

Automated APOE Genotyping Directly from Blood and Buccal Specimens Without DNA Isolation: A Point-of-Care-Ready Approach

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BACKGROUND

Apolipoprotein E (APOE) genotype is a critical determinant of risk for late-onset Alzheimer's disease (AD) and cardiovascular disorders. The $\epsilon 4$ allele confers elevated AD risk, while the $\epsilon 2/\epsilon 2$ genotype is associated with reduced AD risk. Conventional APOE genotyping requires genomic DNA (gDNA) isolation, a time-consuming and technically demanding step that limits accessibility in point-of-care settings. Here, we describe a simple, fully automated protocol for APOE genotyping directly from blood and buccal cell specimens, bypassing gDNA isolation entirely.

MATERIALS & METHODS

APOE Genotyping using the automated Genotyper-8 instrument (Instant Nano Biosensors). Following Figure 1, a small aliquot of the specimen was lysed, and the resulting lysate was added to PCR. After PCR and CE, an electropherogram was produced (Figure 2) with an APOE genotype call for each sample:

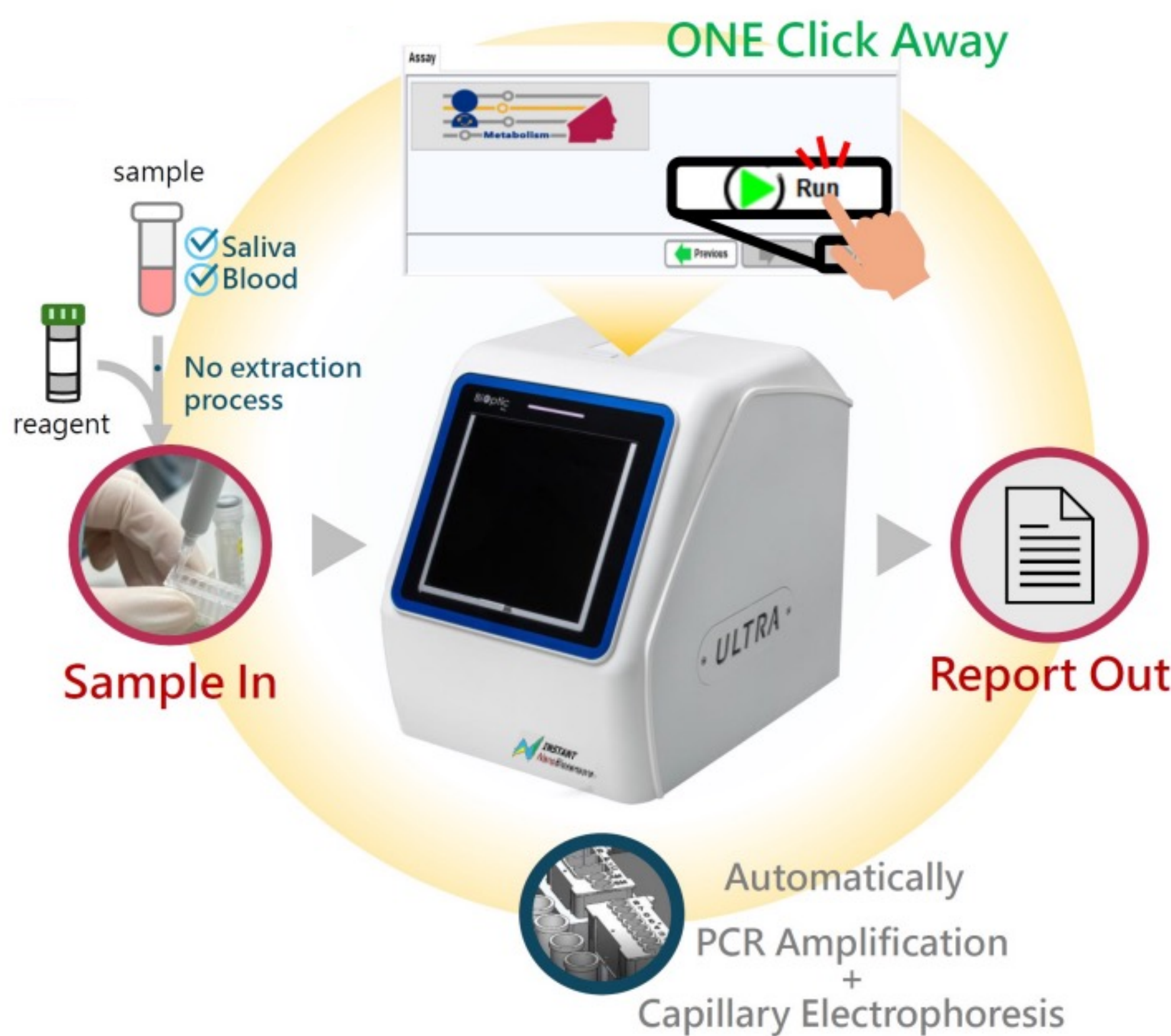


Figure 1. Illustrated procedure for genotyping using the Genotyper-8

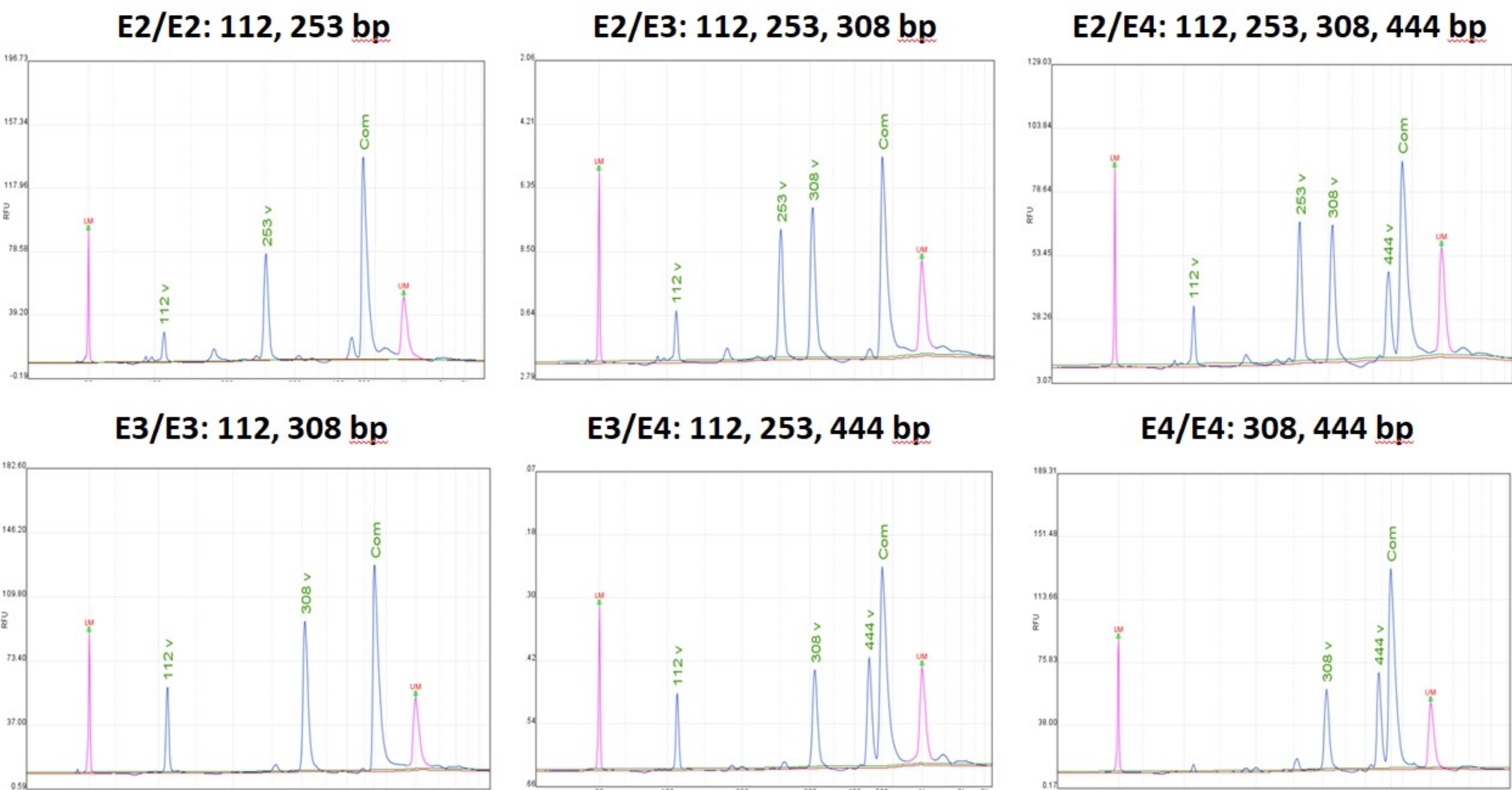


Figure 2. Examples of electropherograms produced for each genotype, along with the size markers and control band.

