

Chapter 3: Finger mark development techniques within scope of ISO 17025

3.1 Acid dyes (acid black 1, acid violet 17, acid yellow 7)

1. History

- 1.1 Fingerprints may be deposited in a number of contaminants at crime scenes, and of all these blood is the most commonly observed. This is possibly because, when present even in small quantities, it is easily seen as it strongly absorbs light throughout the visible spectrum. However, when present in minute amounts, or on dark, patterned or multicoloured confusing backgrounds, the blood may require enhancement to make it more useful for evidential purposes. Additionally, proof that a stain is actually blood rather than an innocuous substance may be important in assessing guilt or innocence, and may even be a matter of life or death in some cases.
- 1.2 The history of proving the presence of blood evidence in forensic investigation dates back over 150 years using chemical means, and further still when microscopical methods are considered. Anton van Leeuwenhoek was said to be the first person to describe and illustrate blood cells in the latter part of the 17th century, although this is disputed.
- 1.3 The earliest tests for blood were of two types, both relying on the presence of the haem group present in the red blood cells. The early tests included those that reacted with haem to produce crystals and those that relied on its catalytic nature. More recently (1999) a third test relying on antibodies has been introduced.
- 1.4 The crystal or confirmatory tests were formulated by Teichmann in 1853 [1], producing crystals of haematin, and by Takayama in 1912 [2], producing crystals of haemochromogen. However, these tests require that the blood be scraped from the surface, and therefore they can only be used where blood is easily observed, and cannot be used speculatively. Having to scrape blood also gives no regard to the forms of physical evidence that may be present, such as fingerprints, footwear impressions or splash patterns.
- 1.5 Catalytic or presumptive tests that attempted to keep much of the physical evidence intact were produced by Van Deen in 1862 based on guaiacum [3], Schönbein in 1863 using hydrogen peroxide [4] and by Adler and Adler in 1904 using benzidine [5]. They also pioneered the use of leuco-malachite green in 1904 [5]; their method being later modified by Medinger in 1933 [6] to make it more sensitive.
- 1.6 In 1901 Kastle and Shedd [7] developed another catalytic test using phenolphthalein, which Meyer in 1903 [8] modified to detect blood. Further investigation by Kastle and Amos in 1906 [9] proved the phenolphthalein to be reacting with haemoglobin present in blood. This test is known as the Kastle-Meyer Test.
- 1.7 Other presumptive tests for blood were developed for forensic use by Ruttan and Hardisty in 1912 using o-tolidine [10]; by Specht in 1937 using luminol (3-amino-phthalhydrazide); [11] and by Gershenfeld in 1939 using o-toluidine [12].

- 1.8 In 1911 Abderhalden and Schmidt [13] reported the development of fingerprints on the bottle label of triketohydrindene hydrate (ninhydrin). This discovery was not exploited for the detection of fingerprints or blood until 1954 when Oden [14] produced his ninhydrin formulation based on acetone. The use of this method for the enhancement of fingerprints in blood revolutionised thinking in this area of forensic investigation. The emphasis was shifted away from presumptive tests for haem, which generally require expert opinion to interpret the test results correctly, to easier to use reagents, which produce intensely coloured products with other components of blood, usually protein or its breakdown products.
- 1.9 Use of the protein dye amido black (acid black 1) quickly became popular with forensic investigators. Its use by the Metropolitan Police Laboratory, in a solvent base of methanol and acetic acid, was discussed at a forensic science symposium in 1961 by Godsell [15]. This formulation, with a change away from the fixing of the mark by the use of heat to immersion in methanol in 1981 [16], along with a water-based formulation of the same dye [17] continued to be recommended for the enhancement of fingerprints in blood by the UK Home Office until 2004 [18] when a new formulation by Sears and Prizeman [19] was adopted.
- 1.10 Many other protein stains for the enhancement of both fingerprints and footwear impressions in blood have also been proposed; coomassie blue (acid blue 83) and Crowle's double stain (acid blue 83 and acid red 71) by Norkus and Noppinger in 1986 [20], fuchsin acid (acid violet 19, Hungarian Red), patent blue V (acid blue 1) and tartrazine (acid yellow 23) by Barnett *et al.* in 1988 [21], benzoxanthene yellow and acid violet 17 by Sears *et al.* in 2001 [22] and acid yellow 7 by Sears *et al.* in 2005 [23].
- 1.11 Although the use of protein dyes became most popular for enhancing fingerprints in blood, research on presumptive enhancement methods continued and in 1976 Garner *et al.* [24] proposed the use of tetramethyl-benzidine (TMB) as a safer and more effective technique than benzidine. Suggestions for other presumptive tests continued; tetraamino-biphenyl (TAB, also known as diaminobenzidine, DAB) in 1989 by Hussain and Pounds [25], fluorescein in 1995 by Cheeseman and DiMeo [26] and leucocrystal violet (LCV) in 1996 by Bodziak [27].
- 1.12 In addition there have been many modifications made to ninhydrin formulations to increase its effectiveness and safety by Crown in 1969 [28] and Morris and Goode in 1974 [29]. Further changes were forced on the fingerprint community because of The Montreal Protocol on Substances That Deplete the Ozone Layer in 1987 and new formulations were proposed by Watling and Smith in 1993 [30] and Hewlett *et al.* in 1997 [31]. The use of transition metal toners to change the colour or make the reaction product between amines and ninhydrin fluoresce have also been proposed by Morris in 1978 [32], Everse and Menzel 1986 [33] and Stoilovic *et al.* in 1986 [34].
- 1.13 It was also suggested that the use of one of several ninhydrin analogues would improve sensitivity and many have been proposed; benzo[f]ninhydrin in 1982 by Almog *et al.* [35], 5-methoxyninhydrin by Almog and Hirshfield in 1988 [36], 1,8-diazafluoren-9-one (DFO) in 1990 by Grigg *et al.* [37] and 1,2 indandione by Ramotowski *et al.* in 1997 [38]. All of these techniques, although primarily

