary DBS extract from the 3-month-old samples and 5 ng or 20 μL of the primary DBS extract from both duplicates of the 22- and 26-year-old samples were evaporated to dryness at 37 °C. We subjected the pellet to MDA followed by measurement of the DNA concentration by real-time PCR using 2 μL 100-fold diluted MDA product in a 6-μL reaction and the 3 TaqMan SNPs as described above, using primary DBS extract and 100-fold diluted MDA product. All samples were analyzed on a panel of 101 mass spectrometry (MS) SNP assays using 10 ng MDA product as template if available.

**Results**

**Pilot Study**

*Size and concentration of DNA.* Standard DNA minipreps from 200 μL of 4 fresh EDTA-whole blood samples (4.6 to 21.8 × 10⁶ lymphocytes/L) yielded 4 to 20 μg double-stranded DNA (dsDNA) [mean (SD) 58% (4.7%) of theoretical yield]. The apparent size of eluted DNA was 21 kb (Fig. 1, lane 1).

The mean DNA yields from 6-mm DBS discs as measured by PicoGreen fluorescence were 14% (67–306 ng), 12% (62–178 ng), 8% (31–134 ng), and 1% (4–8 ng) using the Qia-gen, EZNA, Chelex 100, and alkaline lysis methods, respectively. Quantification by real-time PCR gave similar results for samples isolated with the EZNA and Qia-gen methods (Fig. 2). Extraction with Chelex 100 resulted in small amounts of functional, real-time PCR amplifiable DNA (Fig. 2), and a 20% Chelex 100 concentration did not improve the yield of functional DNA (not shown). DNA extracted by alkaline lysis failed consistently in real-time PCR assays. Optimal template dilution of primary DBS extracts from the EZNA and Qia-gen methods was typically 20- to 2000-fold in real-time PCR assays. Only data within the linear range were used to calculate DNA concentrations (Fig. 3). OliGreen fluorescence data agreed poorly with the PicoGreen and real-time PCR data regardless of extraction method (Fig. 2).

The apparent size of DBS DNA extracted with the Qiagen and EZNA methods was 21 kb, and >21 kb with the Chelex 100 method. The alkaline lysis method produced insufficient DNA to be visible on gel electrophoresis (Fig. 1, lanes 2–5).

**MDA**

We used dilution series of DNA (5 to 0.05 ng) from 3 DBS samples as templates for MDA and measured the DNA

![Fig. 1. Pilot study: fragment size of DNA from whole blood, DBS, and MDA products of DBS.]

*Lane 1: 2 μL DNA extracted from whole blood (262 ng); one-third of DNA from the DBS extracted by lane 2: Qiagen (220 ng); lane 3: EZNA (135 ng); lane 4: Chelex 100 (225 ng); and lane 5: alkaline lysis (9.4 ng) methods; 10 μL of MDA products of DNA from DBS extracted by lane 6: Qiagen (114 ng); lane 7: EZNA (114 ng); lane 8: Chelex 100 (138 ng); and lane 9: alkaline lysis (0 ng) methods.*
concentration of the MDA product by use of real-time PCR. Results are presented in Table 1. The majority of DNA fragments after MDA, using 5 ng template DNA, appeared to be 21 to 3.5 kb in length (Fig. 1, lanes 6–8). Repeated attempts with MDA of alkaline lysis-extracted samples consistently failed to produce any measurable amount of functional DNA (Fig. 1, lane 9).

Snp analyses
To evaluate the minimal amount of template DNA in the MDA reaction necessary to retain biallelic representation, we compared results of the TaqMan analyses (Table 1) and a panel of 254 SNPs analyzed by MS (Table 2) on QIAamp MiniKit-preps of 3 whole blood samples to those of MDA products from the dilution series of DBS DNA from the same samples. Identical TaqMan results were obtained if >0.15 ng MDA template had been used, except for 1 sample extracted by the Qiagen method for which allelic dropout was seen when ≤0.5 ng was used. Using <0.15 ng as MDA template resulted in failed amplification or failed or irreproducible SNP analyses in all samples (Table 1).

The success rate in the MS SNP analyses was 98.0% for DNA from whole blood and 97.9% for MDA product using 5 ng template of DBS DNA extracted with the EZNA method. No allelic dropout was seen in the MDA product from these EZNA-extracted DBS samples. Corresponding rates for MDA products were 97.2% after Qiagen and Chelex 100 extractions. The success rates decreased when lower amounts of template DNA were used in the MDA reaction. Although most genotyping failures were due to missing values, allelic dropout was seen in a small percentage of the samples (Table 2).

