

A Substitute Presumptive Test for Screening of Semen using Sodium *p*-Nitrophenyl Phosphate (NaPNPP)

Daniel Njau (✉ daniel.njau@uonbi.ac.ke)

University of Nairobi School of Medicine <https://orcid.org/0000-0001-7287-7256>

Accadius Lunayo

University of Nairobi; Centre for Biotechnology and Bioinformatics

Johansen Odour

Division of Forensics and Pathology Services; Directorate of Medical Services; Ministry of Health; Republic of Kenya

Research Article

Keywords: Acid Phosphatase, Preliminary screening, Presumptive test, Semen detection, Sexual assault

Posted Date: June 17th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-361624/v1>

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Abstract

Background

In forensic investigation of alleged sexual crimes, presumptive semen detection test methods are commonly used to reach preliminary identification of seminal fluid in questioned samples. These methods are based on the detection of components of semen such as enzyme acid phosphatase (AP). Of these methods, the acid phosphatase identification method still remains the most reliable and widely used presumptive test due to high activity of the AP in seminal fluid.

In standard AP test, Bretamine Fast Blue B (FBB) reagent is used. However FBB has been explicitly classified as carcinogenic. Although FBB has been handled safely over time, there is a need at the moment to develop a simple, readily available, reliable and efficient method for screening the presence of semen in any material collected as evidence in a sexual assault crime.

Given the improved sensitivity of DNA profiling tests that have been introduced in to routine forensic casework over recent years, the need for improved sensitivity at this first stage of detection has never been higher.

Findings

Here we highlight a simple method using readily available reagents in standard biochemical laboratory as a substitute for the standard AP test for seminal fluid identification from a crime scene. This method is based on the hydrolysis of sodium-p-nitrophenyl phosphate at pH 5.5 by the acid phosphatase to produce an intense yellow coloured complex in 15 seconds.

Conclusions

The method presented is sensitive, reliable, efficient and routinely used in standard biochemical and pathology laboratories for spectrophotometric analysis of alkaline phosphatase. It can be easily and readily applied as a preliminary test for identification of semen at a crime scene that involves sexual assault.

Background

Over the years several different methods have been utilized for identification of seminal fluid at a crime scene (Laffan et al. 2011). Although new technologies are being researched and developed, traditional semen identification methods appear to remain the most widely applied in forensic casework (Gamblin and Morgan-Smith 2020). Of these methods acid phosphatase identification method still remains the most reliable and widely used presumptive test (Raju and Iyengar 1964; Rana et al. 2019).

Quantitative and qualitative AP testing is routinely used in biochemical analysis and more importantly in screening for the presence of semen in evidence found at a crime scene. AP is one of the forensically

significant components of semen and the activity of this enzyme is usually very high in seminal fluids (Gamblin and Morgan-Smith 2020). The standard screening AP test for semen involves the use of alpha-naphthyl phosphate as a substrate to produce alpha-naphthanol in the presence of AP which then has to combine with Bretamine Fast Blue B (Orthodianisidine / diazo blue dye) to form a violet / purple coloured complex within 2 minutes (Raju and Iyengar 1964).

In standard AP test, Bretamine Fast Blue B (FBB) reagent is used. However FBB has been explicitly classified as carcinogenic. Although FBB has been handled safely over time, there is a need to highlight sensitive, reliable, efficient and readily available methods for screening the presence of semen in any material collected as evidence in a sexual assault crime to address this gap. This is particularly important since evidence has shown that in order to get a good confirmatory DNA result, it is critical for the seminal fluids to be collected within a specific timeframe, preferably within the first 24-48h (Gonçalves et al. 2017).

The substitute presumptive test presented here is sensitive, reliable, and efficient. The method utilizes readily available reagents routinely used in standard biochemical and pathology laboratories for spectrophotometric analysis of alkaline phosphatase (McComb et al. 1979). This method can be easily and readily applied as a substitute preliminary test for identification of semen at a crime scene that involves sexual assault. The method is based on the hydrolysis of sodium-*p*-nitrophenyl phosphate (NaPNPP) at pH 5.5 by the AP in semen to *p*-nitrophenol, a yellow chromophore as shown in Figure 1.

Figure 1. The reaction between sodium-*p*-nitrophenyl phosphate (NaPNPP) and Acid Phosphatase

Methods

A buffered substrate consisting of 0.41g of citric acid, 1.125g of sodium citrate and 1.65mg of sodium-*p*-nitrophenyl phosphate (NaPNPP) in 100ml distilled water is prepared at a pH of 5.5. The semen is extracted from material collected at a crime scene or swab by dampening a Whatman filter paper with distilled water and then pressed on the surface of the suspected stain on the cloth and left in position for approximately 10 minutes.

