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Forensic techniques in blood macules: an updated review

Técnicas criminalísticas en máculas de sangre: una revisión actualizada

Patricia Alexandra Padilla Ortiz
Degree in Health Sciences in Clinical and Histopathological Laboratory. Diploma in Clinical Hematology, Postgraduate, Universidad Nacional de Chimborazo. papadilla.fslc@unach.edu.ec, https://orcid.org/0000-0001-7603-1119

Verónica Paulina Cáceres Manzano
Master in Criminalistics. Professor at the National University of Chimborazo, Faculty of Health Sciences, Clinical and Histopathological Laboratory. vcaceres@unach.edu.ec, https://orcid.org/0000-0001-5710-5661

ABSTRACT

Bloodstain forensic techniques rely on the ability to examine a crime scene and collect physical evidence to enable analysis. Often, investigators use ultraviolet and infrared lights to detect subtle bloodstains that are not visible to the naked eye. Once identified, these bloodstains are collected for further analysis. Bloodstain analysis involves comparing the patterns of the bloodstain to other patterns at the crime scene. Thus, an updated systematic review of techniques and methods employed in the analysis of blood macules at crime scenes was conducted. Ten studies were identified between 2020 and 2022, corresponding to publications indexed in the Scopus and Scielo databases and master's theses. It is concluded that the application of different methods related to science such as mathematics, physics, electrochemistry is the proposed way to improve criminalistic techniques.

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RESUMEN

Las técnicas criminalísticas en máculas de sangre se basan en la habilidad para examinar una escena del crimen y recopilar evidencia física que permita el análisis. A menudo, los investigadores usan luces ultravioletas e infrarrojas para detectar manchas sutiles de sangre que no son visibles a simple vista. Una vez identificadas, estas máculas de sangre se recolectan para su análisis posterior, el análisis de la mácula de sangre involucra la comparación de los patrones de la mácula con otros patrones de la escena del crimen. Es así, que se llevó a cabo una revisión sistemática actualizada sobre técnicas y métodos empleados en el análisis de máculas de sangre en escenas del crimen. Se identificaron 10 estudios entre el 2020 y 2022, que corresponden a publicaciones indexadas las bases de datos Scopus, Scielo y tesis de maestría. Se concluye que la aplicación de diferentes métodos afines a la ciencia como matemáticas, física, electroquímica es el camino que se propone con el fin de perfeccionar las técnicas criminalísticas.

Keywords / Palabras clave

Forensic techniques, blood stains, forensics, forensic investigation.

Técnicas criminalísticas, máculas de sangre, forense, investigación forense.

Introduction

Criminalistic techniques are a set of methods and procedures used in criminal investigation to collect, evaluate and process evidence obtained at the scene of a crime. These techniques seek to establish the relationship between the facts and the identification of the perpetrators. Criminalistic techniques encompass a wide variety of skills and knowledge related to criminal investigation. These include evidence collection, crime scene analysis, fingerprint and fingerprint identification, computer forensics analysis, ballistics analysis, DNA examination, and blood examination(Martín Nuñez, 2016).

In the same vein, practitioners can use these techniques to collect evidence, establish chain of custody and present evidence in court. Advances in technology have changed the focus of forensic techniques. For example, fingerprint identification, once done manually, is now done through facial recognition technology. Computer forensics has also improved crime scene analysis and evidence collection. As

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technology advances, professionals must keep up with the latest developments. This helps ensure that professionals are prepared to handle a wide variety of situations, from low-profile crimes to murders and rapes(Sriraman, 2021; Weber & Lednev, 2020)

Along the same lines, blood identification has been one of the most important forensic techniques, since it makes it possible to link the incident to a specific person. This technique is based on the analysis of the characteristics of the blood, such as its color, chemical composition and type, which are unique to each person. This makes it possible to identify the origin of the blood and establish a connection between the incident and the alleged perpetrator(Moreno Lopera, 2016; Quispe & Flores, 2014).

