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The sensitivity and specificity of the RSID™-saliva kit for the detection of human salivary amylase in the Forensic Science Laboratory, Dublin, Ireland

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ABSTRACT

We demonstrate here that the RSID™-saliva test can be used as a test for human salivary α-amylase on samples routinely examined in forensic casework. We show that the RSID™-saliva test detects salivary α-amylase at lower concentrations than the Phadebas® Quantitative test, that the RSID™-saliva test does not cross-react with forensically important human fluids and that the RSID™-saliva test can be successfully integrated into the whole swab semen extraction method.

1. Introduction

The forensic detection of human saliva can be a very powerful tool in the investigation of crime. In particular, in the investigation of cases of a sexual nature where the detection of saliva can demonstrate contact between the complainant and the accused. In cases of stranger sexual attacks the presence of human saliva can lead to a DNA profile from a suspect, as epithelial cells found within saliva are a potential source of DNA.

Salivary α-amylase is produced in the salivary glands and its physiological role is the digestion of starch, beginning in the mouth [1]. In humans two main isozymes of α-amylase exist, salivary α-amylase and pancreatic α-amylase. Both α-amylases's have been identified in many different body fluids [2–5]. Historically this has led to difficulties in reporting the presence of salivary α-amylase in forensic case work. Current methods available for the detection of saliva have a number of drawbacks, most importantly is specificity and sensitivity [6–8] and they can be difficult to integrated into DNA profiling techniques [8,9]. The presumptive identification of saliva has conventionally been preformed by the detection of amylase using techniques such as the Phadebas® paper assay [2] or Red-starch paper [9] followed by the Phadebas® Quantitative test. These systems rely on the ability to identify the enzyme activity of α-amylase, a constituent of saliva and cannot distinguish between these two different α-amylase isozymes or the α-amylase present in plants, bacteria and fungi [1]. The lack of mobility associated with these tests also limits their use to the laboratory. The current systems for salivary α-amylase detection in the Forensic Science Laboratory, Dublin are the presumptive Phadebas® paper assay followed by the Phadebas® Quantitative test (Magle Life Sciences, Lund, Sweden).

The RSID™-saliva test is a lateral flow immunochromatographic strip test designed to detect the presence of human salivary α-amylase. The test, which uses two anti-human salivary amylase monoclonal antibodies, detects the presence of salivary amylase, rather than the activity of salivary amylase as seen with other tests.

2. Materials and methods

2.1. Samples

Human saliva from three individuals was collected, combined and used within 12 h of collection. For sensitivity tests, serial dilutions of human liquid saliva were prepared using PBS (Sigma), or human blood or human urine as diluents for mixed body fluid tests. 50 μl was pipetted onto cotton swabs and allowed to dry. Semen and blood were obtained from a local hospital. Penile swabs of the coronal sulcus (the groove or furrow between the shaft and the head) of the penis and the glans (the head of the penis) were collected. Vulval swabs and faecal samples (anal swabs) and sweat samples (underarm swabs after exercise) were collected fresh and frozen until required. Vaginal secretions were sampled from female volunteers who wore a new pair of panties for their working day and visibly stained areas were excised and tested. Swabs of faecal material from the nappies of five children ranging in age from 8 months to 24 months were tested. Animal saliva samples (buccal swabs) from guinea pig, cat, dog, mouse and sheep were used for species specificity. Case work samples of vulva swabs from complainants processed through the full swab semen extraction method [10], were further tested for the presence of human salivary α-amylase. Controls included; positive

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human buccal swabs, negative unused swabs, swabs of fresh neat urine, swabs of semen or blood.

2.2. Methodologies

The manufacturers (single tube-Stain ID Integrated into STR analysis, RSID™-saliva, April 2007) protocol was used initially in this investigation (described as Section 2.3). Two other methods (Sections 2.3 and 2.4 described below) were developed after difficulties were identified with the above protocol for some of the forensic samples examined in this paper. Body fluids (liquid saliva diluted in PBS, urine or blood) were prepared at the following dilutions;1/10, 1/100, 1/200, 1/300, 1/400, 1/500, 1/600, 1/700, 1/800, 1/900, 1/1000.

2.3. Method 1

The single tube-Stain ID Integrated into STR analysis protocol.

50 μl of the body fluid preparations were pipette onto swabs and left to air-dry. Swab (one half) was placed into 1.5 ml microcentrifuge tubes. 300 μl of RSID™-saliva extraction buffer was added to the tube contained the cutting and agitated by vortexing for approximately 15 s. Samples were incubated for 1–2 h at room temperature to extract. 20 μl of the extracted solution was then added to a new microcentrifuge tube containing 80 μl of running buffer (20% final volume of extracted sample). This solution (100 μl) was then loaded on to a RSID™-saliva cassette. Results were read at 10 min.

2.4. Method 2

Swabs (one half) were placed into 1.5 ml microcentrifuge tubes. 300 μl of RSID™-saliva extraction buffer was added to the tube containing the swab cutting and agitated by vortexing for approximately 15 s. Samples were incubated for 1–2 h at room temperature. 12 μl of the extract solution was then added to a new microcentrifuge tube containing 108 μl of running buffer (10% final volume of extracted solution). 100 μl was then loaded on to a RSID™-saliva cassette. Results were read at 10 min.

2.5. Method 3

The whole swab semen extraction method [10] used in the Forensic Science Laboratory generates a 300 μl final volume of supernatant. 40 μl of this supernatant was pipetted into 1.5 ml microcentrifuge tubes containing 260 μl RSID™-saliva extraction buffer. Samples were incubated for 1–2 h at room temperature. 12 μl of the extract solution was then added to a new microcentrifuge tube containing 108 μl of running buffer. 100 μl was then loaded on to a RSID™-saliva cassette. Results were read at 10 min.

