

*Original Research Paper*

**Amel Gene Profiling from Toothbrush for Sex Determination  
Among Tanzanians in Surabaya, East Java.**

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**ABSTRACT :**

**Introduction :** Forensic identification depends significantly on evidence detected at the crime scene. Toothbrush continues to prove its usability to aid the identification for sex determination. Its contributory power continues to be confirmed to the different population at the varied time.

**Materials and Methods :** The present study reports the use of toothbrush in determining sex through Amel gene method by DNA band contrast at 106bp and 112bp among Tanzanians on ten volunteers.

**Results :** Electrophoresis band contrast confirmed six samples to be of male origin and three sample for the female. One sample presented undetectable DNA band contrast by showing the blank image on F1 sample profiled.

**Conclusion :** Toothbrush as source of biological samples and Amelogenin gene through DNABand contrast analysis is helpful in sex determination among Tanzanians. This finding not only evidently proposes but also contributes to bench marking applicability of Amel gene analysis as a successful and reliable method of sex determination to localized population (Tanzanians) for strengthened evidential data for forensic service.

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**INTRODUCTION :**

Sex determination remains an essential parameter to the revelation of unknown live or dead<sup>[1]</sup>. Gene profile through DNA analysis has proved a reliable technique for this purpose<sup>[2]</sup>. Toothbrush like any other evidence that might be found at a crime scene, can help in identification of an individual by analysis of the harbored material<sup>[3-6]</sup>. This technique is based on Locard's exchange principle, that anything in violent contact may be retained or taken back and can later used as trace evidence<sup>[7]</sup>. To attain sex determination of such recovered sample, a number of methods are studied one of which is through the use of Amel gene (categorized in a molecular group of methods)<sup>[8]</sup>. AMEL X gene (female sex-determining DNA chromosomal marker) is present in 106 bps (base pairs) and AMEL Y (male sex-determining DNA chromosomal marker) is present in 112bps of the DNA hence homologous (XX) in female and heterogonous allele in male. This gene form Amelogenin which become detected as protein

when individualizing sex<sup>[8-9]</sup>. The method is widely accepted in forensic sex identification with a tolerable percentage error <sup>[10]</sup>. If the results are doubtful, it is suggested to run the SRY gene (sex region gene on the Y chromosome that is necessary and sufficient for male sex determination) or/and Y-STR markers (specialized class of short tandem repeats on the male Y-chromosome often used in forensics, paternity, and genealogical DNA testing) as parallel methods in order to confirm and resolve ambiguity<sup>[11-12]</sup>. Despite the marginalized ambiguity, its usefulness remains profound into varied populations and continue to be suggested among techniques in sex determination <sup>[8,13,14]</sup>.

Social offenses, crime and disasters casualties accelerate immensely in costing and endangering global life among Tanzania inhabitants. Solving the mystery, forensic identification responds to involved individual, couple, family or mass in terroristic, catastrophic or destructive events. Forensic scientists at the front line are obliged to bring the

meaning of all thought “evidence” in identification to sexing gender of the involved for the law-suit purpose or resolving trepidation of the grieving<sup>[15,16]</sup>. In mutilated, destructed, decomposed or burned cases, toothbrush substantiates DNA profile of genetic materials logged with cells worn during brushing. Therefore; this study aimed at exploring the usefulness of toothbrush and Amelogenin in determining sex among Tanzania population. This will mark continued efforts in deriving experience from a different population and strengthening and improving forensic services and human resource personnel in line with tremendous changes that Tanzania is taking recently <sup>[17]</sup>.

**MATERIALS AND METHODS :**

After taking approval from Ethics committee and consent from Ten Tanzanians (six male and four female) participants, the present study was conducted at the Human Genetic Laboratory of the Airlangga University. Toothbrushes were collected after being used (as instructed) regularly for one week and sent to the laboratory for DNA analysis.

**Experimentation Process :**

Analytical process started by soaking of the toothbrushes into distilled water for an overnight in order to recover the logged biological samples (worn cells) from bristles at room temperature. Recovered biological samples were extracted by an organic method using DNA zol (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) at alternated Voltex, incubation and centrifugation<sup>[18-19]</sup>. Resulted pellet was resuspended to form 50 µl final volume which was then measured by Ultraviolet-visible Spectrophotometer (UV-1601, PC, Shimadzu, Japan) to obtain concentration and purity. Amplification of sex fragment was by PCR mix (Promega Corporation, Madison, USA) using common amelogenin gene (Promega Corporation, Madison, USA) at a set of (forward: 5'-CCCTGGGCTCTGTAAAGAA-3' and reverse: 5'-ATCAGAGCTTAACTGGGAAGCTG-3') primers as recommended<sup>[20]</sup>. Amplified DNA of Amel genes (106bp and 112bp) were visualized as band contrast using Gel Polyacrylamide 6% gel (cat. K562, GoTaq®, Green Master Mix, Promega Corp, Madison, USA) formed by electrophoretic migration.

