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Evaluation of amylase testing as a tool for saliva screening of crime scene trace swabs

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ABSTRACT

Amylase testing has been used as a presumptive test for crime scene saliva for over three decades, mainly to locate saliva stains on surfaces. We have developed a saliva screening application for crime scene trace swabs, utilising an amylase sensitive paper (Phadebas® Forensic Press test). Positive results were obtained for all tested dried saliva stains (0.5–32 μL) with high or intermediate amylase activity (840 and 290 kU/L). Results were typically obtained within 5 min, and all samples that produced DNA profiles were positive. However, salivary amylase activities, as well as DNA concentrations, vary significantly between individuals. We show that there is no correlation between amylase activity and amount of DNA in fresh saliva. Even so, a positive amylase result indicates presence of saliva, and thereby presence of DNA. Amylase testing may be useful for screening in investigations where the number of DNA analyses is limited due to cost, e.g., in volume crime.

1. Introduction

Saliva is the most common DNA source at crime scenes in property (volume) crime [1], and can be retrieved from a range of objects using moistened cotton swabs [2,3]. However, a police force in the United Kingdom reported that 57% of presumed saliva stains from bottles and cans did not produce acceptable DNA profiles [1]. This may partly be explained by the lack of pre-testing.

The starch-digesting enzyme amylase is present in high concentrations in human saliva [4]. In forensic investigations, amylase activity testing has been used to locate saliva stains on surfaces for over three decades [5,6]. Presently, a screening method based on amylase testing could be useful to lower the number of negative DNA samples from crime scenes. However, to use such a method some things need to be considered. It is known that salivary amylase activity varies greatly between individuals [4,7–9], and also varies somewhat over time within individuals [4,8]. Furthermore, people have different propensities to shed epithelial cells [10], affecting the amount of DNA in saliva. The correlation between amylase activity and amount of cells/DNA in saliva therefore needs to be investigated. Here, we measured the amylase activity levels and determined the corresponding DNA concentrations of saliva collected from 10 males.

Additionally, we used the Phadebas® Forensic Press test (Magle Life Sciences, Lund Sweden), originally intended to locate saliva stains on surfaces [11], to develop an application for quick saliva screening of crime scene trace swabs. The presence of amylase is indicative for human saliva, but not proportional to the amount of DNA in a crime scene stain. However, amylase screening could be useful in volume crime, where the number of performed DNA analyses for each case may be limited due to cost issues.

2. Materials and methods

2.1. DNA concentrations in presumed saliva stains from real crime cases

At SKL, analysis of volume crime DNA samples with DNA concentrations below 0.025 ng/μL is discontinued, due to the low probability of obtaining complete DNA profiles. To determine the fraction of presumed saliva/secretion stains with DNA concentrations below this limit, we evaluated the outcomes of the DNA analyses of all (n = 17,587) presumed saliva/secretion stains analysed in routine crime cases at SKL during 2007. The main sample types were cigarette butts and swabs from bottles, cans etc. DNA concentration data was obtained from the laboratory

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information management system (LIMS) Forum DNA (Ida Infront, Linköping, Sweden). DNA extraction was performed using Chelex® beads (Bio-Rad Laboratories, Hercules, CA, USA) [12], with the addition of Centricon® (Millipore, Billerica, MA, USA) purification [13] for visibly dirty samples. DNA quantification was performed using the Quantifiler® human assay (Quantifiler kit's user manual, Applied Biosystems, Foster City, CA, USA, 2003) on a ABI 7300 (Applied Biosystems).

2.2. Correlation between amylase activity and DNA concentration in fresh saliva

Saliva samples were retrieved from 10 healthy male volunteers, 22–35-years old, at least 1 h after eating or drinking other beverages than water. All samples were obtained at 10 am, since amylase activity is known to vary during the day [14]. The amylase activity was measured using the Phadebas Amylase test® (Magle Life Sciences) following the manufacturer's instructions (Phadebas Amylase test user manual, Magle Life Sciences, 2008). Three replicates were analysed for each sample. One month after the first analysis, new saliva samples were collected from volunteers #1, #2 and #10, and the amylase activity was re-measured, in triplicates.

Three replicates of 32 μL of saliva from each of the 10 volunteers were analysed for DNA concentration (see Sections 2.3.4 and 2.3.5). The correlation between salivary amylase activity and salivary DNA concentration was investigated using Spearman's rank correlation test.