We compared the sensitivity and the robustness of the RSID™-saliva kit against the Phadebas® Quantitative test (Magle Life Sciences) as per the manufacturers protocol. Samples were analysed using the Perkin–Elmer Lamda 35 UV/vis spectrophotometer. The Phadebas® Quantitative test is considered to give a positive result for salivary α-amylase activity at OD 620_{nm} >0.3 (Metropolitan Police Manual, 1973) [11].

2.6. Reading results

100 μl of sample are pipetted into the sample window (S), and results read at 10 min. The presence of two red lines, one in the test area 'T' and one in the control area 'C' indicates a positive result. A red line in the control 'C' area only indicates a negative result. The absence of a red line at the 'C' indicates an invalid test (Fig. 1).

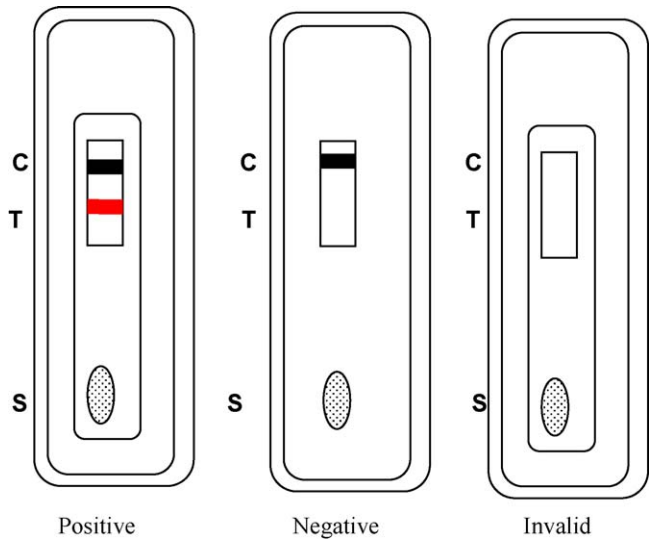

Fig. 1. Three RSID™-saliva cassettes showing the expected results from a positive reaction, negative reaction and an invalid reaction for salivary amylase. In cases where the operators disagreed on the presence or absence of a red line in the test area 'T' the test was identified by a 'R' in the results sheet.

3. Results and discussion

3.1. The sensitivity of the RSID™-salvia test

Swabs of liquid saliva/PBS serial dilutions up to a 1000-fold dilution extracted through Methods 1 and 2 resulted in a limit of detection of up to a 500-fold dilution and a limit of detection to a 100-fold through Method 3. Parallel samples extracted through Methods 1, 2 and 3 assayed using the Phadebas® Quantitative test resulted in a limit of detection of up to a 100-fold dilution (Table 1). Pang and Cheung [8] has shown that RSID™-Saliva kit can detect up to a 10,000-fold dilution (0.1 nl/μl) of commercially lyophilized human saliva and up to a 20,000-fold dilution (0.5 ng/μl) human salivary amylase respectively. This equates to a limit of detection from the liquid saliva combined from three individuals in this study to approximately 5 nl/μl (1/500) of human saliva or 50 ng/μl (1/500) of human salivary amylase for the RSID™-saliva test compared to approximately 9 nl/μl (1/100) of human saliva or 90 ng/μl (1/100) of human salivary amylase for the Phadebas® Quantitative test. Our results are in agreement with Pang and Cheung [8] and demonstrate that the RSID™-saliva test is more sensitive detecting Human salivary amylase than the Phadebas® Quantitative test. No variation in the level of sensitivity of the RSID™-saliva test was observed when similar dilutions were tested using Method 1, on nylon, denim or cotton fabrics (data not shown). Gutowski and Henthorn [12] have reported variations in the detectable levels of salivary amylase activity on dried saliva stains on different fabrics, using the Phadebas® Quantitative test.

The level of detection from liquid saliva/blood mix increased to a 1000-fold dilution (Table 2). The high protein concentration of blood has been attributed to a reduction in the measurable activity of amylase [13]. However given blood homeostasis is tightly controlled; the natural buffering capacity of blood appears to improve the sensitivity of the RSID™-saliva test.

Table 1
The limit of detection of the RSID™-saliva test versus the Phadebas® Amylase assay.

Diluent (PBS)	RSID™-saliva result methods			Phadebas® OD 620_{nm} result methods			
	1	2	3	1	2	3	
Pos. buccal	+	+	+	5.02	3.80	1.03	(+)
Neg. (PBS)	–	–	–	0.00	0.00	0.00	(–)
1/10	+	n.t.	+	4.60	n.t.	4.73	(+)
1/100	+	+	+	0.49	0.50	4.52	(+)
1/200	+	+	–	0.04	0.02	0.15	(–)
1/300	+	+	–	0.07	0.16	0.20	(–)
1/400	+	+	–	0.14	0.21	0.08	(–)
1/500	+	+	–	0.07	0.16	0.08	(–)
1/600	–	–	–	0.05	0.11	0.07	(–)
1/700	–	–	–	0.04	0.11	0.12	(–)
1/800	–	–	–	0.02	0.09	0.10	(–)
1/900	–	–	–	0.00	0.00	0.06	(–)
1/1000	–	–	–	0.00	0.00	0.04	(–)

Serial dilutions of liquid saliva were prepared in PBS tested as per Methods 1, 2 and 3. (+) positive result, (n.t.) not tested, (–) negative result, OD 620_{nm} (+>0.3),