



DNA typing after α -amylase test

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1. Introduction

Saliva traces in forensic casework can be found in many different samples such as cigarette butts, stamps or balaclavas (Fig. 1). In these cases, we are able to test for saliva presence by α -amylase testing [1] (BNP-Amylase test, Sclavo), an enzymatic method that utilises blocked *p*-nitrophenyl-maltoheptaoside (BpNPG7) as substrate. The α -amylase (1,4- α -D-glucan glucanhydrolase) catalyses the endohydrolysis of α -1,4 glucosidic linkages to produce *p*-NP-labelled fragments (*p*-NPGn). Glucoamylase (exo-1,4- α -D-glucosidase) and maltase (α -glucosidase) quickly hydrolyse nonreducing ends of the chains, liberating *p*-nitrophenol; the absorbance of *p*-nitrophenol (*p*-NP), stoichiometrically formed, can be measured at 405 nm. The rate of its liberation is directly proportional to the α -amylase activity in the sample.

This test is rapid, highly sensitive and allows forensic scientists to determine exactly and localise saliva traces. When we have a positive response with the α -amylase test, we extract the DNA directly from the same region of the substrate with a modified phenol–chloroform method. We performed over 200 tests with reference saliva samples on cigarette butts, stamps and balaclavas. No differences were observed between α -amylase tested versus nontested samples in DNA recovery and successful STR typing.

2. Materials and methods

Six different substrates were utilised in this study: cigarette butts, toothbrushes, stamps, chewing gum, balaclavas and glass surfaces. Each sample was obtained from laboratory

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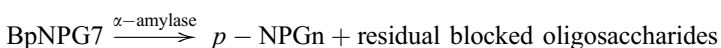
E-mail address: gplago@tiscalinet.it (G. Lago).



Fig. 1. Balaclavas, α -amylase test.

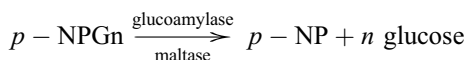
personnel and was divided in two. On one set, direct DNA extraction was performed, the second set was previously α -amylase tested.

To detect α -amylase activity, we used the BNP-Amylase test [1]. This is a total enzymatic method that utilises blocked *p*-nitrophenyl-maltoheptaoside (BpNPG7) as the substrate. This unique soluble substrate, labelled with *p*-nitrophenol (*p*-NP), has a blocked nonreducing terminal group preventing the hydrolysis by exoenzymes such as glucoamylase and maltase. The hydrolysis of the substrate can be kinetically measured by the increase in colour caused by liberation of the *p*-NP. The ready-to-use reagent is stable and has a short lag phase. α -Amylase (1,4- α -D-glucan glucanhydrolase) catalyses the endohydrolysis of α -1,4 glucosidic linkages to produce *p*-NP-labelled fragments (*p*-NPGn) with a different glucose number (from 2 to 5) and residual blocked oligosaccharides:



Glucoamylase (exo-1,4- α -D-glucosidase) and maltase (α -glucosidase) quickly hydrolyse in sequence, the terminal 1,4-linked α -D-glucose residues from nonreducing ends of

the chains (only nonblocked, contrary to the BpNPG7 substrate), liberating *p*-nitrophenol:



The absorbance of *p*-nitrophenol (*p*-NP), stoichiometrically formed, can be measured at 405 nm. The rate of its liberation is directly proportional to the α -amylase activity in the sample.

Extraction was carried out essentially as described in Sambrook et al. [3]. The samples were digested at 37 °C overnight in 500 μ l extraction buffer (10 mM Tris–HCl, pH 8, 100 mM NaCl, 10 mM EDTA, 2% SDS, 39 mM DTT) in single tube with 15 μ l of proteinase K (20 mg/ml). The DNA was phenol–chloroform-extracted and ethanol-precipitated. Finally, the samples were resuspended in 50 μ l of sterile deionized water.

Quantitation of human genomic extracted DNA was performed using the ACES 2.0 Human DNA quantitation system (GIBCO BRL). A small amount (5 μ l) of the extract was quantified by direct comparison with DNA standards of known concentrations, using a biotinylated human-specific DNA probe from the alpha satellite locus D17Z1, followed by ECL detection as described by Walsh et al. [2].