In addition, technology has contributed to improved evidence collection techniques, through the use of tools for detecting fingerprints, fingerprints, DNA, greenhouse gases, and other crime-related materials(Lee et al., 2020; Sharma & Kumar, 2018). This has enabled investigators to collect more accurate evidence and present it in a court hearing. Moreover, data analysis systems have been developed that allow investigators to collect and analyze information quickly and accurately(Moreno Hernández, 2020). Thus, it has been possible to improve the investigative process as investigators are able to identify patterns of behavior, track the movement of suspects and generate criminal profiles. In general, criminalistic techniques have advanced significantly in recent years. Under this perspective, in this article, we present the methods and techniques employed in cases and studies conducted in the period 2018-2022 globally through an updated review of relevant documentation.

Materials and Methods

This research was conducted under a search strategy that consisted of the selection of two relevant bibliographic databases; Elsevier's Scopus and Scielo, which group documents from peer-reviewed journals. Google Scholar was also used as a specialized search engine for doctoral theses and master's theses, in order to include any document relevant to the research. The inclusion criteria for this review were based on the points detailed below.

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- Review and original articles on the subject that appear in a journal indexed in Scopus or Scielo published from 2020 to 2022, in Spanish or English.
- Master's and doctoral theses in Spanish and English published between 2020-2022.
- Open access documents.

Twenty documents were found with the inclusion characteristics, then a reading of the summary of each document was made to select the most appropriate for the research, so that 10 final documents were selected, the search terms and filters used are specified in Table 1.

Table 1. *Database and search terms*

Database	Search terms	Filters	Total results	Selected
Scopus	"techniques bloodstains" AND "forensic blood stains" OR "Blood and crime".	Open Access 2020-2022	95	7
Scielo	"techniques bloodstains" AND "forensic blood stains" OR "Blood and crime".	Open Access 2020-2022	1	2
Academic Google	"techniques bloodstains thesis" AND "forensic blood macules" OR "Blood and crime".	2020-2022	57	1

Source: Authors, 2022

Results

The right to life of the unborn child is a controversial issue, which currently generates social, legal and legislative debates between those who defend the right to life of the conceived and those who approve

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and support the practice of abortion. For this reason, this document reviews the different positions adopted by various authors and the Cosmovision of Human Rights organizations.

The Inter-American Court of Human Rights, on several occasions in its rulings, has condemned the violation of the rights of the unborn, as well as highlighted the actions taken by governments in pursuit of the protection of the unborn. Thus, in Advisory Opinion OC/17-2002, on the Juridical Condition and Human Rights of Children, this body states that rights are also applicable to the unborn, placing the principle of the best interest of the child first.

In other words, the development and exercise of the rights of the nasciturus must be considered as guiding criteria to elaborate norms that contain orders related to the life of minors. The best interest of the child will always be to live, therefore, for the States to decide on the right to life from conception becomes a sensitive issue, which may violate supranational agreements on Human Rights. From this angle, the Latin American Center for Human Rights (2011) proposes that, when making a decision on the right to life from conception, States should "recommend both punitive measures for any act aimed at destroying the life of the unborn and preventive measures".

As stated in art. 1.2 of the American Convention on Human Rights, every human being is a person, consequently, the nature of the unborn must be recognized as a person or human being, which makes him/her a subject of rights in the Inter-American System. There is no doubt then, that the States parties to the Human Rights agreements must recognize the right to life of every person from the moment of conception, according to a good faith interpretation of the terms contained in the signed and ratified treaties.

Indeed, Sanchez, and Taype (2018), argues that the nasciturus is not a subject of law at least until birth, then it must be understood that the rights for the conceived are not null, but are subject to the resolutory condition of birth, in other words, these rights are in a passive state, until they can be activated once the individual is born. Therefore, following this premise, it can be affirmed that every human being is a person, therefore, he/she is a holder of human rights in any of the stages of his/her integral life.

With regard to Ecuador, the right to life is constitutionally protected from conception. Article 45 of the Constitution states that "the State shall recognize and guarantee life, including care and protection from

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conception". Therefore, the constitutional mandate considers that the unborn is a human life, regardless of its stage of development.

