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Research Article

Sensitivity and Intertextile variance of amylase paper for saliva detection

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Summary

Contemporary forensic science hinges on DNA analysis to link an individual to a crime scene. Sources of DNA include bodily fluids, including saliva. Amylase is a primary enzyme in human saliva and thus, if detected, indicates possible presence of human saliva. Amylase paper can be used to map apparent saliva and thus provide a source from which DNA can be extracted and analyzed. In this study, the sensitivity of amylase paper was tested, firstly, using dilutions of an amylase standard and subsequently also tested using fresh human saliva. Three trials total were conducted, the first two using an amylase standard and a third using fresh saliva. The first two trials demonstrated firstly that detection of amylase is dependent on the material upon which amylase is deposited. The third trial demonstrated that amylase levels in human saliva may drop significantly somewhere around 48-72 hours. All trials were consistent in the concentration of amylase that Seratec Amylase Paper will detect.

More Information

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Introduction

As it currently stands, DNA is the strongest evidence to link an individual to a particular crime scene. Its specificity leaves little to no doubt as to whom the DNA belongs. Sources at a crime scene are in the form of bodily fluids: usually blood or semen, but saliva as well carries a significant concentration of DNA and therefore can prove to be an invaluable piece of evidence. However, unlike the vibrant color of blood and visibility of dried semen, saliva is much harder to see with the naked eye. Currently the detection of saliva involves looking for its main component, amylase. Amylase is the primary enzyme in saliva and is responsible for digesting starches. It is important to have a method of detecting or mapping saliva, especially in cases in which a victim has been orally assaulted. One method of this is an alternating light source, ALS, which uses UV light which causes the saliva stain to fluoresce, enhancing its visibility. However, an ALS will cause other bodily fluids to fluoresce and saliva cannot be distinguished from these [1], it is necessary to use a more specific test. Another method of this is to use amylase paper. The paper is laced with starch; amylase will digest the starch in the paper. When dyed with iodine, the location in which the starch has been digested will appear differently than the rest of the dyed paper. This allows for a mapping of the saliva stain. When mapped, one is then able to make an extract of the saliva and use this to extract DNA. The primary purpose of this study was to assess the sensitivity of the amylase paper by diluting amylase concentrations and testing the paper's ability to still detect amylase. While part of the study utilized an amylase standard, it is important to note that amylase concentrations in fresh saliva will differ greatly. While the amylase standard, concentrated, contains 1 kU/mL, the average concentration in human saliva is somewhere around 93 IU/mL [2]. Therefore, we also used fresh human saliva to get a more realistic assessment of the sensitivity of the paper.

Results

Materials and methods

- Seratec amylase paper (Gottingen, Germany)
- Iodine
- Distilled water
- Denim sample
- Cotton sample
- Polyester sample
- Amylase standard
- Saline solution (for dilution)

Amylase paper was obtained from Seratec (Gottingen, Germany) and stored at room temperature until use. Sections large enough to cover the stained areas were cut out. Fabric, which included cotton, denim, and polyester, was obtained from Jo-Ann Fabrics and washed in a washing machine

in cool water, then dried in a dryer at a delicate setting. Amylase standard, catalog number 11111, 1 kU, was obtained from Sirchie (Youngsville, North Carolina). Fresh saliva was collected from five student volunteers, combined, and immediately serially diluted with saline.

Trial 1

1. A serial dilution of the amylase standard (using saline solution) was made of the amylase standard in the following concentrations:
    a. 1 kU/1mL
    b. 500 U/mL
    c. 250 U/mL
    d. 125 U/mL
    e. 62.5 U/mL
    f. 31.25 U/mL
2. Each dilution was added as a stain using 20 microliters to a denim sample, a cotton sample and a polyester sample (Tables 1,2).
3. Stains were mapped over the following time periods:
    a. 2 hours
    b. 24 hours
    c. 48 hours
    d. 1 week
    e. 2 weeks
    f. 3 weeks

Trial 2

1. Only denim was used in this trial
2. The following dilutions of the amylase standard were used (Table 3)
    a. 1 K IU
    b. 500 IU/mL
    c. 250 IU/mL
    d. 125 IU/mL
    e. 62.5 IU/mL
    f. 31.25 IU/mL
    g. 15.6 IU/mL
    h. 7.8 IU/mL
    i. 3.9 IU/mL
    j. 2 IU/mL
    k. 1 IU/mL
    l. 0.5 IU/mL
3. The dilutions were added to the denim sample in 30 microliter stains.
4. Stains were mapped at the following times:
    a. Immediately
    b. 24 hours
    c. 48 hours
    d. 1 week
    e. 2 weeks
    f. 3 weeks

Table 1: Table demonstrating time versus sensitivity of paper on cotton sample.

