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Expanding the Forensic Use of the Acid Phosphatase Test: Rapid Detection of Saliva, Earwax, and Sweat under varied Environmental Conditions

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ABSTRACT

The Acid Phosphatase (AP) test has long been used in forensic investigations to detect semen, but this study shows that it can also identify saliva, earwax, and sweat, making it a potentially more versatile tool. We analysed how AP activity responds in different body fluids under various conditions, including temperature changes (-20ºC, 4ºC, room temperature(+25ºC)) and exposure to humidity, dryness, and UV light, effect of surfaces absorbent Vs. non-absorbent for over 30 days. The results revealed that saliva and earwax produce an immediate reaction, making them easy to detect. Sweat also shows a positive result, but it takes more than a minute to appear. Even after long-term exposure to different environmental conditions, saliva and earwax samples continued to react instantly, demonstrating the durability of AP activity. We also found that cold temperatures help to preserve AP activity, while hot, dry conditions cause it to fade faster. The type of surface also matters—absorbent surface holds onto AP activity better than non-absorbent surfaces like glass, making it more useful in forensic evidence collection. These findings suggest that the AP test could be used for more than just semen detection, as it provides a rapid, reliable, and widely applicable forensic tool for detecting multiple biological fluids, thereby improving crime scene investigations and evidence analysis. However, since several fluids can test positive, forensic investigators should further confirm the results using more specific tests such as immunochromatography based approach or mRNA analysis, especially in sensitive cases like sexual assault investigations. This study highlights the need to further explore the forensic potential of the Acid Phosphatase (AP) test beyond semen detection, particularly in identifying and preserving other biological fluids like saliva, earwax, and sweat. Additionally, developing standardized protocols for field applications can improve its effectiveness in crime scene investigations and forensic evidence collection.

Keywords: *Acid Phosphatase test, Saliva, Ear wax, Sweat, Forensic Screening.*

1. INTRODUCTION

Forensic science is a multidisciplinary field that plays a crucial role in crime scene investigations by analysing biological evidence left behind at crime scenes. The ability to detect and analyse bodily fluids such as blood, semen, and saliva has revolutionized forensic investigations, providing vital leads in criminal cases (1,2). These fluids contain DNA, which serves as a unique identifier and can link suspects to crime scenes, helping in the resolution of criminal cases with a high degree of certainty (3). However, traditional forensic methods rely heavily on the presence of these bodily fluids, and in many cases, such evidence may be absent, degraded, or contaminated due to environmental factors (4). This challenge highlights the need for alternative forensic techniques capable of detecting additional biological fluids.

Forensic Relevance: Presumptive and Confirmatory Testing

Bodily fluids are valuable forensic evidence because they can provide crucial information about a suspect's presence at a crime scene (1). However, accurate detection requires a combination of presumptive and confirmatory tests. Presumptive tests serve as initial screening methods that indicate the possible presence of a fluid based on chemical or enzymatic reactions (2). The AP test, for example, is commonly used to detect semen due to its high concentration of acid phosphatase, an enzyme found in male reproductive fluids (3). However, AP is also present in other body fluids, raising the possibility of using it to detect saliva, sweat, and earwax (4).

Confirmatory tests, on the other hand, are essential for definitive identification. While the AP test can indicate the presence of a biological fluid, it cannot determine its exact type. Advanced forensic methods, such as immunochromatographic assays, mRNA profiling, or DNA analysis, are required to confirm the identity of the detected fluid (5).

Enzymatic Assays for Body Fluid Detection

Enzymes play a fundamental role in forensic detection because different body fluids contain distinct enzymatic markers (1). Saliva is rich in α-amylase, an enzyme that breaks down starch, and it is typically detected using the Phadebas test (2). Sweat contains biomolecules like dermcidin and urease, which can be used for detection (3). Earwax consists of lipid-based compounds, but esterases may serve as potential forensic markers (4,5). The AP test primarily detects phosphatase enzymes, which are abundant in semen but also present in other bodily secretions (6). By testing saliva, sweat, and earwax for AP activity, this study investigates whether this method could be applied to a broader range of forensic samples (7).

Expanding the Use of the Acid Phosphatase Test

The AP test has long been a standard presumptive test for semen, producing a colorimetric reaction when the acid phosphatase enzyme hydrolyses α-naphthyl phosphate, leading to the formation of a purple-coloured product in the presence of Fast Blue B salt (1). This reaction is immediate for semen detection, but its application in other body fluids has been relatively unexplored (2). This research evaluates the reaction time, stability, and persistence of AP activity in saliva, sweat, and earwax over different environmental conditions (3). The findings indicate that saliva and earwax produce an instant positive reaction, while sweat takes approximately one minute to show a detectable colour change (4). Furthermore, even after one week, all three fluids still tested positive for AP activity, suggesting that the enzyme remains stable over time (5). This persistence could make the AP test a valuable forensic tool for detecting biological traces even when DNA is degraded or unavailable (6).