It so happens that the Ecuadorian Constitution determines the protection of the unborn child from conception, without giving rise to any interpretation, unlike other Constitutions, which simply state that the right to life is inviolable. In this sense, countries such as Canada, India, the United States and Uruguay, do not recognize the unborn child as a subject of the right to life, which has allowed the creation of public policies that allow the undue manipulation of the conceived, for example, the legalization of abortion.

For Cornejo (2013), this happens because international human rights conventions do not expressly defend the human life of the unborn, but this is deduced from some of the texts enunciated by human rights organizations, which gives rise to an erroneous interpretation that ends up violating the right to life.

In this sense, the Inter-American Court of Human Rights (2011), points out that "the lack of recognition of juridical personality harms human dignity, since it absolutely denies their status as subjects of rights and makes the individual vulnerable to the non-observance of their rights by the State or by private individuals". Similarly, he argues that denying the legal existence of the nasciturus is a clear violation of the right to life, "although children and other legally incapable persons lack the capacity to fully exercise their rights (for example, the right to vote or property rights), they are fully entitled to inalienable and inherent rights of the human person" (Muñoz, 2020). (Muñoz, 2020).

And precisely, the Ecuadorian Civil Code (CCE) exposes a similar premise, stating in Art. 63 that "The rights that would correspond to the creature that is in the mother's womb, if it had been born and lived, will be suspended until the birth takes place. And if the birth constitutes a principle of existence, the newborn shall enter into the enjoyment of said rights, as if it had existed at the time they corresponded to it."

On the other hand, art. 61 of the CCE declares the protection of the life of the unborn, stating that "the law protects the life of the unborn. Consequently, the judge will take, at the request of any person or ex officio, all the measures that seem appropriate to protect the existence of the unborn". Then, the unborn is a subject of law by its legal nature, which emanates from the protection of life, from the moment of conception until birth. (Galvis, 2019)

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Therefore, even if the unborn child does not have civil capacity, this should not be an impediment for it to enjoy its human rights, or to be recognized as a person from the embryonic and fetal stages. So, while fundamental rights are qualified as general principles to achieve legal effectiveness, on the other hand, the human being is being denied the first moments of his life, this incongruence ends up denying the nasciturus his status as a person. This dichotomy results in the difficulty of determining whether the unborn child really has the same rights as the mother, whether these are superior to the will of the mother, or on the contrary, whether its rights are subordinated to the mother's decision.

In view of the above, Calvo (2004) considers that the right to life is "among human rights, the one that has been subject to the most restrictive interpretation, giving way to other rights of lower rank and even to certain legitimate desires that do not deserve the qualification of rights, such as those of the woman to her own body (which is placed before the right to life of the child and thus justifies abortion). In this way, an inversion of values is produced, in which secondary rights are placed before the principal and fundamental right to life".

This conception is a relevant social derivation that responds to the vindication of reproductive freedom and the controversial concept of a woman's freedom to decide over her own body, which is used to justify induced abortion. According to Vial and Rodriguez (2009), this notion of reproductive freedom has been widely accepted by important jurists, who consider that it is a woman's right to choose whether to continue or terminate the pregnancy, putting her needs and desires first.

In this notion of reproductive freedom, one aspect of interest is to consider the mental health of the woman who, not being able to choose and decide whether or not to terminate the pregnancy, would trigger psychic-emotional imbalances or imbalances, making the unwanted pregnancy a hopeless environment and a risk factor for psychological disorders.

Consequently, abortion is a prevailing factor in the psychic affectation of women. Aznar and Cerdá (2014) propose to give their criteria based on the evaluations of the reviews of different articles on this subject, where they conclude that women with induced abortions have between 1.80 and 7.07 higher probability of presenting mental problems, including mentioning that mental health disorders attributable to

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abortion represent between 1.5% and 5.5% of the total mental disorders of women.

In relation to abortion, the deviation of the Law is remarkable, because when this freedom is granted to the woman, the unborn child, who is a person, ceases to be a subject of the Law, loses its transcendence, and becomes an object of the rights of others and of the legal business. To avoid this transgression, the State has the duty to refrain from interrupting the process of natural gestation, establishing a legal system that allows to effectively defend life from conception. (Galvis, 2019).