Archival DBS
Discs of 3 mm diameter were used from all archival DBS. The mean (SD; range) DNA yield from 1 disc was 12.7 (7.1; 5.3–30.4) ng for the 3-month-old samples, 33.8 (9.8; 20.8–48.5) ng for the 22-year-old samples stored at −20 °C, and 24.5 (16.3; 8.0–66.9) ng for the 26-year-old samples stored at room temperature, the latter 2 extracted after proteolysis for 24 h. The mean (SD; range) DNA yield was 17.2 (20.9; 3.0–82.7) ng for the 22- and 26-year-old samples combined, extracted according to the original EZNA protocol, compared with 28.3 (14.6; 8.0–66.9) ng for the 22- and 26-year-old samples combined, extracted after proteolysis for 24 h.

The degree of fragmentation of DNA extracted from archival DBS increased with increasing age of the samples (Fig. 4, lanes 1–3). TaqMan SNP analyses were successful on all archival samples, using primary DBS extract as template.

Although several samples, irrespective of age, produced insufficient DNA for 5 ng to be used as MDA template, the use of duplicates for the 22- and 26-year-old samples provided at least 1 MDA reaction per sample donor with ≥5 ng template DNA. MDA was successful on the 3-month-old samples, with a mean (SD) yield of 11 620 (4624) ng, and the 22-year-old samples, with a mean yield of 6383 (2872) ng. MDA on the 26-year-old samples resulted in a mean yield of 17 (14) ng when measured by real-time PCR. Using PicoGreen for measuring concentration of the 26-year-old samples suggested a mean yield of 5000 (1591) ng. The majority of fragments after MDA appeared to be 21 to 3.5 kb in length regardless of storage length or temperature (Fig. 4, lanes 4–6).

In the TaqMan analyses, 3 of the 26-year-old samples showed allelic dropout when comparing the genotypes determined using the MDA products of 5 ng DBS DNA to those determined using the primary DBS extract; the genotypes were identical for all other archival samples.

We analyzed all archival samples for a panel of 101 SNPs by MS. The concordance rate for the 3-month-old samples was 97.5%, between 2 aliquots of the MDA reactions. The success rate was 97.5% for the 22-year-old samples and 84.7% for the 26-year-old samples. Success rates of the 22- and 26-year-old samples were calculated from samples where ≥5 ng template DNA had been used in the MDA reaction. The discrepancy rate was 0.1% between duplicate aliquots of the MDA products for the 3-month-old samples, and 0.1% and 10.3% between MDA products from duplicate extracted discs from the DBS for the 22- and 26-year-old samples, respectively (Table 2).

When including genotyping results from the samples with <5 ng MDA template, the discrepancy rates between 2 discs of the DBS were 0.4% and 12.7% for the 22- and 26-year-old samples, respectively.
Archival DBS samples stored at −20 °C performed equally well as freshly prepared DBS samples in the TaqMan analyses. In the MS SNP analyses, the 22-year-old DBS samples performed as well as the 3-month-old DBS samples and nearly as well as freshly prepared DBS samples. Missing values were only slightly more common in the DNA from these archival samples, and we found a small percentage (0.1%) of discrepancies between duplicate aliquots of the MDA products of the 3-month-old and between MDA products from duplicate extracted discs from the DBS of the 22-year-old samples, compared with the fresh samples extracted with the EZNA method. Although DNA yields from the archival DBS, using the original EZNA protocol, appeared similar, fragmentation of the DNA increased and performance in SNP analyses after MDA decreased with increasing storage time and temperature. The 26-year-old samples showed discrepancies between the primary DBS extract and the MDA product of the same DNA in the TaqMan analyses and the

### Table 1. Pilot study: DNA yields and TaqMan SNP results from MDA products of 2 typical DBS samples according to template.