RESULTS

Blood and buccal cell specimens from twelve de-identified volunteers (n = 6 per sample type) were processed for APOE genotyping using the automated Genotyper-8 instrument. For concordance validation, gDNA was independently isolated from all 12 specimens using the MagMax DNA Multi-Sample Ultra 2.0 Kit (Thermo Fisher Scientific) and genotyped using DiamiR's New York State CLEP-approved TaqMan assay-based APOE test (comparator method).

Table 1. Results obtained for each blood and buccal swab sample along with the gDNA controls. Two operators performed the experiment on two separate occasions (n=4).

Specimen ID	Specimen Type	Genotyper Results		APOE Status (gDNA / TaqMan)	Genotyping Concordance Between Technologies
		Op1 (n=4)	Op2 (n=4)		
NG09714	gDNA	E4/E4	E4/E4	E4/E4	Concordant
NG16516	gDNA	E3/E4	E3/E4	E3/E4	Concordant
NG21159	gDNA	E2/E3	E2/E3	E2/E3	Concordant
NG21157	gDNA	E3/E3	E3/E3	E3/E3	Concordant
IF-2	gDNA	E2/E4	E2/E4	E2/E4	Concordant
HM-6	gDNA	E2/E2	E2/E2	E2/E2	Concordant
260204Sample1	Blood	E2/E3	E2/E3	E2/E3	Concordant
260204Sample2	Blood	E2/E3	E2/E3	E2/E3	Concordant
260204Sample3	Blood	E2/E3	E2/E3	E2/E3	Concordant
260204Sample4	Blood	E3/E3	E3/E3	E3/E3	Concordant
260204Sample5	Blood	E2/E3	E2/E3	E2/E3	Concordant
260204Sample6	Blood	E3/E3	E3/E3	E3/E3	Concordant
260211Sample1B	Buccal cells	E3/E3	E3/E3	E3/E3	Concordant