In other words, by considering the life of the unborn child as a legal good, it is subject to the will of the parents, as legitimate holders of that legal good, therefore they have the right to freely dispose of the life of the unborn child, which opens the possibility of performing an abortion, as a right of the parents to not have an unwanted child. (Chávez, 2015).

From this point of view, in countries where abortion has been legalized, the norm, the protection of human rights and their guarantees, are only valid for human beings when they are recognized as subjects of rights, that is, after birth, but are not applicable to the unborn, which results in a paradox, since it is precisely human beings in their embryonic and fetal stage that are weaker, and therefore require greater protection and legal protection of all kinds.

Therefore, it is logical that the recognition and protection of human rights cannot be subordinated to the period in which man is born, since the right to life is the main and most fundamental of rights, without which the others would have no reason to exist, which is why it is important to demand respect for life, from its beginning until its natural extinction.

For man, life is a right that must be protected, without distinction between the being who has already been born and the one who is about to be born, and precisely from this precept derives the illegitimacy of abortion. It cannot be ignored that the right to life is the most essential of all human rights, since it is the basis of humanity, nor can it be denied that the human being begins to be such from conception, therefore, the unborn child is invested with human dignity, which gives him/her rights that are enforceable against third parties. (Iglesias, 2016).

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States cannot deny the personhood of the unborn, therefore, their right to life must be constitutionally protected from conception. In this sense, Positive law must recognize the unborn as a subject of rights, leaving aside controversial criteria contrary to what is stated in the Universal Declaration of Human Rights (Garzón, 2017).

As Paulson (2017) states, intentional abortion is a more serious form of execution than the death penalty, since to terminate a pregnancy in order to get rid of the product is to deny the unborn child its legal personality, to deprive it of its right to life and to go against the mandates contained in the declarations of human rights.

Therefore, legalizing abortion is tantamount to denying the right to life of a particular group of persons, in this case unborn children, which constitutes discrimination on the basis of age or birth prohibited by the American Convention in Article 1.1, the Additional Protocol to the American Convention on Human Rights in the Area of Economic, Social and Cultural Rights (Article 3), the Convention on the Rights of the Child in its Preamble and Article 2(1), and the Declaration of the Rights of the Child.

Thus, in several countries, the legislation that sustains laws prohibiting abortion bases its criteria on the right to life of the unborn child, without considering the complementary interpretation that should be made of the rights of other women.

For example, the Convention on the Rights of the Child and the International Covenant on Civil and Political Rights establish that life is an inherent right of the human person, including the unborn child, while international treaties on women's human rights, such as the Convention on the Elimination of All Forms of Discrimination against Women, determine that all discrimination in areas such as health, family relations and family planning must be eradicated.

In this sense, the arguments that defend prenatal life under the premise that it is protected by international human rights treaties generate an even greater controversy, since they consider the rights of the unborn child, but leave aside the rights of women as a whole, which goes against the principle of equality and non-discrimination.

Therefore, it is not by chance that anti-abortionists defend the theory that the embryo or fetus is a human life, therefore, they prioritize the right to be born of the conceived, before the woman's right to decide freely about her body and maternity. In this context, the different pro-

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life groups demand the tightening of laws and oppose the legalization of abortion, regardless of the reason for which the woman decides to terminate the pregnancy.

Under this scheme, Ugarte (2006) argues that abortion is equivalent to homicide, since it violates the right to life of the fetus. According to Pérez (2019), this position is based on the position of the Catholic Church, for which, interrupting pregnancy is an attempt against human life, therefore, it cannot be admitted under any circumstances and must be punished by law.

The Catholic Church bases its fundamental criterion on the anthropological nature of man, in the opinion of Jara (2013) "the natural moral law evidences and prescribes the purposes, rights and duties based on the bodily and spiritual nature of human persons. The Creator is the only one who can direct and regulate their life and acts and, more specifically, to use and dispose of one's own body". Consequently, for Catholicism, life is sacred and only God determines the beginning and end of it, therefore, under no circumstances, no human being can claim the right to end the life of an innocent human being.