<table>
<thead>
<tr>
<th>MDA template, ng</th>
<th>DNA yield, ng</th>
<th>MTHFR c0.1298A&gt;C</th>
<th>MTHFR c0.677C&gt;T</th>
<th>APOE c0.334T&gt;C</th>
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</thead>
<tbody>
<tr>
<td>Whole blood*</td>
<td>AC CT TT</td>
<td>TT</td>
<td>AC</td>
<td>CC</td>
</tr>
<tr>
<td>5</td>
<td>2560</td>
<td>AC</td>
<td>CT</td>
<td>TT</td>
</tr>
<tr>
<td>1.5</td>
<td>3240</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>0.5</td>
<td>2600</td>
<td>AC CT</td>
<td>TT</td>
<td>—</td>
</tr>
<tr>
<td>0.15</td>
<td>1050</td>
<td>AC CT</td>
<td>TT</td>
<td>—</td>
</tr>
<tr>
<td>0.05</td>
<td>360</td>
<td>Failed CT</td>
<td>Failed</td>
<td>—</td>
</tr>
<tr>
<td>Qiagen DBS</td>
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<td></td>
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<td>5</td>
<td>3780</td>
<td>AC</td>
<td>CT</td>
<td>TT</td>
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<tr>
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<td>AC</td>
<td>CT</td>
<td>TT</td>
</tr>
<tr>
<td>0.5</td>
<td>4060</td>
<td>AC</td>
<td>CT</td>
<td>TT</td>
</tr>
<tr>
<td>0.15</td>
<td>3340</td>
<td>AC</td>
<td>CT</td>
<td>TT</td>
</tr>
<tr>
<td>0.05</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>EZNA DBS</td>
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<td></td>
</tr>
<tr>
<td>5</td>
<td>3120</td>
<td>AC</td>
<td>CT</td>
<td>TT</td>
</tr>
<tr>
<td>1.5</td>
<td>3920</td>
<td>AC</td>
<td>CT</td>
<td>TT</td>
</tr>
<tr>
<td>0.5</td>
<td>2320</td>
<td>AC</td>
<td>CT</td>
<td>TT</td>
</tr>
<tr>
<td>0.15</td>
<td>660</td>
<td>AC</td>
<td>CT</td>
<td>TT</td>
</tr>
<tr>
<td>0.05</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Chelex DBS</td>
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<td>AC</td>
<td>CT</td>
<td>TT</td>
</tr>
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<td>1.5</td>
<td>3920</td>
<td>AC</td>
<td>CT</td>
<td>TT</td>
</tr>
<tr>
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<td>CT</td>
<td>TT</td>
</tr>
<tr>
<td>0.15</td>
<td>660</td>
<td>AC</td>
<td>CT</td>
<td>TT</td>
</tr>
<tr>
<td>0.05</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*Not subjected to MDA.

### Table 2. MS SNP results of whole blood DNA and MDA products of DBS samples according to template.

<table>
<thead>
<tr>
<th>Samples</th>
<th>MDA template, ng</th>
<th>No. of MS analyses</th>
<th>Concordance, %</th>
<th>Missing values, %</th>
<th>Discrepancies, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood DNA</td>
<td>NA</td>
<td>762</td>
<td>98.0</td>
<td>2.0</td>
<td>NA</td>
</tr>
<tr>
<td>Pilot samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chelex</td>
<td>0.15–1.5</td>
<td>1270</td>
<td>86.3</td>
<td>9.1</td>
<td>5.1</td>
</tr>
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<td>Qiagen</td>
<td>0.5</td>
<td>762</td>
<td>88.2</td>
<td>8.1</td>
<td>4.0</td>
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<td>EZNA</td>
<td>0.5</td>
<td>762</td>
<td>93.0</td>
<td>5.8</td>
<td>1.3</td>
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<tr>
<td>Chelex</td>
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<td>254</td>
<td>97.2</td>
<td>2.4</td>
<td>0.4</td>
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<td>Qiagen</td>
<td>5</td>
<td>762</td>
<td>97.2</td>
<td>2.7</td>
<td>0.1</td>
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<tr>
<td>EZNA</td>
<td>5</td>
<td>762</td>
<td>97.9</td>
<td>2.1</td>
<td>0.0</td>
</tr>
<tr>
<td>Archival samples</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>26 years at room temperature</td>
<td>5</td>
<td>1010</td>
<td>84.7</td>
<td>5.6</td>
<td>10.3</td>
</tr>
<tr>
<td>22 years at −20 °C</td>
<td>5</td>
<td>1010</td>
<td>97.5</td>
<td>2.4</td>
<td>0.1</td>
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<tr>
<td>3 months at −20 °C</td>
<td>1.3–7.6</td>
<td>1010</td>
<td>97.5</td>
<td>2.4</td>
<td>0.1</td>
</tr>
</tbody>
</table>

*Percentage of concordant genotypes in duplicate.

b Discrepant results/MS analyses missing values.

c NA, Not applicable.

