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Emerging spectrometric techniques for the forensic analysis of body fluids

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ABSTRACT

Body fluids are evidence of great interest in forensics because they allow identification of individuals through the study of DNA. After reviewing the tests and the methods that are currently being used by forensic practitioners for the detection of body fluids (e.g., blood, semen, saliva, vaginal fluid, urine and sweat), and after showing their main drawbacks and limitations, this work focuses on the review of emerging spectrometric techniques applied for the forensic analysis of body fluids. These techniques include the use of ultraviolet-visible, infrared (IR), Raman, X-ray fluorescence and nuclear magnetic resonance spectroscopy and mass spectrometry for investigating blood, semen, saliva, urine, vaginal fluid or sweat. Although all these spectrometric techniques seem to have a high potential to differentiate body fluids prior to DNA extraction, IR and Raman spectroscopy have shown the most promising results for discriminating stains from body fluids.

Contents

1. Introduction

Body fluids are exceptionally useful forensic evidence because they provide information that allows police to discover their corresponding owner by analyzing the DNA (deoxyribonucleic acid) content. Since DNA testing has acquired huge relevance for solving crimes, the detection of biological evidence at the crime scene has become one of the priorities of law-enforcement officers during ocular inspection. According to Locard's principle, "every contact leaves a trace", so a small part of the offender usually stays in the place while a small part of the place goes with the offender (e.g., in homicide, resulting from confrontation between victim and aggressor, residues from the victim are probably found on the attacker and *vice versa*) [1]. After trace detection, the forensic process continues with evidence analysis to obtain information about the source (identity) or activity (why the trace is where it is) and how it might relate to a proposed crime scene.

Technically, a biological fluid comes from a living being. In the forensic field, the biological fluids of interest are body fluids, which come from a human being, especially those from the attacker. Body fluids generated by human beings include blood, semen, saliva, vaginal fluid, urine, sweat, breast milk, tears and mucus. Undoubtedly, blood, semen and saliva are found in larger amounts than the

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others at crimes scenes. Consequently, these body fluids have been the most studied samples [2–4].

Tests currently used to analyze body fluids are classified according to their specificity in two different categories (presumptive and confirmatory) [2]. Presumptive tests provide a large number of false positives (i.e., the test is positive although the body fluid is not present in the sample). Presumptive tests are unspecific to a single body fluid, so a positive response is due only to the suspicion that the fluid may be present in the stain. Thus, it is always necessary to apply a confirmatory test to confirm the presence of a body fluid in a stain because, by contrast, confirmatory tests are specific to identify a particular body fluid. A positive response certainly ensures the presence of the searched body fluid in the stain under examination.

Presumptive and mainly confirmatory tests used in body-fluid identification are based on each body fluid having a unique composition, which is the result of specific components of each body fluid and the difference in the relative ratio of common components found in several body fluids [2]. As Lednev and Virkler indicate [2], urea is found in urine, semen and sweat, its concentration in urine being larger than in either of the other two fluids. Table 1 shows the principal components present in blood, semen, saliva, vaginal fluid, urine and sweat [2].

Table 2 shows the most relevant tests currently used to detect or to confirm blood, semen, saliva, vaginal fluid, urine and sweat. Table 2 contains the current available tests to detect blood, semen, saliva, vaginal fluid, urine and sweat. The columns indicate, respectively, test name, type of test (chemical, spectroscopic, microscopic, crystal test, immunological, chromatographic or electrophoretic), specificity (presumptive or confirmatory), main characteristics of the test and references. All the tests pursue the detection of specific components, ratios of components or characteristics, such as fluorescence, to detect presumptively or to confirm (identify) each body fluid.

As seen in Table 2, the six body fluids can be presumptively detected by different tests, most of them chemical. However, there are available confirmatory tests solely for blood and semen, which involve immunological interactions, microscopic visualization of specific components or formation of specific crystals by chemical reaction. In addition to the lack of confirmatory tests for saliva, vaginal fluid, urine and sweat, there are other disadvantages, such as:

(1) most confirmatory tests (for blood and semen) are destructive; and,
(2) it is necessary to apply different tests to confirm each type of body fluid; this limitation requires division of a sample into several parts, and a portion of the sample having to be kept for possible future analyses.

To date, there is no test or method used by forensic practitioners able to detect and to confirm a body-fluid source without destroying the evidence [2,5]. This situation makes it necessary to develop confirmatory and non-destructive methods applicable to different types of body fluids. Nowadays, there are two principal lines of active research in body-fluid identification. One is dedicated to the development of mRNA (messenger ribonucleic acid) markers [6–12] based on the different mRNA sequences in each body fluid. The other is based on the use of spectrometric analytical techniques.

In this work, our goal is to provide a critical review of the works using emerging spectrometric techniques to analyze body fluids.

2. Emerging spectrometric techniques

Several analytical techniques can be applied to determine the presence in a stain of any of the different types of body fluid included in this review article.

In general, classical analytical chemistry is based on colorimetric assays, the use of many reagents and implementation of a large number of methods, usually specific for only one single analyte. As consequence it leaves room for spectrometric methodologies that are characterized by speed, and absence, or minimum use, of reagents and frequently applicable to the analysis of many analytes at a time. Spectrometric analysis results in a characteristic spectrum, which contains the information related to the chemical composition of the sample under analysis [13,14]. Since the composition of each body fluid is different, it is possible to differentiate each type of body fluid by using these techniques.

In recent years, spectrometric techniques underwent strong development in many different fields. Regarding forensic sciences, spectrometric techniques were used for analyzing drugs [15–20] and explosives [15,21–25] and they are still used at forensic laboratories nowadays. However, approved analyses of body fluids used at forensic institutions have continued unchanged, based on the use of classical chemical assays. Their evolution into modern analytical methods has not happened yet. However, some research groups are investigating and developing novel methods for the analysis of body fluids. Specifically, ultraviolet-visible (UV-Vis), infrared (IR), Raman, X-ray fluorescence (XRF) and nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) are being investigated in this field.

As common data to all studies, body-fluid stains analyzed were all dried stains unless otherwise indicated, in order to test conditions similar to those in real forensic crime scenes, and, for some techniques (e.g., IR spectroscopy), this condition also avoids the problem of the huge IR signals from water.

2.1. Ultraviolet-visible spectroscopy

Illumination with UV-Vis light was the first spectroscopic method used for forensic detection of body fluids. It is based on the two processes that substances subjected to UV-Vis radiation may undergo, absorption or fluorescence emission. Under a UV-Vis source, most body fluids (e.g., semen, saliva, and urine) emit fluorescence [26], which is visibly detected as intense brightness of the sample. Blood is not fluorescent at UV-Vis radiation, but it presents a characteristic absorption band in the UV region, which, in good lighting

Table 1
Major components of body fluids.

Blood	Semen	Saliva	Vaginal fluid	Urine	Sweat
Hemoglobin	Acid phosphatase	Amylase	Acid phosphatase	Urea	Chloride
Fibrinogen	Prostate-specific antigen	Lysozyme	Lactic acid	Creatinine	Sodium
Erythrocytes	Spermatozoa	Mucin	Citric acid	Uric acid	Urea
	Choline		Urea		
	Spermine		Vaginal peptidase		
	Semenogelin				
	Urea				

{Adapted from [2]}