From this perspective, Cornejo (2018) argues that the legal criminalization of abortion, incorporates ideological or moral reasons to prohibit the termination of pregnancy, for example, there is the conviction that the nasciturus is a person with equal dignity to the mother, and to attempt against his life, not only violates a superior right, but, it is done in a conscious and voluntary way.

Morality is an intrinsic process of the subject constructed in such a way that different subjective, environmental and cultural elements are incorporated, so that the choice of abortion involves a confrontation of the being himself between what he wishes and what he should do. Luigi Ferrajoli quoted by García (2006) in his article Questions of life and death mentions "the principle of utility and that of separation between law and morality that does not end in an integration of these complex systems but rather in an opposition between moral values and the protective function of criminal law" (p.184). And in the morality play, the clandestinity of abortion is absorbed in the morality of the professional who practices it and we leave the gap open in concomitance of the participants.

Another argument sustained by abortion's detractors is the consideration of the fetus and embryo as a human being. This thesis

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seems logical, so much so that several legislations accept it as a basis for the criminalization of abortion. As García (2017) mentions, "the personality of the fetus nullifies any justification of abortion" (p.189), in other words, from this position, abortion could not be justified under any circumstances, even if the woman's life is in danger or she has been sexually violated.

From the perspective of the Declaration of Human Rights, the unborn child is considered a person from conception, therefore, it is a subject of rights, interests and claims, so it is not only a matter of exalting a life, but also of protecting its legitimate rights. Therefore, when it comes to affirming or denying the legal existence of the unborn child, the discussion becomes very complex.

Consequently, by giving the unborn child the same value as the mother, interrupting a pregnancy is a decision that attempts against a life, therefore, abortion would be on the same level as homicide, which is why it is penalized by several legislations. But, from a theoretical point of view, affirming that the embryo and the fetus are a person, shakes the arguments of those who are in favor of abortion, so they are forced to use medical knowledge in order to counteract this statement, often used as an argument difficult to refute.

Other arguments used in defense of abortion are measured by the freedom of the woman, her state of necessity, the preservation of the life or health of the mother, in spite of this, these affirmations lose force when the rights of the mother are contrasted with those of the unborn child.

In the opinion of Finnis (2016), the nasciturus is a weak being that must be protected by the State against the aggressions of others, since, just as it cannot be admitted that the life of an innocent person is sacrificed to save another, neither should it be permissible to deliberately end the life of the fetus to save the life of the mother or at the mother's wish.

Those who are against abortion state that because of its status as a person, the unborn child deserves legal protection and protection even before birth; however, this is not a sufficient premise for it to be considered as a unique person. In fact, those who are in favor, defend that the fetus is not yet a human person in the full sense, therefore, it cannot be considered as a subject of law or superimpose this on the rights of the mother.(Bellieni, 2019)

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It should be noted that the defenders of abortion consider that it is a woman's right, therefore, its recognition guarantees compliance with human rights treaties and agreements. In this way, abortion is justified with arguments that defend women's freedom, which range from "the supposed property of women over the fruit of their sexual relations, to the atavistic condition of subjugation, which alone would legitimize any abortion. In addition, certain key criteria are cited from other documents that, although not selected for the review, provide data or concepts that support the results of the chosen research.

Table 2. *Selected and reviewed documents*

N.	Title	Reference
1	Towards a likelihood ratio approach for bloodstain pattern analysis	(Zou & Stern, 2022)
2	Finding blood in the dark: A comparison of infrared imaging devices for the detection of bloodstains on dark fabrics based on their resolution.	(Hart et al., 2022)
3	Quantifying forensic investigations involving bloodstain pattern analysis within the UK	(Home et al., 2022)
4	Accuracy and reproducibility of conclusions by forensic bloodstain pattern analysts	(Hicklin et al., 2021).
5	Identification of the origin area of hematic spots on a crime scene: theoretical analysis.	(Valdivieso-González et al., 2020).
6	Presumptive and confirmatory blood tests: teaching chemistry in forensic hematology.	(Figueroa Martínez et al., 2022).
7	Occult bloodstains detection in crime scene analysis	(Indalecio-Céspedes et al., 2021).
8	Forensic blood stain aging using reverse transcription real-time PCR	(Manasatienkij & Nimnual, 2021)
9	Bloodstain pattern analysis as scientific evidence in the judicial process. State of the matter in spain and lege ferenda proposal.	(Moreno, 2022)
10	Forensic electrochemistry: time since deposition (TSD) estimation of degrading bloodstains using differential pulse voltammetry	(Tiessen, 2021)

