B1 A Comparative Analysis of Globally Used Forensic Semen Detection Methods and Their Differing Applications

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The goal of this presentation is to inform attendees of the effectiveness of differing applications of commonly utilized forensic semen detection methods employed globally.

This presentation will provide the forensic science community with insight into the wide variety of different methods of applying semen identification tests, such as the acid phosphatase test for screening and extraction methods for spermatozoa staining, that are utilized in forensic laboratories worldwide.

The ability to detect and identify the presence of seminal fluid can be crucial to an investigation. Methods used to detect seminal fluid are common across the world; however, the application of these methods varies greatly on a global level. For example, while some laboratories favor the Alternate Light Source (ALS) for locating potential stains, other laboratories favor the Acid Phosphatase (AP) press test, as the ALS has been shown to be unreliable and not specific. The AP press test is common in Europe; however, there is a lack of research into how this method is applied, with varying approaches taken in different laboratories. Further, for the confirmatory identification of semen, the most commonly utilized method is microscopic visualization of spermatozoa; however, it is essential to extract potential stains from substrates, such as fabric, in order to perform microscopic examination. A number of different methods of extraction have been identified but have not been compared to date. Therefore, the goal of this study was to first investigate the differing methods of applying AP for presumptive testing and, second, to compare and contrast the differing extraction methods from a variety of substrates.

Following Institutional Review Board (IRB) approval, semen was collected with informed consent from healthy volunteers. For each experiment, 100µL of semen was deposited onto white cotton fabric in dilutions from neat to 1:1,000. The differing AP application methods examined included: (1) wetting both the substrate and test paper versus just the test paper, including examination of the potential transfer of spermatozoa to the test paper; (2) application of the two AP reagents (sodium α-naphthyl phosphate and Fast Blue B) as a combined formulation versus sequential application; (3) application of the AP reagents directly onto the substrate versus indirect application using test paper; and, finally, (4) evaluation of the reliability of the two-minute cutoff for the AP reaction. The differing extraction methods examined include five methods used globally and were performed on five substrates (cotton, denim, polyester, wool, and cotton swabs) for each of five dilutions, neat to 1:1,000. To evaluate the extracted stains, the extract was seeded on a microscope slide, stained with Christmas tree stain, examined under a microscope, and scored.

The results of this study investigating the differing AP application methods revealed: (1) wetting both the test paper and the substrate greatly enhances the positive AP reactions obtained, particularly through the dilutions, with no observable transfer of spermatozoa to the test papers; (2) the sequential application of the AP reagents provides stronger and faster color reactions; (3) similarly, the direct application of the reagents onto the substrate provides greater sensitivity and faster/stronger reactions, when compared to the indirect application onto the test paper; and, finally, (4) the two-minute cutoff for the AP reaction was insufficient time for positive reactions to be observed with dilutions above 1:5,000. The results of this study investigating the differing extraction methods demonstrated one particular method — utilizing two stacked Eppendorf tubes — to extract the most spermatozoa from four of the five substrates, across all dilutions.

This research highlights the potential impact on results obtained when using differing semen screening and identification tests identified across the globe. These results emphasize the need for more research into the varying application methods used. It is crucial for forensic laboratories to be made aware of the variety of these methods and the potential to improve the effectiveness and sensitivity of their testing.

Acid Phosphatase, Extraction, Christmas Tree Stain

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