260211Sample2B	Buccal cells	E4/E4	E4/E4	E4/E4	Concordant
260211Sample5B	Buccal cells	E2/E3	E2/E3	E2/E3	Concordant
260211Sample6B	Buccal cells	No amplification	No amplification	E2/E3	Not called
260211Sample7	Buccal cells	E3/E3	E3/E3	E3/E3	Concordant
260213Sample8	Buccal cells	E3/E3	E3/E3	E3/E3	Concordant

APOE genotypes generated by the Genotyper-8 from blood and buccal cell lysates demonstrated 100% concordance with TaqMan-based genotypes from matched gDNA isolates across all specimens.

Blood specimens represented $\epsilon 2/\epsilon 3$ and $\epsilon 3/\epsilon 3$ genotypes; buccal cell specimens represented $\epsilon 2/\epsilon 3$, $\epsilon 3/\epsilon 3$, and $\epsilon 4/\epsilon 4$ genotypes. Repeatability testing across two operators demonstrated consistent genotype calls and reproducible electropherogram profiles.

CONCLUSIONS

The Genotyper-8 automated platform enables accurate, reproducible APOE genotyping directly from blood and buccal cell specimens with minimal sample preparation.

This streamlined workflow reduces hands-on time and pre-analytical variability, with a total turnaround time of approximately two hours.

Together, these findings support future deployment in point-of-care and decentralized testing environments, advancing accessibility of clinically actionable APOE genotyping for AD risk assessment in both clinical and research settings.