**A : DNA extraction process using DNAzol**



**Spectrophotometer machine used to MEASURE extracted DNA**



**PCR machine used to polymerase targeted STR loci... thermal cycler**



**Electrophoretic reaction by the use of Vertical electrophoresis (acrylamide gel) to contrast AMEL gene**



**E: Toothbrushes used Colgate toothpaste used**





G: Centrifuge machine used



**RESULTS :**

**Amel Gene profile sex detection :**

The Polymerase Chain Reactions ( P C R ) through electrophoresis visualization of the profile identified correctly nine out of ten (Table 1).

Amelogenin gene detection presented two bands for male samples and one band for female samples (Figure 1). However one sample didn't respond to any of the sex gene categories during electrophoresis ionic segregation (no band appearance). **Based upon it, participants were divided into three categories :**

**True positive male (M3 – M8) :** Amelogenin identified by 106 and 112bp in reference to 100bp

**True positive female (F2, F9 & F10) :** Amelogenin identified by 106bp in reference to 100bp

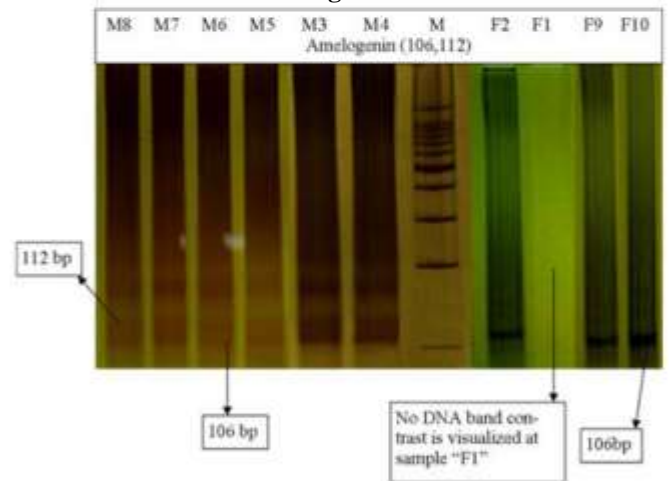
**Null (F1) :** no observed band presented on a profile

The null presenting sample (F1) category on further examination was found accumulated with green toothpaste remains of Sodium Monofluoro phosphate (Compound constituted in toothpaste) which is supported and proven to have contributory damage and degradation effect to DNA molecules<sup>[21-24]</sup>.

**Table 1: Determined Sex by Amel Gene Profile of 106bp and 112bp DNA Band**

Sample code	Amel gene	Sample Sex
F1	Null (-)	Null (-)
F2	106bp	Female
M3	106bp & 112bp	Male
M4	106bp & 112bp	Male
M5	106bp & 112bp	Male
M6	106bp & 112bp	Male
M7	106bp & 112bp	Male
M8	106bp & 112bp	Male
F9	106bp	Female
F10	106bp	Female

**Figure 1: Determined Male sex on M3 – M8, Female on F2, F9 & F10 with the blank F1 sample with reference to Amel gene-sex determinants.**



**DISCUSSION :**

Amelogenin gene is scientifically proved as a marker for sex determination. Present study has shown its applicability to Tanzania population which will help in solving forensic inquiries. Our findings are similar to studies by Indian authors<sup>[8,13,14]</sup>. The sample profiled from toothbrush logged cells successfully responded to the testing of Amelogenin as similar to study by Kashyap et al<sup>[10]</sup>. The finding is also supported by the fact that Amel profiling still work better except in

destructive and inhibitory media (toothpaste in this case).

In this study, all toothbrushes used responded to amelogenin sex determination except one that presented an undetectable DNA band on the electrophoresis profile as portrayed in **Figure1**.

The undetectable sample implies and agrees with the effect of fluorine ingredient in the toothpaste as established by similar other studies<sup>[21-22]</sup>. Otherwise, the usage of toothbrush despite the predisposed and potential degradation, contaminants and inhibitory factors, remain reliable and preferred physical evidence at crime scene for recovering DNA samples. Toothbrush like many other sources of collecting evidence weighs similar importance and value in terms of usefulness, reliability, and applicability among Tanzanians.

#### **CONCLUSION :**

The toothbrush continue to be an important source to solve forensic inquiries especially related to identification and individualization. It has yielded enough quality DNA that successfully resulted in good DNA profiling. Amelogenin gene analysis has presented a convincing and promising finding for sex determination.

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