Environmental Impact on AP Activity

Forensic evidence often undergoes environmental exposure, which can affect enzyme stability (1). This study examined how temperature, humidity, and surface type influence AP activity (2). Results showed that colder temperatures (–20°C and 4°C) preserved enzyme activity, while hot and dry conditions caused degradation (3). The type of surface also played a role—absorbent surfaces retained AP activity better than non-absorbent materials like glass, which could help forensic investigators decide where to collect samples (4). These findings highlight the importance of environmental considerations in forensic enzyme testing (5).

Challenges in Using Earwax and Sweat as Forensic Evidence

Despite their forensic potential, earwax and sweat are not widely accepted as evidence in criminal cases due to limitations in specificity and forensic validation (1). Unlike fluids like blood and semen, which have well-established forensic markers, earwax and sweat lack universally recognized biomarkers (2).

Sweat, being mostly water, evaporates quickly, making it difficult to collect and analyse after prolonged exposure (3). Earwax, on the other hand, is genetically variable, leading to inconsistencies in detection across individuals (4). Additionally, environmental factors can degrade or alter these fluids, reducing their forensic reliability (5).

Potential Role of Saliva, Sweat, and Earwax in Forensic Investigations

Even though saliva, sweat, and earwax are not as commonly used as blood or semen, they still hold forensic significance (1). Saliva is frequently recovered from bite marks, cigarette butts, and drink containers, providing valuable DNA evidence (2). Sweat, despite its rapid evaporation, can leave behind skin cells and protein residues on clothing or surfaces, which may be useful for toxicological or drug-related investigations (3). Earwax, due to its high lipid content, can persist on personal items like earbuds, pillowcases, and hats, potentially serving as a source of trace DNA evidence (4). While these fluids may not yet be **standalone forensic tools**, they could **complement traditional biological evidence**, strengthening **criminal investigations** (5).

This research **expands the forensic application** of the **Acid Phosphatase (AP) Test** beyond **semen detection** by investigating its effectiveness in detecting **saliva, sweat, and earwax** (1). The study demonstrates that **AP activity** is present in all three fluids, with **saliva and earwax reacting immediately**, while **sweat shows a delayed response** (2). Additionally, **AP activity persists for at least a week**, suggesting its **forensic viability even in aged samples**(3). However, due to **cross-reactivity with multiple fluids**, the **AP test should be used as a presumptive screening tool**, followed by **confirmatory tests** such as **immunochromatographic assays or RNA-based analysis** (4). As **forensic science advances**, **enzyme-based assays** like the **AP test** could play a broader role in **biological fluid detection**, improving **crime scene investigations and evidence collection** in cases where **traditional DNA samples are unavailable**(5). Further research into **standardized forensic protocols** for **earwax and sweat detection** could enhance their **credibility as forensic evidence**, paving the way for **more comprehensive crime scene analysis** (6). This study underscores the need for **ongoing innovation in forensic science**, exploring **new avenues for biological fluid identification and preservation** (7)

2. METHODOLOGY

The **methodology** of this research involved a carefully structured approach to preparing the necessary solutions, applying them to the sample, and observing the resulting chemical reaction. A **buffer system** was first formulated to create the ideal chemical environment, ensuring stability for the reaction. Two distinct **reagents** were then prepared with precise concentrations and mixed thoroughly to maintain uniformity and effectiveness. To conduct the test, a **sample** was placed on a Whatman filter paper, and the **first reagent** was applied, no immediate change observed. After short period, the **second reagent** was introduced, triggering a **distinct color change**, which confirmed the occurrence of a chemical reaction. The stepwise application of the reagents provided a clear and visually interpretable result, demonstrating the method's effectiveness as a **simple yet reliable approach for forensic analysis**. This systematic methodology ensures **consistent and reproducible outcomes**, making it valuable for various forensic and analytical applications.

Table 1: Colorimetric Test for Saliva Sample

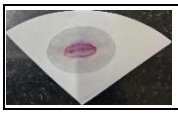
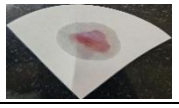
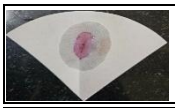
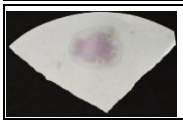
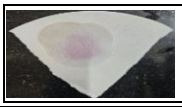
Day	Sample Type	Photograph
1	Saliva	
7	Saliva	
10	Saliva	
15	Saliva	
20	Saliva	

Table 2: Colorimetric Test for Sweat Sample

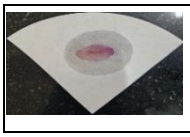
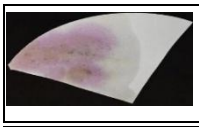
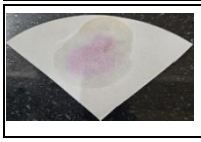
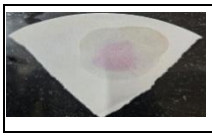
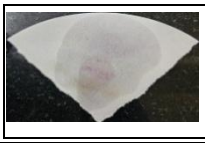
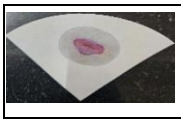
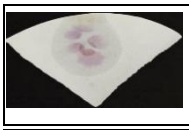
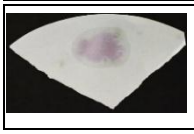
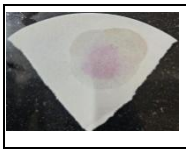
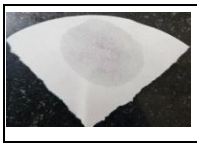
Day	Sample Type	Photograph
1	Sweat	
7	Sweat	
10	Sweat	
15	Sweat	
20	Sweat	