Source: Authors, 2022

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Zou & Stern (2022)developed their research on bloodstain analysis, emphasizing that for over a hundred years, bloodstain analysis has been a forensic tool used to reconstruct crime scenes and provide testimonial evidence. Bloodstain pattern analysis (BPA) experts evaluate the shapes, sizes, orientations and locations of bloodstains to develop hypotheses about how the event occurred. As well as deducing the spatial location of the bloodshed. However, in the last decade, the accuracy and reliability of BPA have been questioned. The latest approaches to GEP are primarily based on techniques learned in courses and seminars, as well as on experience and experimentation. A study published by Hicklin et al. (2021) analyzed bloodstain patterns where experts disagreed on the underlying mechanism, and in other cases where most analyses concluded the lack of a correct method. Under these premises the authors of the study evaluated the origin of a bloodstain pattern using the coefficient of probabilities as a forensic tool. For this purpose, two conflicting hypotheses were considered. The stain patterns were represented as a set of ellipses with features of location, size and orientation. They also applied a bivariate Gaussian model to estimate the distribution of the features according to the hypothesis and thus approximate the probability of a pattern. To test the model they used data with 59 impact patterns and 55 shot patterns. The results suggest the feasibility of using the likelihood ratio for bloodstain pattern analysis, but also point to challenges that will need to be addressed for future use.

The following article entitled "Finding blood in the dark: A comparison of infrared imaging devices for the detection of bloodstains on dark fabrics based on their resolution" explored the use of infrared (IR) light to identify bloodstains on dark fabrics, which is a widely used search technique in forensic investigation. This light is used to enhance existing white light visual search techniques, and many commercial products are available for this purpose, as well as the possibility of using standard DSLR cameras converted to IR. In this research, several infrared systems of different resolutions were evaluated in comparison to white lights for locating bloodstains on eight different types of dark tissue. These systems included options such as handheld webcams, to cameras designed to perform more detailed blood searches. The results showed that all IR systems were capable of detecting bloodstains, with significantly higher numbers compared to white light. The higher resolution systems were able to locate significantly more bloodstains than the lower resolution systems(Hart et al., 2022).

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In the study on "Quantifying forensic investigations with bloodstain pattern analysis in the United Kingdom" by. Home, Norman, Palmer, Field & Williams (2022)(2022), points out that, the details of cases are not public knowledge because being violent they are hidden from the general public and academia. But in this case, they conducted an institutional collaboration and a UK police unit for the evaluation and quantification of the records of 78 criminal investigations between 2012 and 2020, in which Bloodstain Pattern Analysis (BPA) was employed. The most common methods of assault and weapons used were identified, as well as the frequency of different blood pattern classifications. These findings explored impact patterns and software to calculate area of origin (AO), wound pattern was only recorded in 22% of scenes, with blunt force trauma being the most common form of assault. This paper recommends a revision of the BPA terminology to include additional commonly encountered patterns that are not defined by the current standard.

On the other hand, Hicklin et al. (2021)conducted an analysis of 75 bloodstain pattern analysts' studies of 192 bloodstain patterns selected to be broadly representative of operational casework, resulting in 33,005 question responses and 1,760 short text responses. Concluding that they were often erroneous and conflicted with those of other analysts. In samples with known causes, 11.2% of responses were erroneous. The results show limited reproducibility of the findings: 7.8% of responses contradicted others. Discrepancies in the meaning and use of GAP terminology and classifications suggest the need for improved standards. Semantic differences and conflicting interpretations contributed to errors and disagreements, which could have serious implications if they were to occur in casework.

