

## Age Estimation of a Dried Bloodstain Using Different Techniques – A Review Article

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### Abstract:

*Bloodstains can be a crucial evidence in reconstructing the events that would have occurred during the crime. This information always strengthens and verifies the statement of witness and also narrow down the number of suspects. Currently there are many techniques available to establish the age of the bloodstains. However, there is no single reliable method which can be used in the field of forensic science to determine the exact time elapsed since the crime was committed. This review paper summarizes also the important techniques that have been used by many scientists for the determination of age of a blood stains at different conditions. By analyzing all the techniques one can select the best suited technique for his analyses, as different techniques have different accuracy rate, in addition to this some are destructive and some are nondestructive techniques. Therefore on comparing all these techniques one can able to find the best method according to the various environmental conditions and thus contributing the important role in investigation and for the court of law.*

**Key Words:** Age estimation, Blood stains, skeletonized stains, Hemoglobin, HPLC, Electron Paramagnetic Resonance Spectroscopy, Reflectance Spectroscopy, Atomic Force Microscopy, Hyper Spectral Image Analysis, Electron Spin Resonance Spectroscopy, Oxygen electrode.

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## Introduction

Blood is most commonly and frequently found at the crime scene and is the most important and useful physical evidence for the forensic investigators (Li et.al. 2011). This evidence is used for the DNA-profiling to authenticate the suspect's identity and its pattern reveals the direction of spattering in order to reconstruct the crime. Blood pool can easily be collected, preserved and examined by the analyst but sometimes investigators encounters the dried blood stains also. These dried bloodstains have a great value when used for the determination of age so as to find out the relative time of the trauma or bleeding because this information may help the investigators to determine, whether the stain is related to a crime and also the approximate time when the crime was committed (Edelman et.al. 2012). The blood begins to dry whenever it's exposed to the external environment and the time required by it is directly proportional to the size, volume, nature of the surface and the involvement of other factors. Under normal condition of temperature and humidity the small, lighter and thin bloodstain pattern on non-porous surfaces dries easily and doesn't take much time (Within few minutes), while under same conditions, the greater size or large volume blood stains requires longer period of time to get dry. The drying of blood stains begins around the periphery and then proceeds towards the central portion. The flaking central portion and the intact periphery or the rim of dried blood stain produces a characteristic skeletonized bloodstain. Age estimation of blood stain provides valuable and significant information and therefore found to be one of the important application during crime scene investigation (Inoue et. al. 1992).

Ageing of blood can be determined on the basis of color changes with the passage of time. As Fresh blood usually bright red in color because of the presence of oxyhemoglobin, after few minutes of deposition it appears dark red to bluish brown color because of the conversion of Hemoglobin (Hb) to Met-hemoglobin (met-Hb) and ultimately after few hours it appears dark brown color due to presence of hemichrome. So oxidation of  $\text{HbO}_2$  results into the color change of blood which can be used for age determination of blood stain (Bremmer, 2012). This method is not reliable because we cannot find the accurate time of deposition as the result by visual analysis can vary person to person therefore to authenticate the results, different scientists developed different techniques to achieve the best results.

It is reported that, for more than 100 years many scientists used several methods and techniques for the determination of age from dried blood stain and they

also proved that these methods are reliable and can be applicable for the forensic purposes (Li et.al. 2011). The various compounds present in a blood stain and depending of that there are numerous methods that have been established for this purpose, some of them are electron paramagnetic resonance spectroscopy, reflectance spectroscopy, atomic force microscopy, oxygen electrode, hyper spectral image analysis, electron spin resonance spectroscopy etc. (Inoue et. al. 1992).

The aim of this study is to summarize all the methods, techniques, time duration (up to which it provides correct result) and its reliability which is used by different scientist for the age determination of blood stains. This review study also evaluates the imprecision and unreliable results of many techniques and also covers the recent development and the future challenges and other factor that influences the result.

## Review and Literature

**Patterson (1960)** worked on a well-known fact that the blood changes its color from red to brown with respect to time but there was no authentic literature for the rate at which these changes occurred. There are methods such as spectrophotometric analysis to determine the color but it was found to be destructive and its results was not accurate as it is altered due to the dirt or dye which comes in addition with the stain during extraction from the surface. He also found that sometimes extraction procedure also accelerates the color change of the stain. So to overcome these problems he used photoelectric colorimeter of high sensitivity and accuracy. This method was a non-Destructive method and gives results based on the surface color of the bloodstain, the reading provided by colorimeter can easily be converted to the C.I.E system of color specification. After performing experiment he observed that the greater part of the change in blood stain takes place within first three days after that the changes are minimal. Finally after analyzing the results he concluded that the temperature, humidity and lighting plays an important role and have a great effect on rate of color change.