Simultaneous amplification of the 13 CoDIS STR loci was performed using AmpFISTR® Profiler Plus (Applied Biosystems) and GenePrint® PowerPlex 1.2 (Promega). STR amplification as well as the gender determination marker, Amelogenin, was conducted in 12.5 μ l final reaction volume containing 0.5 ng of genomic DNA, PCR Reaction Mix, Primer Set Solution and 0.3 μ l of AmpliTaqGold® DNA polymerase (5 U/ μ l stock). Amplification was conducted on a Perkin Elmer GeneAmp PCR System 9600 thermal cycler using standard protocols [4].

Analysis of the amplified products was performed as follows: an aliquot of 1 μ l of each PCR reaction was mixed with 0.3 μ l of ABI Gene Scan-500 Internal Lane Size Standard (labelled with 6-carboxy-X-rhodamine) and 2.7 μ l of denaturing loading buffer. After denaturation at 95 °C for 2–3 min, the samples were snap-cooled in ice, and 1- μ l aliquots were loaded on a 4% (19:1) acrylamide/bisacrylamide gel containing 6 M urea (36-cm well-to-read glass plate format), which had been pre-run at constant voltage for 30 min and equilibrated to 51 °C. Electrophoresis was conducted for 2.25 h at constant voltage (3000 V, 50 mA) in 1 \times TBE using an ABI PRISM 377 DNA Sequencer with the laser set at 40 mW [5]. Allele sizes were determined with Gene Scan Analysis v.2.1 software. Automatic allele designation was achieved by means of Genotyper v.2.1 software (Applied Biosystems) [5].

3. Results and discussion

This study demonstrates that consistent DNA typing results can be obtained from post- α -amylase-treated substrates suspected to have saliva stains.

The α -amylase test is a rapid method used in forensic analysis for screening substrate surfaces suspected to have saliva stains. Saliva can be found on cigarette butts, tooth-

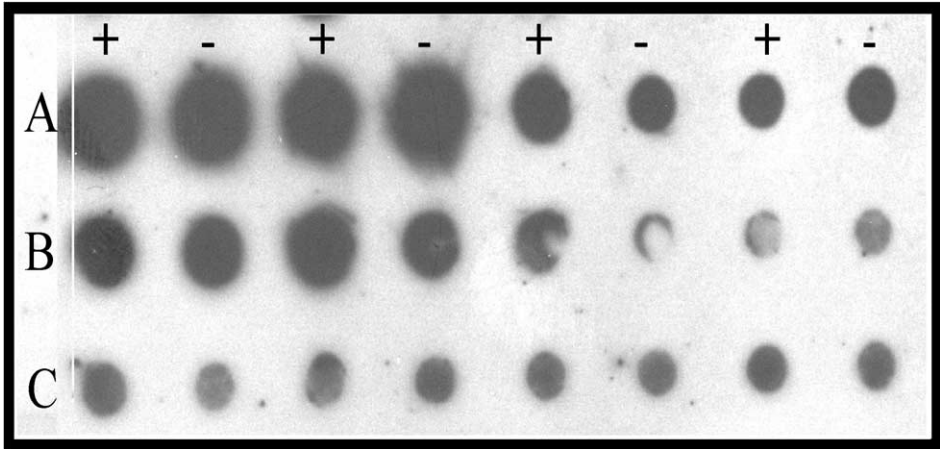


Fig. 2. Slot Blot Analysis.

brushes and balaclavas used in robbery cases (Fig. 1). To confirm the saliva nature, we tested these stains with the α -amylase test and, if positive, we directly extracted the DNA. To evaluate the effect of α -amylase testing, we performed a set of reference samples from the most common forensic cases such as cigarette butts, stamps and sleeves used as balaclavas.