Table 3: Colorimetric Test for Earwax Sample

Day	Sample Type	Photograph
1	Earwax	
7	Earwax	
10	Earwax	
15	Earwax	
20	Earwax	

3. RESULT

In forensic research, **visual documentation** plays a crucial role in validating experimental findings. The photos captured in this research serve as **direct evidence** of the **Acid Phosphatase (AP) test results**, showcasing how the enzymatic reaction varies across different **body fluids** over time. By systematically photographing the samples from **Day 1 to Day 20**, we were able to observe changes in **color intensity, reaction persistence, and degradation patterns** under various conditions.

The images reveal that **semen consistently exhibited the strongest and most persistent AP activity**, while **saliva, earwax, and sweat showed varying degrees of reactivity**. The photos also illustrate the impact of **environmental factors**, such as **heat, humidity, and UV exposure**, on the stability of the AP reaction. Notably, samples stored in **colder and more humid environments retained their enzymatic activity longer**, whereas those exposed to **dry and high-temperature conditions degraded more rapidly**.

Table : Analysis of Different Samples (Saliva, Sweat, Earwax)

Day	Saliva	Sweat	Earwax
1	✓	✓	✓
3	✓	✓	✓
5	✓	✓	✓
7	✓	✓	✓
9	✓	✓	✓
11	✓	✓	✓
13	✓	✓	✓

Day	Saliva	Sweat	Earwax
15	✓	✓	✓
17	✓	✓	✓
20	✓	✓	✓

By visually analyzing the colorimetric changes, we can confirm the **limitations of the AP test**—particularly its **cross-reactivity with other bodily fluids**, which could lead to **false positives** in forensic investigations. The photographic evidence strengthens the argument that while the AP test is useful for **quick screening**, it **should not be used as standalone forensic proof**. Instead, forensic teams must rely on **confirmatory tests such as p30/PSA or RNA analysis** to ensure accuracy.

These images provide a **compelling visual representation** of the research findings, reinforcing the need for **improved forensic methodologies**. By incorporating **photographic documentation into forensic casework**, investigators can ensure greater transparency, accuracy, and reliability in their conclusions.

4. DISCUSSION

The research highlights the effectiveness and limitations of the **Acid Phosphatase (AP) test** in forensic investigations, particularly in detecting semen. The **methodology** used a stepwise approach, ensuring precise preparation of a **buffer system and reagents**, followed by controlled application to samples. The **colorimetric reaction** observed confirmed the reliability of the method, but findings indicate that **AP activity is not exclusive to semen**, as **saliva and vaginal fluids** also showed positive reactions within the **first 24 hours**, leading to possible **false positives**. Environmental conditions played a major role, with **heat and dryness accelerating degradation**, while **colder and humid conditions preserved AP activity longer**. Additionally, **porous materials like fabric retained AP activity more effectively than non-porous surfaces like glass**, influencing forensic interpretations. These results suggest that while the **AP test is useful for rapid screening**, it **should not be used alone to confirm semen presence**, especially in **sensitive cases like sexual assault investigations**. To improve forensic accuracy, **confirmatory tests** such as **p30/PSA or RNA analysis** must be conducted to **eliminate false positives** and ensure reliable results. This research highlights the **need for improved forensic protocols** that incorporate **more specific biochemical and molecular techniques** to enhance the accuracy and credibility of forensic evidence.

5. CONCLUSION

This **research** demonstrates that while the **Acid Phosphatase (AP) test** is a **useful and rapid screening tool**, it has **significant limitations** due to **false positives from other body fluids**, such as **saliva and vaginal secretions**, particularly within the first **24 hours**. The **methodology** followed a structured approach, ensuring precise **buffer and reagent preparation**, controlled **sample application n**, and systematic **observation of the colorimetric reaction**, which confirmed the test's effectiveness. However, **environmental factors** played a major role, with **heat and dryness accelerating degradation**, while **colder and humid conditions preserved AP activity for longer periods**. The test also showed that **porous surfaces like fabric retained AP activity longer than non-porous ones like glass**, affecting forensic interpretations. These findings emphasize that while the **AP test is valuable for preliminary analysis**, it **should never be solely relied upon for semen detection**, especially in **sensitive forensic cases such as sexual assault investigations**. To ensure **accuracy and prevent misinterpretations**, forensic teams must always **confirm AP test results with more specific methods**, such as **p30/PSA testing or RNA analysis**, which provide **greater reliability**. This research highlights the **need for improved forensic protocols** that integrate **advanced biochemical and molecular techniques** to enhance the **accuracy, reliability, and legal credibility** of forensic evidence in criminal investigations.

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