Continuing with the articles analyzed, the paper Identification of the area of origin of blood stains at a crime scene: theoretical analysis, which analyzed quantitatively the patterns of stains impregnated on hard, smooth, non-porous surfaces without irregularities, was found. It also investigated the trajectory of a drop of blood generated by a cutting wound is one of the most relevant areas of study in the criminal sphere. Taking into consideration that the trajectory can be characterized through the laws of physics, so that the more detailed the model, the more accurate the estimation of the position of origin of the drop of blood found at a crime scene. From experimental measurements, this position was determined, which can offer the judge a clear view of the events that

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occurred, and therefore serve as additional evidence in the criminal investigation.(Valdivieso-González et al., 2020)..

On the other hand, the article on Presumptive and confirmatory blood tests: teaching chemistry in forensic hematology is developed, which focused on determining the presence of the heme group of hemoglobin, based on the fact that forensic medicine involves hematology and serology to determine whether the biological fluids present at a crime scene are of human origin, how they were deposited and, if possible, who placed them there. They state that, testing is critical to identify and trace the source of biological fluids. The authors show in Figure 1 the steps involved in an investigation of an alleged crime. At the top are the steps that must be taken to process a suspected crime scene. This includes preserving the scene, searching for and observing evidence, taking photographs and describing the findings. After identifying the evidence, presumptive tests were conducted to determine whether or not the evidence is positive. If the tests are positive, the evidence is collected and labeled before being transported to the laboratory for confirmatory testing; if the tests are negative, an assessment is made as to whether further analysis is possible to obtain a conclusive result. It is necessary for Forensic Science experts to have an understanding of the fundamentals of presumptive and confirmatory tests that are applied in the investigative process. Therefore, emphasis is placed on the recognition of the knowledge of chemistry as applied in forensic education. In addition, a detailed description of reactions, procedures and scenarios is offered, in order to provide teachers with the necessary information to generate didactic strategies for teaching chemistry through the use of presumptive or confirmatory tests from a forensic framework, which is the contribution of these investigators (Figueroa Martínez et al., 2022).

Figure 1. Representation of forensic investigation.

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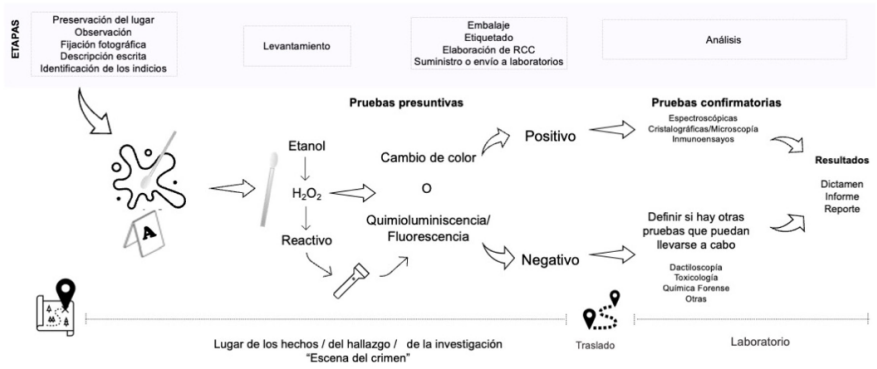

Source: (Figueroa Martínez et al., 2022)

The following study first provides a brief introduction to forensic crime scene investigation, which states that locating bloodstains on dark materials is a challenge, and there are many discrepancies about which method is the most appropriate for detecting bloodstains, depending on the contaminants present and the occurrence of false positive or negative results. Thus, they conducted research that compared different orientation tests for detecting blood on various materials, including contaminated samples and treatments. Human blood was tested on car floor mats, dark tiles, unlacquered wood surface and slippers with recent and old stains. Samples with blood, paint, bleach, all-purpose dish cleaner, juice, fruit and milk were prepared and combined with different protocols. By using forensic lights with different wavelengths and filters, no latent stains were detected and varied false negatives and positives appeared for contaminants and treatments. In addition, bleach was the main contaminant, affecting most of the tests. Therefore, the results of this study confirmed that luminol is the most effective test for detecting fresh or latent bloodstains, since no type of contamination affected its reliability.(Indalecio-Cespedes et al., 2021)

