Comprehensive Genetic Testing for Deafness from Fresh and Archived Dried Blood Spots

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Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

Abstract
Comprehensive genetic testing has become integral in the evaluation of children with deafness, but the amount of blood required to obtain DNA can be prohibitive in newborns. Dried blood spots (DBSs) are routinely collected and would provide an alternative source of DNA. Our objective was to evaluate the use of DBSs for comprehensive genetic testing for deafness. DNA derived from fresh and archived DBS samples was compared with DNA from whole blood. We performed next-generation sequencing of all known deafness genes in 4 DBS samples: 2 positive controls, an unknown sample, and a negative control. The DBS-derived DNA was of sufficient quantity and quality for clinical testing. In the 2 positive control samples, pathogenic variants were identified; in the negative control, no pathogenic variants were found; and in the unknown sample, homozygous deletion of the OTOA gene was identified as the cause of deafness. This pilot study shows that comprehensive genetic testing for deafness is feasible with fresh and/or archived DBSs.

Keywords
dried blood spot, deafness, hearing loss, genetics, genomics, GJB2

Received January 6, 2018; revised July 5, 2018; accepted August 8, 2018.

In the United States and other developed countries, the majority of congenital deafness is nonsyndromic and due to a genetic cause. Genetic testing for deafness is a cornerstone in the evaluation of newborns and children with hearing loss and, depending on clinical features, has a diagnostic rate exceeding 60%, which is higher than that of other tests. For this reason, recent guidelines indicate that genetic testing should be performed after history, physical, and audiometric evaluation among children with deafness.

Genetic deafness is an extremely heterogeneous condition, with 107 genes implicated to date in nonsyndromic hearing loss (http://hereditaryhearingloss.org). The advent of targeted genomic enrichment with massively parallel sequencing has provided the ability to sequence all known deafness genes simultaneously. However, this method typically requires several milliliters of blood for DNA isolation, which may be implausible in the neonatal period. For example, a 3-kg neonate has an estimated total blood volume of 100 mL; the maximum allowable volumes that can be taken in 1 blood draw and over a 30-day interval are 6 mL and 12 mL, respectively.

Dried blood spots (DBSs) represent another potential source of DNA. These samples contain on average 50 μL of blood per spot and are routinely collected in the first 48 hours of life as part of the newborn screen. In many states, DBSs are retained for years and therefore represent a large repository of genetic data for possible study. DBSs have been used for single-gene or pathogenic variant evaluation for deafness, as well as for whole exome and genome sequencing and genetic newborn screening. The goal of this pilot study was to determine if comprehensive genetic testing for deafness could be performed with DNA from fresh and archived DBSs.

Methods
Samples were collected from 4 subjects (Table 1), including 2 positive controls (subjects 1 and 2), an unknown (subject 3), and a negative control (subject 4). For subject 4, DNA was derived from fresh DBSs obtained <48 hours...

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This article was presented at the 2017 AAO-HNSF Annual Meeting & OTO Experience; September 10-13, 2017; Chicago, Illinois.

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prior to DNA extraction via finger stick. For subjects 1 to 3, samples were derived from whole blood obtained via venipuncture and placed on standard Guthrie cards in 2002 for long-term archival storage (at room temperature and in the dark) until the date of extraction, October 2016.

DNA was extracted with 24 mm² of saturated DBS paper (2 strips, 6 × 3 mm). The Qiagen (Germantown, Maryland) DNeasy column-based isolation method was used with the following alterations: incubation with proteinase K was performed for 3 hours in a 56°C shaker with 2 final elutions, each with 25 μL of LoTE buffer (low tris-ethylenediaminetetraacetic acid). DNA quality was assessed with gel electrophoresis and spectrophotometry. We determined DNA yield from fresh DBSs with multiple extractions from separate DBS cards for subject 4.

The KAPA Biosystems (Wilmington, Massachusetts) Hyper Prep library preparation method for low DNA input was used with 400 ng of DNA input. Targeted genomic capture with massively parallel sequencing was then performed to sequence 152 genes implicated in hearing.3 Sequencing metrics, including total mapped sequencing reads and coverage, were compared with data from 27 subjects who underwent comprehensive genetic diagnosis with DNA isolated from whole blood according to our standard protocol.3 Due to the small sample size, statistical comparison of total sequencing reads was performed with nonparametric testing (Mann-Whitney U, with P < .05 considered significant). The study was approved by the Institutional Review Board of the University of Iowa, and informed consent was obtained.

### Results

For the fresh DBSs from the negative control subject, 12 separate extractions resulted in an average of 250 ng of DNA extracted per blood spot (95% CI, 211-290 ng). From the 3 archived DBS control subjects, extraction resulted in an average of 1059 ng of DNA (95% CI, 965-1150 ng). This amount of DNA was of sufficient quantity and quality to generate ~12,000,000 total mapped reads per sample, which exceeds our established threshold of >7,750,000 total mapped reads per sample that is required for clinical diagnostic testing. The total mapped reads per sample for DBSs was not significantly different from the total mapped reads per sample generated with whole blood as the DNA source, indicating effective amplification and mapping (P = .589; Table 2). In addition, the 10× coverage exceeded the quality threshold of 99.52% for sample analysis.