Discussion

Archival DBS samples stored at −20 °C performed equally well as freshly prepared DBS samples in the TaqMan analyses. In the MS SNP analyses, the 22-year-old DBS samples performed as well as the 3-month-old DBS samples and nearly as well as freshly prepared DBS samples. Missing values were only slightly more common in the DNA from these archival samples, and we found a small percentage (0.1%) of discrepancies between duplicate aliquots of the MDA products of the 3-month-old and...
highest percentage of missing values and discrepancies between MDA products from duplicate extracted discs from the DBS in the MS SNP analyses. These samples also performed poorly in real-time PCR after MDA, and the amount of MDA product was estimated by PicoGreen fluorescence. This overall poor performance could be due to the low molecular weight of the DNA extracted from these samples, probably caused by degradation of the DNA during room temperature storage as previously suggested (21). A larger amount of template from these samples could be used in MDA reactions to compensate for the degradation. Unfortunately, the small amounts of DNA yielded from the DBS did not allow for enrichment of large fragments or for repeated MDA reactions in our study. The PicoGreen assay is not optimal for MDA products, as even nontemplate controls sometimes produce considerable dsDNA owing to primer-on-primer amplification products. Nonetheless, the apparent success rate in MS SNP analyses of nearly 85% for the 26-year-old samples indicates that considerable amplification must have occurred during the MDA reaction, which was confirmed by gel analysis.

Maintenance of biallelic representation in SNP assays required the use of a minimum of 5 ng DNA as template in the MDA reaction, similar to our findings in a previous study (22). This could be routinely obtained from the archival samples if the duration of the proteolysis step in the EZNA extraction method was increased.

If routine protocols using a minimum of 5 ng intact DNA as MDA template and 2 or 10 ng MDA product in TaqMan or MS SNP analyses, respectively, are followed, a 3-mm archival DBS disc should be sufficient for ~7000 TaqMan SNP analyses or ~39 000 (28 assays/multiplex) or ~56 000 (40 assays/multiplex) MS SNP assays (assuming an ~16 ng DNA yield per 3-mm disc and ~4700 ng yield per MDA reaction). The yield of 1 MDA reaction would also be more than sufficient for most whole-genome scan platforms.

The Qiagen, EZNA, and Chelex 100 methods all generated DNA from DBS of sufficient quality to perform well in SNP analyses after MDA, contrary to reports of previous studies in which DBS DNA were found inappropriate for MDA (21). Although very small amounts of template DNA were greatly amplified by MDA, generating enough DNA for a large number of SNP assays, allelic dropout increased with decreasing amounts of MDA template.

The fact that the Chelex 100-extracted DNA was quantifiable using PicoGreen and was a suitable target for MDA indicates that this method extracted substantial amounts of DNA, but the extracts contained impurities that inhibited real-time PCR. The high molecular weight of the DNA in the Chelex 100 extract could indicate that this method produces more intact DNA than the other methods. An alternative interpretation could be that the sample impurities that were clearly visible in the gel-well might have caused clogging and delayed the DNA in entering the gel.

The agreement of results of PicoGreen fluorescence and real-time PCR quantification indicate the presence of intact DNA with little inhibition in the Qiagen and EZNA primary DBS extracts. OliGreen consistently overestimated the DNA content of extracts and is not recommended. The DNA yields from DBS are altogether too low to allow reliable quantification by ultraviolet Abs 260/280.

When genotyping high-quality DNA, the samples usually separate into neat, tight clusters according to genotype in the 7900 HT scattergram, with little variation in the FAM:VIC fluorescence ratios. Allelic discrimination of MDA products of heterozygous samples was more difficult, with greater variation in fluorescent ratios. The reference material was therefore used as an objective method to confirm the genotype of these samples.

In summary, for optimal DNA analyses from DBS, we recommend storage of DBS at ~20 °C to prevent degradation of DNA, prolonged proteolysis steps during DNA extraction for optimal yield, the use of >5 ng intact DNA as MDA template, and solubilization of MDA product by 10-fold dilution with TE buffer for at least 6 h. Our recommendations for quality control guidelines include
(a) quality-assured storage and documentation of samples before analysis, (b) tracking of sample identity throughout all processes, and (c) DNA extraction by a validated method. Validation includes (a) evaluation of fragment size on agarose gel electrophoresis, (b) quantification of dsDNA by PicoGreen fluorescence and comparison with real-time PCR in more than 1 dilution to reveal inhibitors and to confirm the presence of intact functional human DNA, and (c) performance of MDA on extracted DNA and evaluation of the product by real-time PCR. Using these guidelines, archival DBS samples can provide sufficient DNA for successful large-scale genetic epidemiological studies.

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