**Sakurai et.al. (1989)** used Electron Spin Resonance technique to date human blood samples. This method uses the fact that the free radicals are generated when hemoproteins such as hemoglobin and myoglobin denatures in the blood stain due to the natural radiations. To investigate these proteins levels in the blood stain with respect to time he used this method. During the examination he observed peaks/signals, resulted due to the presence of some paramagnetic species such as Ferric Species or free radicals and the spectra of these signals was recorded at liquid nitrogen

temperature (77K) in a JES FE-IXG Spectrometer for about 270 days. After analyzing the results they concluded that this technique provides good results up to 270 days further more the better correlation with the time was found up to 120 days. Therefore ESR is a useful technique to determine the age of the blood stain.

**Inoue et. al. (1992)** for the first time utilizes the reverse phase high performance liquid chromatography (HPLC) for age estimation of blood stains. The method took advantage of the fact that the ratio of alpha-globin to heme is gradually decreases with time and therefore the peak area detected from the extracts of dried blood increases with time on chromatogram. It was found that this method was applicable up to 20 weeks old blood stains after that the precision gradually decreases due to the extra peaks appeared before and after the major peaks.

**Matsuoka et. al. (1995)** estimated the age of bloodstain by using the oxygen electrode. In this technique the oxygen content in the blood was determined by using the oxygen electrode which is immersed in water and the total hemoglobin was determined by colorimetry. Then the ratio of oxyhemoglobin to the total hemoglobin was used for estimating the age. They monitored the results for about 10 days and found that at different temperatures the decay rate of oxyhemoglobin ( $\text{HbO}_2$ ) is different.

**Anderson et. al. (2005)** used real time reverse transcriptase PCR to measure the ratios between different types of mRNA i.e mRNA and rRNA (18 S rRNA to b-actin mRNA). This method concludes that, as the blood dried over a period of time, the level of these mRNA also changes in a linear fashion under controlled conditions. The time period over which one can get successful results by this method was found to be upto 150 days.

**Fujita et. al. (2005)** used electron paramagnetic resonance (EPR) technique for the purpose of estimating the age of the blood stain. This technique measures the amount of hemoproteins which denatured under controlled environment. This method based on four major components i.e. ferric high spin, ferric non heme, ferric low spin and free radicals produced in blood stain and shows respective four striking EPR signals as  $g = 6.2$  ( $g_6$ ),  $4.3$  ( $g_4$ ),  $2.27$  (H) and  $2.005$  (R) out of which  $g_6$  represents the level of met-Hb whereas H represents Hemichrome level (HC) in the blood. They found that the age of a bloodstain could be determined by the ratio of H/ $g_4$  signal. By using this criteria they got positive or suitable results for the dried blood stains which are not older than two months, beyond this period it is impossible to determine the exact age of blood stain.

**Strasser et. al. (2007)** presents a new tool for determining the age of bloodstains. They used Atomic Force microscopy to get the high resolution imaging of erythrocytes (RBC) of blood samples. This method was used to detect the changes, occurred in the elasticity of the erythrocytes as the function of time. Here the high resolution microscope consists of a cantilever and its tip (nanoindenter) scans the surface of the blood and provides a nanometer resolution image. The elasticity were recorded based on the Force- distance curves on different areas of a blood stain. After analyzing all the results they found that the elasticity of the blood was decreased with time or age of the sample.

**Hanson and Ballantyne (2010)** developed a method to estimate the time since deposition of bloodstain by the help of UV-VIS spectrophotometric analysis of hemoglobin. This method measures the wavelength of hemoglobin with maximal absorption around 412 nm but after detailed study of Soret band of hemoglobin they found that there was a blue shift (toward the shorter wavelength) with the increase in age of the bloodstain. This shifts can be easily distinguish between the blood that were deposited minutes, hours, days and weeks.

