Body fluid identification in forensics

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INTRODUCTION

Biological samples found at the crime scenes play pivotal roles in forensic investigations by providing valuable evidence (1). Since the DNA profile of every individual is considered to be unique (except in the case of identical twins), DNA typing of biological samples can prove whether a suspect was involved in a crime, and even can exonerate innocent persons who have been wrongfully convicted (2). Besides, determination of the type and origin of biological samples found at crime scenes can provide important clues for crime scene reconstructions by supporting a link between sample donors and actual criminal acts. For more than a century, numerous types of body fluid identification methods have been developed, such as chemical tests, immunological tests, protein catalytic activity tests, spectroscopic methods and microscopy. However, these conventional body fluid identification methods are mostly presumptive, and are carried out for only one body fluid at a time. Therefore, the use of a molecular genetics-based approach using RNA profiling or DNA methylation detection has been recently proposed to supplant conventional body fluid identification methods. Several RNA markers and tDMRs (tissue-specific differentially methylated regions) which are specific to forensically relevant body fluids have been identified, and their specificities and sensitivities have been tested using various samples. In this review, we provide an overview of the present knowledge and the most recent developments in forensic body fluid identification and discuss its possible practical application to forensic casework. [BMB Reports 2012; 45(10): 545-553]

Determination of the type and origin of the body fluids found at a crime scene can give important insights into crime scene reconstructions by supporting a link between sample donors and actual criminal acts. For more than a century, numerous types of body fluid identification methods have been developed, such as chemical tests, immunological tests, protein catalytic activity tests, spectroscopic methods and microscopy. However, these conventional body fluid identification methods are mostly presumptive, and are carried out for only one body fluid at a time. Therefore, the use of a molecular genetics-based approach using RNA profiling or DNA methylation detection has been recently proposed to supplant conventional body fluid identification methods. Several RNA markers and tDMRs (tissue-specific differentially methylated regions) which are specific to forensically relevant body fluids have been identified, and their specificities and sensitivities have been tested using various samples. In this review, we provide an overview of the present knowledge and the most recent developments in forensic body fluid identification and discuss its possible practical application to forensic casework. [BMB Reports 2012; 45(10): 545-553]

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between different types of body fluids because of their high specificity and fit with current forensic casework application.

This review briefly describes current and previous techniques of body fluid identification that are being used in forensic laboratories, and evaluates the advantages and disadvantages of each method. We will also focus on new methods of identification that have been developed in the past decade, specifically mRNA based assays, miRNA based assays and DNA methylation based assays. The review concludes with a discussion of the potential application of these new methods for forensic case works.

CURRENT TECHNIQUES

Alternate light source methods

The simplest way to detect body fluid stains that are difficult to see with the naked eye is to use an alternate light source (ALS) such as ultraviolet light. Because it is a routine procedure to search a crime scene for latent stains of body fluids using ALS, there are various commercial ALS devices, such as Wood’s Lamp (WL), specific for semen detection. WL which emits wavelengths ranging about 320-400 nm is safe and handy, but the specificity is somewhat low, thereby carrying a high risk of false positive results for other fluids (6). Another ALS device, Bluemaxx™ BM 500 demonstrated 100% sensitivity to semen stains (7), and Polilight® can detect several body fluids, including semen (8). A newly developed device, Lumatec®, Superlight 400 emits light from 320 to 700 nm and is able to detect stains both in darkness and in daylight (9). Using this device, semen was best detected using a range of 415-490 nm, and saliva was also detectable in 60% of cases. However, poor results were obtained when it was applied to dark fabrics and to fabrics which had been washed, although it had been reported that different types of fabrics showed similar results (9, 10).

Chemical tests

Chemical tests are usually based on the color change or chemiluminescence of a particular reagent when it comes into contact with a particular body fluid. Luminol is frequently used in identifying blood stains, particularly when the perpetrator has attempted to clean up the blood, thus rendering it invisible to the naked eye (11, 12). Due to the emission of light as a result of an oxidation of luminol enhanced by iron in hemoglobin and its derivatives in blood, trace amounts of blood can be detected by chemiluminescence of a blue-green color (13, 14). However luminol has been known to react with other substances, including copper containing chemical compounds, certain bleaches, saliva, and various animal and vegetable proteins. The luminol test remains popular due to the relatively high sensitivity and specificity in comparison with other screening tests. However, it is limited to use in dark environments and also has disadvantages regarding the duration of illumination (about 30 seconds) and sometimes has detrimental effects on subsequent DNA analysis (15-17). Bluestar® Forensic is another latent blood stain reagent based on chemiluminescence, and it gives more sensitive and stable results without damaging DNA and thereby allows for subsequent genotyping of stains (16, 18).

Catalytic tests

Catalytic methods are based on the enzyme activity which catalyzes the reaction of a variety of substrates to produce visible color changes. There are several different catalytic tests which are commonly used to presumptively identify blood based on the peroxidase-like activity of heme group (13, 14). The heme group of hemoglobin possesses a peroxidase-like activity which catalyzes the breakdown of hydrogen peroxide, and the oxidizing species formed in this reaction can then react with a variety of substrates to produce a color change (19). Among the substrates in common use are benzidine and various substituted benzidines, ortho-tolidine, leucolamachite green, leucocrystal violet and phenolphthalein, also known as the Kastle-Meyer test (20, 21).

The most commonly utilized of these tests is the benzidine test, where the presence of blood is indicated by blue colored products. However, there are several substances which can generate false positives for this test, such as chemical oxidants and fruit and vegetable peroxidases (22). Moreover, benzidine is known as a carcinogen (23), and accordingly this has largely been replaced by tests using phenolphthalein/hydrogen peroxide. The test using phenolphthalein is also known as the Kastle-Meyer test, which is a very popular presumptive test for blood. The stain in question is collected with a swab or filter paper, and phenolphthalein reagent and hydrogen peroxide are then applied to the sample. If the sample contains hemoglobin, a result produces a pink coloring only after the addition of hydrogen peroxide, by oxidation of phenolphthalein into phenolphthalein. Although false-positives are reported in the presence of chemical oxidants and vegetable peroxidases, the test can detect blood as dilute as 1 part in 10,000. In addition, this test is nondestructive to the sample, which can then be kept and used in further tests, including DNA analysis (20, 21, 24, 25).

The acid phosphatase (AP) test is one of the most common tests for semen detection. AP is an enzyme secreted by the prostate gland that is present in large amounts in seminal fluid (26). The level of AP activity is 500 to 1,000 times higher in human semen than in any other body fluid. AP can catalyze the hydrolysis of phosphates, which results in the formation of a product that will react with the color developer (27). Therefore, in the presence of Alpha-Naphthyl acid phosphate and Brentamine Fast Blue, AP will produce a dark purple color (27). However, the test for AP is highly presumptive because vaginal secretions and other body fluids also contain detectable levels of this enzyme (13).

A catalytic test for the detection of saliva is based on the enzymatic activity of alpha-amylase. Saliva is rich in alpha-amylase, an enzyme that hydrolyses polysaccharides into smaller