DEVELOPMENT AND VALIDATION OF BUCCAL SWAB COLLECTION METHOD FOR DNA TESTING FOR PATERNITY TESTING

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I. Introduction

One significant advantage of DNA typing as compared to classical serological testing is that tissue other than blood can be used to obtain DNA. The use of buccal swabs has several advantages over the collection of blood samples. Buccal swab collection is a noninvasive method of tissue collection which is less painful and traumatic than venipuncture. It is a safe alternative for collection of tissues from newborns, children, and adults who cannot have blood drawn for medical or religious reasons. Furthermore, it reduces the risk involved in shipping liquid blood by eliminating the risk of tube breakage. Finally, buccal swabs are resistant to extreme temperatures which can affect the results from liquid blood.

The purpose of this presentation is to present the development and validation of our buccal swab procedures. This study will present the method of extracting DNA from the buccal cells collected from the swab. Additionally, it will discuss the variation in the amount of DNA collected from buccal swabs as well as compare the allele size measurements of DNA obtained from buccal swabs and blood. A summary of buccal swab cases will be presented with regards to observed heterozygosity, exclusion rates, turnaround time, and retest frequencies.

II. Development and Validation of Buccal Swab Test

A. Choice of swab collection device:

GDI investigated four different types of brushes: cervical brush, dacron swab, cotton swab, and a toothbrush. The cotton swab (Baxter Scientific Products S/P Sterile Cotton Tip Applicator catalogue #A5002-5) was chosen as the specimen collection device due to consistent high yield of DNA and its safe and nonabrasive texture.

B. Specimen Collection Kit:

8 buccal swabs are collected per individual. Two swabbings of each quadrant of the cheek are collected . The eight swabs are placed in a paper envelope that is attached

to the Buccal Swab Mailer Kit. Each envelope is sealed and initialed by the individual and the phlebotomist to maintain the chain of custody. The buccal swab kit is mailed to Genetic Design Inc. by 2 day express mail.

C. Isolation of DNA from the cotton swab:

The cotton material is cut off of each swab and collected into a 15 ml conical tube. Cell lysis buffer (.01 M Tris, .01 M EDTA, .1M NaCl, 2% SDS, and Proteinase K) is added to the cotton and incubated for 1 hour at 56° C. The cell lysate is recovered from the cotton by centrifugal filtration. The DNA is purified using phenol/chloroform extraction followed by ethanol precipitation. DNA concentration is determined by UV absorbance at 260/280 for RFLP testing and by the Life Technologies Human Quantitation blot for PCR analysis. Following purification and quantitation of DNA, the samples are further processed for either RFLP or PCR analysis.

D. Variation of DNA concentration:

Buccal Swabs were collected from nine individuals over a fourteen day period. Eight buccal swabs were collected per individual on days 1,5,10, and 14. The DNA was isolated and processed for RFLP analysis for the loci D10S28 and D2S44. Individual RFLP alleles are analyzed for size and amount of DNA by comparing the density of the alleles to quantitation standards. The amount of DNA varied from 2.3 micrograms to 14 micrograms of DNA per eight buccal swabs. An average of 8.6 micrograms of DNA is obtained from 8 buccal swabs.

E. Comparison of RFLP sizes between DNA isolated from buccal swabs and whole blood:

1. Comparisons of DNA RFLP results between whole blood and buccal swabs were analyzed. The standard deviation of measurement in allele size when comparing blood size measurements from the same individual versus buccal size measurements is 0.60% of the allele size, which is similar to the standard deviation from two different blood specimens from the same individual (0.66%).
2. The size of the obligate maternal allele from the child's buccal cells are compared with the size of the allele obtained from the mother's whole blood. One hundred comparisons were made between the obligate maternal allele of the child (buccal) and that allele detected in the mother (blood) and analyzed for the loci D10S28 and D2S44. The correlation of allele measurements between the obligate maternal

allele isolated from buccal swabs of the child and the corresponding maternal allele isolated from whole blood is 0.9993. Furthermore, the absolute percent difference between the child's allele and the corresponding mother's allele is .84% with a standard deviation of .56%. The average percent difference between the child's allele (buccal) and the mother's allele (blood) is -0.37% of the maternal allele size (Standard deviation is 0.83). Finally, there was no correlation between the percent difference of correponding alleles of the mother and child and the size of the allele. These results are similar to what is observed when the mother's and child's alleles are both derived from blood samples.

III. Case Observation

A. Age of Samples:

DNA from buccal swabs that had been stored for over 6 months at room temperature was successfully analyzed for RFLP results corresponding to the locus D2S44. DNA from buccal swabs that had been stored frozen for over one year was successfully analyzed using PCR results for DQ alpha and the loci corresponding to D1S80, SE33, CYP-19, YNZ22,APO-B, and Col2A1.

B. Production History:

Genetic Design Inc. performed its first buccal swab during 1991 on an geriatric individual that could not be bled for medical reasons. Since then we have processed over 1000 cases using buccal swabs. Currently we are processing over 100 buccal swab cases per month (mother, child,and alleged father) using RFLP analysis of the loci D2S44, D10S28, D17S26, and D4S139 with an average turnaround time of 17 days and a reswab rate of less than 1%. The observed heterozygosity frequencies for the four loci tested using buccal swabs were similar or identical to the frequencies observed for cases tested with blood samples. Of the 1000 cases tested, 32% were exclusions and 68% were inclusions.

IV. Summary and Conclusion

A. Buccal cells are suitable for RFLP and PCR.

B. Genetic results from buccal swab are the same as blood.

C. Eight buccal swabs yield an average of 8.6 micrograms.

D. More than 1000 cases analyzed (less than 1% reswab).

E. SAFE, painless and effective.