The paper is then removed and sprayed with the AP test reagents from the buffered substrate prepared using a chromatographic reagents sprayer. An intense yellow colour appears on the paper at the position where the stain has been extracted in less than 15 seconds depending on the amount of stain. As a negative control an unstained area on the known semen dilution strip doesn't exhibit any changes in colour within the 2 minutes threshold upon addition of these AP working solution.

Results

Using 0.05mL of a neat sample over a 12 hour interval for 48 hours, the results for the test are shown in Fig. 2.

Figure 2. The results for the Acid Phosphatase test over 48 hours at a 12 hours interval

In the presence of sodium ions, *p*-nitrophenol produces the yellow coloured complex; which indicates the presence of semen. Since AP is not exclusive to human semen, it can only be used as a presumptive test for the presence of semen.

Discussion

In a standard AP test, a rapid colour change to purple is expected within 30 seconds to indicate a presumptive positive result for the presence of semen. An inconclusive result that indicates the possible presence of semen is shown by a colour change from 31–59 seconds. A presumptive negative result is indicated by no colour change at or after 60 seconds (Virkler and Lednev 2009).

However, as the method is currently implemented by operational forensic science laboratories allows 2 min for a reaction to be obtained beyond which a negative result is declared (Skalleberg and Bouzga 2016). Furthermore, recent research has shown that using the AP standard presumptive test semen could be detected in excess of 15 min depending on the dilutions using the press and test method (Lewis et al. 2012; Redhead and Brown 2013). The proposed substitute method falls within the timeframe that is indicated by standard operational forensic science laboratories.

Generally cases involving sexual assault are characterized by very low disclosure rates, reporting, prosecution, and conviction (Magalhaes et al. 2015). Due to the prevalence of sexually motivated crime, any improvements or alternatives to current practices and techniques would be welcomed.

Given the improved sensitivity of DNA profiling tests that have been introduced in to routine forensic casework over recent years, the need for improved sensitivity at this first stage of detection has never been higher (Laffan et al. 2011). It is therefore paramount to utilize all available robust and reliable methods for detecting semen during the forensic casework. Furthermore, AP test is one of the most common tests used by forensic laboratories for the detection of semen. Availability of this test and within the appropriate time frame as evidence indicates would be a significant addition to any forensic casework at hand.

This substitute method presented herein is sensitive, reliable, efficient and readily available in standard laboratories. The method can additionally be used in routine forensic laboratory and experimental work owing to the health and safety concerns of the Bretamine Fast Blue B. As standard practice indicates, further confirmatory test such as DNA profiling should be carried out adhering to the standard confirmatory procedures.

Abbreviations

NaPNPP - Sodium -*p*- Nitrophenyl Phosphate

AP - Acid Phosphatase

FBB - Bretamine Fast Blue B

DNA - Deoxyribonucleic Acid

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Declarations

Ethics approval

This research study was conducted retrospectively from data obtained for general forensics purposes. Ethical approval was waived by the forensics lab ethics committee at the division of forensics and pathology ministry of health in view of the retrospective nature of the study and all the procedures being performed were part of the routine studies.

Consent for publication

Not Applicable

Availability of data and material

Not Applicable

Competing Interests

The authors have no conflicts of interest or competing interests to declare

Funding

The authors did not receive support from any organization for the submitted work.

Authors' contribution

All authors contributed to the study conception and design. Material preparation, data collection were performed by 'DN' and 'JO'. Analysis was performed by 'DN' and 'AL'. All the authors participated in the manuscript write up and approved the final manuscript.

Acknowledgements

Not Applicable

Figures

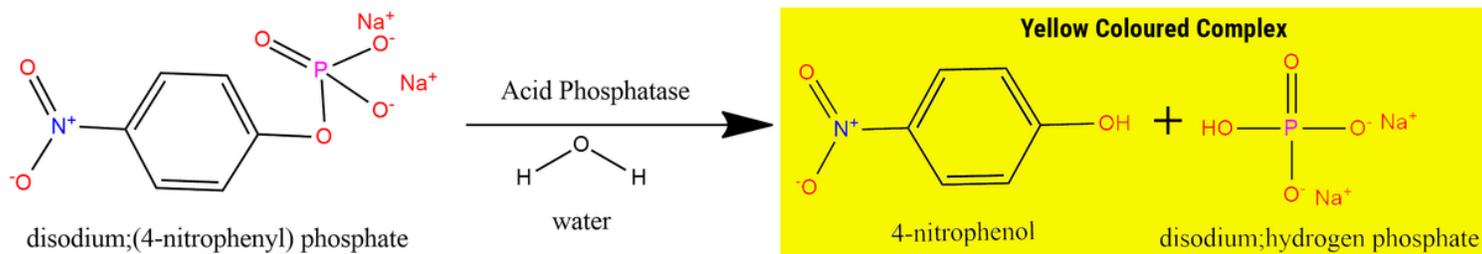


Figure 1

The reaction between sodium-p-nitrophenyl phosphate (NaPNPP) and Acid Phosphatase