Continuing with the review of the selected documents, the research of Manasatienkij & Nimnual (2021) evaluated the efficacy of age estimation of blood stains on cotton fabric using real-time PCR coupled with a multidimensional prediction model. Tissue-specific mRNA (HBB) as well as two internal genes (GAPDH and B2M) were analyzed over a period of 150 days. The formula for estimating the age of the blood spot was $205+4.794(2-(2-(\Delta CTGAPDH-2.14))+50.293(2-(\Delta CTHBB+8.117))-5.778(2-(\Delta CT\ HBB + 8.117))$.

For the researchers of this study, it is essential to determine the age of bloodstains for forensic investigations to establish the time interval since

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the crime or accident. The procedure of this research pointed to blood-specific RNA (HBB and GAPDH) as markers of bloodstain aging, while using a housekeeping gene (B2M) as a reference marker. However, it is important to note that B2M is not suitable for old bloodstain examinations. In addition, the results of this study are in agreement with those of a previous study noting that the margin of accuracy for age estimation of blood spots was no more than 3 weeks for those younger than 6 months. Since this research was conducted under controlled conditions, it should be noted that these results may not be the same in tropical regions where sunlight and humidity levels are very extreme. (Manasatienkij & Nimnual, 2021).

The next selected study on the methodology of blood macule analysis is the one carried out by Moreno (2022) in Spain, emphasizes the importance of bloodstain analysis for the judicial system that provides an opportunity to have impartial and truthful evidence, which facilitates objective decision making by the jury. This research concludes that bloodstain patterns is a scientific discipline, and as such, reports should be governed by different scientific techniques and methods. It also points out that it is necessary to have rules that adequately regulate the introduction of bloodstain pattern analysis as a forensic discipline, in a fair, objective and guarantor-like manner. They also suggest that it is necessary to regularize the figure of the expert in this field and that training must be effective and efficient for the performance of their functions.

The last review article explores the use of differential pulse voltammetry as a technique to analyze degrading blood spots. This protocol was used to measure changes in electrochemical response in a two-week time series experiment using 9 biological replicates under 5 environmental conditions. Linear mixed models suggested that peak height and area ratios for Hb redox reactions were significantly correlated with time ($p < 0.033$). Absolute dating and principal component analysis demonstrate significant changes ($p < 0.043$) at the 96-hour time point and present opportunities for forensic TSD predictions. Overall, changes in Hb redox peaks over time provide information on oxidative changes and cellular degradation in blood spots. Being a master's thesis proposes a research avenue for TSD of blood spots. Electrochemical methods have many useful features for forensic analysis and these first steps towards their application to TSD estimates open the door to future research. Electrode preparation methods would need to be optimized and sensitivity to environmental changes would need to be better understood. The future

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of forensic science depends on new analytical techniques, such as electrochemistry, to determine with forensic-level accuracy and precision the age of a bloodstain. The combination of several analytical methods for TSD of bloodstains is expected to generate multivariate statistical analyses that will help answer the question of the age of a bloodstain.(Tiessen, 2021).

Conclusions

The initial purpose of this research was to identify new techniques and methods of blood macule analysis for forensic purposes present in current research, it is unlikely that all documents have been correctly processed. Although the systematic search supported by inclusion and exclusion parameters allows for a current overview of the subject, it is necessary to clarify that there may be more information that has not been explored in this study.

All the authors investigated agree on the importance of the study of blood macule patterns for criminalistic investigation and that there are inconsistencies in the analyses and studies carried out, which implies that it is not an entirely reliable technique. On the other hand, using scientific methods that are based on physical laws, calculation software, mathematics, electrochemical methods and statistical analysis leads to the development of new forms of blood macule analysis that, in addition to their reliability, have a minimum margin of error and can be effective in the analysis of a crime scene.

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