**Li et.al. (2011)** described a novel method for age determination of dried bloodstain. They used reflectance spectroscopy, a non-destructive method. It involves the use of micro spectrophotometer (MSP) that measures the visible reflectance spectrum of hemoglobin component present in the blood, deposited on a white tile. They monitored the results up to 37 days and the wavelength at which the spectra was generated were 442nm and 585nm under controlled conditions. To overcome the effects of baseline variation and sample scattering the spectra were preprocessed and to test the accuracy of results a supervised statistical classification model (leave- one-out-cross validation) was used.

**Bremmer et. al. (2011)** proposed diffuse reflectance spectroscopy (DRS) method to estimate the age of blood stains by determining the optical property of material. This method uses the advantage of the fact that the color of bloodstain changes with time from red to brown. The changed color can be quantified by optical spectroscopy. They have determined the levels of three major derivatives of hemoglobin in the blood such as oxyhemoglobin, met- hemoglobin and hemichrome as its fraction also changes with time. They found the unique combinations of these three component all the time in the blood samples over the period of 60 days.

**Edelman et. al. (2012)** used hyper spectral imaging to evaluate the feasibility of this technique for the

determination of age of blood stains. In this method they recorded the visible reflectance spectra of bloodstains and this spectra comprised of relative fractions of oxyhemoglobin, Met- hemoglobin and hemichrome with were later compared with the reference dataset. The results were analyzed up to 200 days. By this study it was found that the absolute error of age estimation increased with that of time. Further, to test the practical applicability of this method they simulated a crime scene where the number of bloodstains were deposited of several ages.

**Li et.al. (2013)** used visible wavelength hyper spectral image analysis to determine the age of dried bloodstain up to 30 days of time interval. In this method they generate the hyper spectral image of the dried blood stain and then selected some reflected spectra within the image which were then subjected to spectral preprocessing followed by linear discriminant analysis. This analysis was made under controlled conditions with an overall error of  $\pm 1.17$  days.

### Discussion and Conclusion

**(Patterson, 1960)** first time used the reflectance measurements to estimate the age by using the spectrophotometer. **(Bremmer et.al., 2011)** also worked on reflectance spectroscopy over a period of 60 days under controlled conditions. The results obtained shows that they could able to find the correct age up to 55 days with the uncertainty of 14 days. **(Inoue, 1992)** worked on HPLC technique found this method suitable for the determination of age up to 20 weeks old bloodstains. This was the first time when some use this technique for age estimation. **(Fujita et.al., 2005)** used electron paramagnetic resonance spectroscopy, a non-destructive method for estimating the age up to 270 days under controlled environment. The results give a linear correlation of denatured proteins with the increased age but the accuracy of results found uncertain if the blood was influenced by or exposed to environmental factors such a absorbents, fluctuated temperature or sunlight. **(Anderson et. al., 2005)** worked on different component of blood i.e. the mRNA levels which gradually changes with the time. They found that the age of blood stain could be

obtained up to 150 days by using real time reverse transcriptase PCR. This method offers the following advantages over other approaches such as enhanced detectability of small samples, simultaneous isolation of DNA and RNA from the same sample. **(Hanson et. al., 2010)** used UV-VIS spectrophotometer and found that the blue shift of the hemoglobin changes with the increase of time and directly influence by the ambient relative humidity and temperature. The method was extremely sensitive as it requires only 1 $\mu$ l of dried blood stain for analysis. **(Edelman, 2012)** used hyper spectral imaging system to estimate the age of bloodstains over the period of 200 days but they found that this technique gives absolute error rate with increased age of blood.

After analyzing all the papers it was found that there are numerous techniques have been used for age determination of bloodstains and all techniques are still in the experimental phase in addition with the inaccurate age estimation after a limit. On comparing all the techniques it was found that RNA method can be used for long term determination as compare to other techniques and the least destructive or invasive technique was Reflectance spectroscopy as it involves light source and a spectrometer which never destroys the sample. In addition to this the method was comparatively economical and gives good results for the samples on white background. The samples on colored background can be analyzed by hyper spectral imaging. HPLC and oxygen electrode method can be used for short term old bloodstains.

Some techniques are sensitive to estimate the age of bloodstains for short term changes and some are for long term changes. All the applied techniques, used only those blood samples which were dried at controlled conditions but as we know the results could be affected or influenced by environmental factors. So there is a need for further improvement which could provide highly précised and accurate results even if the bloodstains dried under different conditions. It is important to investigate the effect of other variables such as temperature, humidity, illumination, stain thickness and substrate so as to implement its use into forensic practice and eventually in court.

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