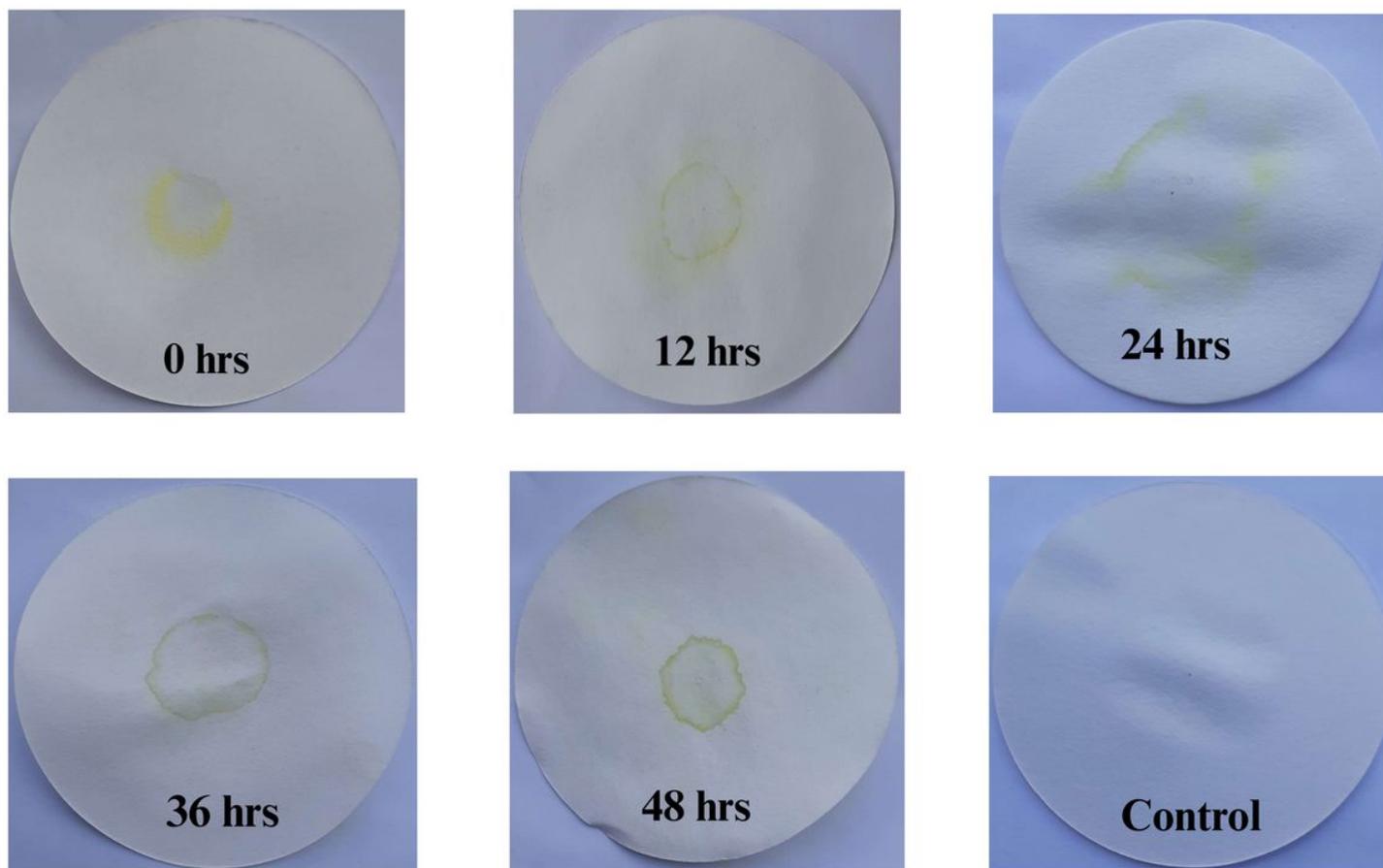


Figure 2

The results for the Acid Phosphatase test over 48 hours at a 12 hours interval