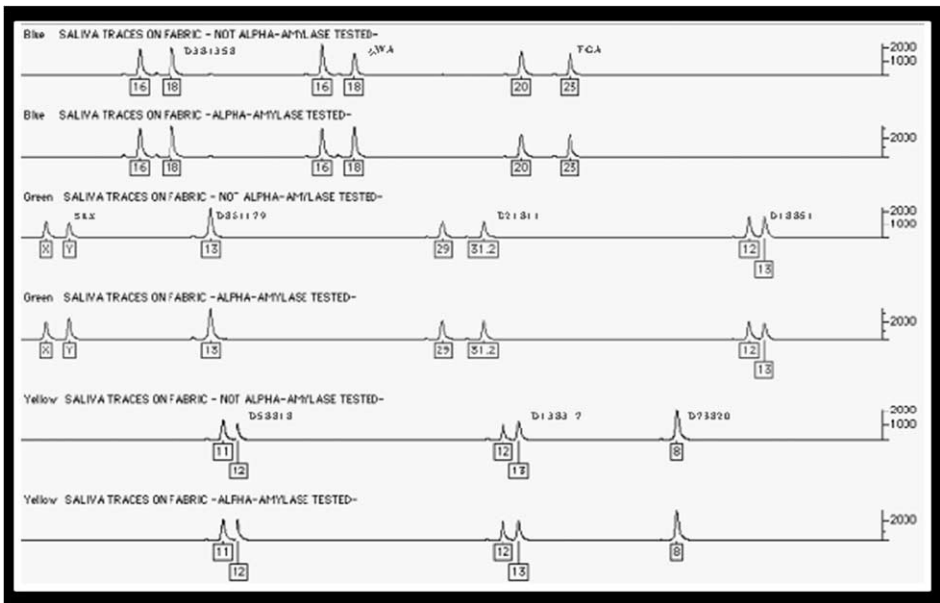


Fig. 3. Applied Biosystem AmpFISTR® Profiler Plus.

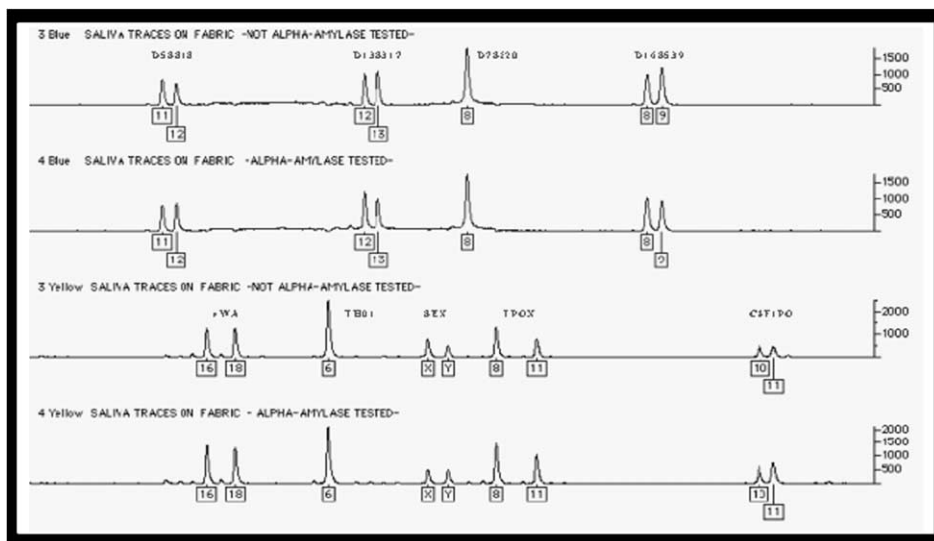


Fig. 4. Promega GenePrint® Powerplex 1.2.

DNA was extracted from both treated and nontreated samples. In order to evaluate DNA recovery, we performed Slot Blot Analysis (Fig. 2). No significant differences were observed, showing that the α -amylase test has no effect on DNA recovery.

All samples were subsequently typed for the 13 CoDIS STR loci using AmpFISTR® Profiler Plus (Applied Biosystems) and Promega GenePrint® PowerPlex 1.2 systems and analysed on an ABI 377 DNA Sequencer.

The results obtained (Figs. 3 and 4) clearly indicate that the α -amylase test had no effect on amplification or successful typing of the samples.

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