Time vs Dilution (in IU/mL)	1K	500	250	125	62.5	31.25
24 hours	+	+	+	+	-	-
48 hours	+	+	+	-	-	-
1 week	+	+	+	-	-	-
2 weeks	+	+	-	-	-	-
3 weeks	+	+	-	-	-	-

Table 2: Table demonstrating time versus sensitivity of paper on polyester sample.

Time vs Dilution (in IU/mL)	1K	500	250	125	62.5	31.25
24 hours	+	+	+	+	+	+
48 hours	+	+	+	+	+	+
1 week	+	+	+	+	+	+
2 weeks	+	+	+	+	+	+
3 weeks	+	+	+	+	+	+

Table 3: Table demonstrating time versus sensitivity of paper on denim sample.

Time vs Dilution (in IU/mL)	1K	500	250	125	62.5	31.25
24 hours	+	+	+	-	-	-
48 hours	+	+	+	-	-	-
1 week	+	+	+	-	-	-
2 weeks	+	+	+	-	-	-
3 weeks	+	+	-	-	-	-

Time vs Dilution (in IU/mL)	1K	500	250	125	62.5	31.25	15.6	7.8	3.9	2	1	0.5
Immediately	+	+	+	+	+	+	+	+	+	+	-	-
24 hours	+	+	+	+	+	+	+	+	+	-	-	-
48 hours	+	+	+	+	+	+	+	-	-	-	-	-
1 week	+	+	+	+	+	+	-	-	-	-	-	-
2 weeks	+	+	+	+	+	+	-	-	-	-	-	-
3 weeks	+	+	+	+	+	+	-	-	-	-	-	-

Trial 3

1. This trial involved use of real human saliva samples.
2. 5 samples were collected. 100 microliters from each sample were taken and all were averaged together in a centrifuge tube to account for differences in amylase concentration per person.

3. The following dilutions were made:
    a. Neat (350 IU/mL)
    b. 175 IU/mL
    c. 87.5 IU/mL
    d. 43.75 IU/mL
    e. 22 IU/mL
    f. 11 IU/mL
    g. 5.5 IU/mL
    h. 2.75 IU/mL
4. Stains were mapped at the following times
    a. Immediately
    b. 24 hours
    c. 48 hours
    d. 72 hours

Time vs Dilution (in IU/mL)	Neat	175	87.5	43.75	22	11	5.5	2.75
Immediately	+	+	+	+	+	+	+	+
24 hours	+	+	+	+	+	-	-	-
48 hours	+	+	+	+	+	-	-	-
72 hours	+	+	+	+	-	-	-	-

Discussion

Upon completion of the first trial, it was evident that the material upon which amylase is deposited will affect the detection of it by amylase paper. Even at equivalent concentrations and time lapsed, there was a difference in detection of amylase. This trial demonstrated that denim is the most effective material in retaining amylase, or at least is the most effective material for a positive test result by amylase paper. Cotton and polyester, on the other hand, did not result in positive test results at lower concentrations or longer amounts of time passed. The cotton material appeared to level off in amylase detection around 250-500 IU/mL. The polyester material appeared to level off around 250-500 IU/mL as well.

The second trial, in which only denim was used once it was determined that denim was the strongest material for amylase detection, demonstrated that detection leveled off around 31.25 IU/mL, around the one week mark. However, this trial had the most sensitive result with a positive test result for amylase at a dilution as weak as 2 IU/mL, when the test was run immediately after depositing on the material.

The third trial, in which the standard was not used but instead saliva from participants, suggests that amylase levels may sharply decline in human saliva around the 24 hour mark. The data demonstrate that the amylase paper detected dilutions as weak as 2.75 IU/mL immediately after the deposit of the saliva onto the material, however, within 24 hours the paper detected a dilution only as weak as 22 IU/mL, evidence for the degradation of amylase in human saliva after 24 hours.

Of all three trials, Trial 3 is the most representative of a true application of amylase paper. Given that stains on a fabric found at a crime scene will more often than not be neat saliva stains, this trial demonstrated most closely the ability and strength of amylase paper to detect the saliva at the scene. In conclusion, it should be noted that amylase paper detects only amylase which is found in a variety of bodily fluids, not the saliva itself; Therefore it is a presumptive test but this paper by Seratec Membrane Technology is still useful in that it is inexpensive and an effective presumptive test to assist in the detection of saliva and hopefully useful DNA at a scene.

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