In the 2 positive controls (subjects 1 and 2), the expected pathogenic variants were identified (Table 1), and in the negative control (subject 4), no causative variants were found. Subject 3, the unknown, was found to have a homozygous deletion of the OTOA gene, which is diagnostic of DFNB22.

### Conclusion

We noted differences between fresh and archived DBSs in regard to quantity of DNA isolated, which may reflect the fact that the archived DBSs were derived from blood from venipuncture and archived, while the fresh DBSs were

### Table 1. Detailed Results for Subjects Studied.

<table>
<thead>
<tr>
<th>Subject</th>
<th>DBS Type</th>
<th>DNA Isolated, ng</th>
<th>Previous Diagnosis</th>
<th>Comprehensive Genetic Testing Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Archived</td>
<td>1066</td>
<td>GJB2 c.35delG / GJB2-GJB6 deletion</td>
<td>GJB2 c.35delG / GJB2-GJB6 deletion</td>
</tr>
<tr>
<td>2</td>
<td>Archived</td>
<td>974</td>
<td>GJB2 c.35delG / GJB2-GJB6 deletion</td>
<td>GJB2 c.35delG / GJB2-GJB6 deletion</td>
</tr>
<tr>
<td>3</td>
<td>Archived</td>
<td>1136</td>
<td>Negative GJB2 testing</td>
<td>OTOA homozygous gene deletion</td>
</tr>
<tr>
<td>4</td>
<td>Fresh</td>
<td>400</td>
<td>—</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Abbreviation: DBS, dried blood spot.

### Table 2. Comparison between DBS and Whole Blood (Control) for Comprehensive Genetic Testing for Deafness.

<table>
<thead>
<tr>
<th></th>
<th>DBS (n = 4)</th>
<th>Whole Blood (n = 27)</th>
<th>Quality Threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total mapped reads</td>
<td>12,918,646 (11,400,000-14,400,000)⁶</td>
<td>12,165,987 (11.300,000-13,000,000)</td>
<td>&gt;7,750,000</td>
</tr>
<tr>
<td>Mapped reads, %</td>
<td>95.3 (94.3-96.3)</td>
<td>97.2 (96.7-97.7)</td>
<td>—</td>
</tr>
<tr>
<td>Reads overlapping target, %</td>
<td>52.0 (50.7-53.3)</td>
<td>63.2 (61.8-64.6)</td>
<td>—</td>
</tr>
<tr>
<td>Mean read depth</td>
<td>754 (671-837)</td>
<td>579 (535-623)</td>
<td>—</td>
</tr>
<tr>
<td>Target covered 10×, %</td>
<td>99.97 (98.6-101)</td>
<td>99.82 (99.5-100)</td>
<td>&gt;99.52</td>
</tr>
<tr>
<td>High-quality genetic variants</td>
<td>2272 (2170-2380)</td>
<td>2463 (2430-2500)</td>
<td>—</td>
</tr>
<tr>
<td>High-quality coding NS/SS genetic variants</td>
<td>27 (17.2-36.8)</td>
<td>22 (21.2-22.8)</td>
<td>—</td>
</tr>
</tbody>
</table>

Abbreviations: DBS, dried blood spot; NS, non-synonymous; SS, splice-site.

⁶Values in parentheses are 95% CIs. Quality threshold levels are determined as part of our clinical genetic testing laboratory’s standard operating procedure.

⁷No significant difference between DBS and whole blood (P = .589 determined with nonparametric testing with the Mann-Whitney U test).
derived from finger-stick blood. Blood from venipuncture is likely to contain more nucleated white cells and therefore more DNA. The total mapped reads per sample with DBSs exceeded quality thresholds for variant analysis and was not significantly different when compared with total mapped reads per sample with whole blood.

In this pilot study, we show that comprehensive genetic testing for deafness can be performed from archived and fresh DBSs. Importantly, screening for cytomegalovirus, which is the most common cause of nongenetic congenital deafness, can also be performed from DBSs. Using DBSs for this dual purpose could improve the current newborn hearing screen.

**Author Contributions**

A. Eliot Shearer, study design, data collection and data analysis, drafted manuscript; Kathy Frees, data collection, contributed to manuscript; Diana L. Kolbe, data analysis, contributed to manuscript; Richard J. H. Smith, study design, drafted manuscript.

**Disclosures**

Competing interests: A. Eliot Shearer, Kathy Frees, Diana L. Kolbe, and Richard J. H. Smith, Molecular Otolaryngology and Renal Research Laboratories—members of a not-for-profit laboratory that offers comprehensive genetic testing for deafness.

Sponsorships: None.

Funding source: National Institutes of Health, National Institute on Deafness and Other Communication Disorders—funding for research studies (RO1 grants: DC002544, DC002842, DC012049). No role in design, conduction of research, analysis, interpretation, writing, or approval.

**References**