2.3. Evaluation of the saliva swab screening application

Three experimental series were performed in order to evaluate the usability of the developed saliva swab screening application. First, the sensitivity was studied by screening swabs with different amounts of saliva and different amylase activity levels. Second, the effect of the screening on DNA recovery was investigated. Third, a study with saliva on glass bottles was performed, in order to test the screening tool in a "crime scene like" environment.

2.3.1. Preparing dried saliva stains

2.3.1.1. Sensitivity. Saliva from three of the volunteers (with high, intermediate and low amylase activities, i.e., #1, #2 and #10) was used. Four replicates of 32, 8, 2 and 0.5 μL of pure saliva were evenly distributed on 2 cm × 2 cm areas of clean window glass.

2.3.1.2. DNA recovery. Ten replicates of 8 and 2 μL of saliva (from volunteer #2) were evenly distributed on 2 cm × 2 cm areas of clean window glass.

2.3.1.3. Mock crime scene. Three replicates of 32, 8, 2 and 0.5 μL of saliva (from volunteer #2) were evenly distributed on the rims of clean glass bottles' necks. In addition, three 33 cL mineral water glass bottles were drunk by volunteer #2. To avoid biased analyses, the experimenter was unaware of the saliva amounts on the bottles.

All saliva stains were left to dry at room temperature for 24 h prior to swabbing.

2.3.2. Swabbing

The saliva stains were swabbed using sterile cotton swabs (SelefaTrade, Spånga, Sweden) moistened with 30–60 μL of sterile saline solution. Only the tip of the swab was applied to the surface, in order to focus the absorbed saliva in a small spot. Each 2 cm × 2 cm window glass area was swabbed three times from different directions. The bottles' necks were swabbed in a similar fashion.

2.3.3. Saliva screening of crime scene trace swabs

Directly following swabbing, the tip of the cotton swab was gently pressed against the blue side of the Phadebas Forensic Press test (Phadebas paper), transferring a minute amount of fluid to the paper. 30 μL of physiological saline solution was added to the same spot, and the paper was left to dry in room temperature. The paper was checked for colour changes after 2, 5, 10 and 20 min, respectively. After 20 min, the paper was dry. In the sensitivity test, another 30 μL of saline solution was added to the stain on the paper after drying. This step was repeated once. The colour change is generally detectable on both sides of the Phadebas paper, but is more easily visible on the blue side. Therefore, this side was used for detection throughout the study. The colour changes were denoted as '−' (negative); '+' (positive) or '++' (intensely positive) (Fig. 1).

2.3.3.1. DNA recovery. Saliva screening was performed on five of the ten swabbed samples per saliva amount. The remaining five samples were used as DNA concentration references, i.e., no saliva swab screening was performed. All 10 samples were subjected to DNA analysis.

2.3.4. DNA extraction

DNA was extracted from pure saliva and from the cotton swabs using the Chelex® method [12], with the cotton material kept in the microfuge tubes throughout the extraction process. The final elution volume was 200 μL.

2.3.5. Real-time qPCR

2.3.5.1. Fresh saliva, DNA recovery. A 156 bp fragment was amplified using a previously described real-time qPCR assay

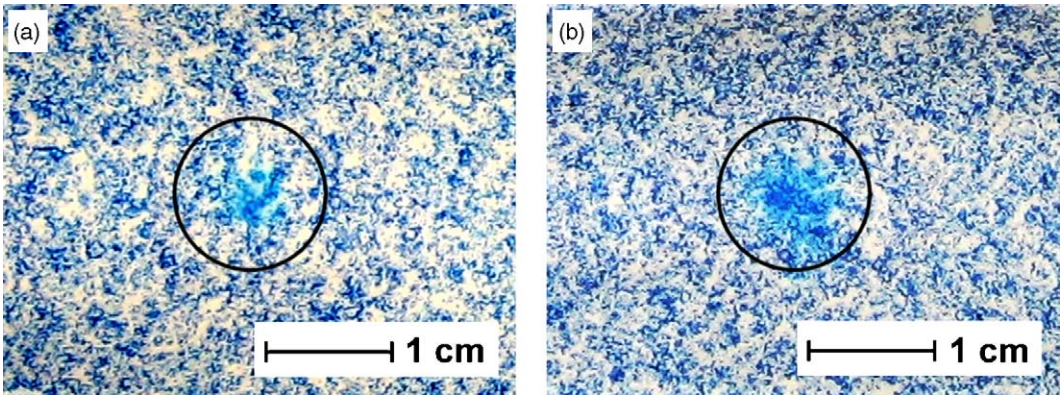

Fig. 1. (a) Positive (+, left) and (b) intensely positive (++, right) colour change from pressing a saliva-containing swab against the Phadebas paper during the saliva screening of swabs.