intended to target with amino acids in latent fingerprints on porous surfaces, will react strongly with the proteins present in blood to coloured and/or fluorescent products.

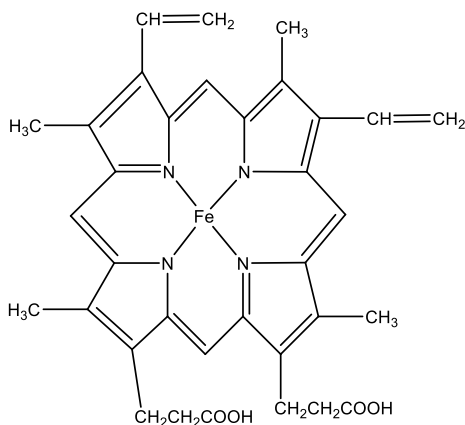
- 1.14 More recently in 1999 Hochmeister *et al.* [39] validated a one-step immunochromatographic test for using anti-human Hb antibodies to prove the presence of human blood. However, this method requires the removal of blood from the surface so it cannot be used to enhance the physical evidence in situ, although if this test could be carried out after the application of the more sensitive protein dyes this would then cover all issues. In 2008 Johnston *et al.* [40] compared several of these tests with luminol and concluded the latter was more sensitive.
- 1.15 It was observed from the earliest times that blood strongly absorbed light and a number of researchers in the mid- to late-19th century tried to use this as a way to identify that a stain was blood. Among them were Hoppe in 1862 [41], who investigated the spectral properties of the colouring matter in blood; Stokes in 1864 [42], who was able to recognise the difference between haemoglobin and oxy-haemoglobin; and Soret in 1883 [43], who characterised the absorption bands of haemoglobin in the violet and ultraviolet (UV) regions of the spectrum. In 1865 Sorby [44] studied the spectra of various haemoglobin derivatives and proposed these as a means of identification for blood stains.
- 1.16 In the late 1970s and early 1980s it was observed by those developing high-intensity light sources that one of their most useful properties was that shorter wavelengths of light in the UV and violet make surfaces fluoresce strongly and this can give extra detail if a fingerprint is in a strongly light-absorbing material [45]. This is an especially valuable method for the enhancement of fingerprints in blood as the haem group absorbs light throughout much of the visible part of the spectrum [46,47].
- 1.17 All these developments meant that by the late 1990s there were so many reagents and formulations existing for the enhancement of blood-contaminated fingerprints and footwear impressions with little or no comparative data that they were causing immense confusion among practitioners. Also the emergence of DNA analysis heaped even more uncertainty over which techniques could or should be used for the enhancement of blood. Vital evidence was likely to be lost by the wrong choices. Therefore the UK Home Office set out to clarify the situation and began a programme of work to review and compare the most commonly used of these techniques [19, 22, 23]. Resulting from this colossal task there were a number of key findings that were incorporated in a comprehensive update to *The Manual of Fingerprint Development Techniques* in 2004 [18], which included the current formulations for acid black 1, acid yellow 7 and acid violet 17.

2. Theory

- 2.1 Blood consists of red cells (erythrocytes), white cells (leukocytes) and platelets (thrombocytes) in a proteinaceous fluid called plasma, which makes up roughly 55% of the whole blood volume. The red cells principally contain the haemoglobin protein, but also have specific surface proteins (agglutinogens)

that determine blood group. The white cells, which form part of the immune system, have a nucleus that contains DNA.

- 2.2 Haemoglobin makes up roughly 95% of red cells' protein content and is made of four protein sub-units each containing a haem group. The haem group is made of a flat porphyrin ring and a conjugated ferrous ion.



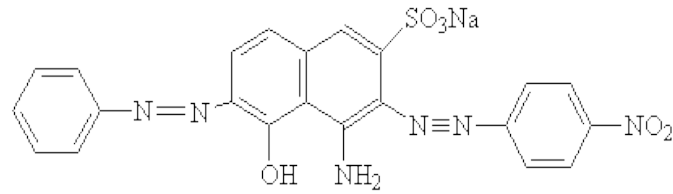
Chemical structure of haem.

- 2.3 As mentioned above, chemical blood enhancement methods fall broadly into two types; those that react with the haem grouping and those that interact with proteins or their breakdown products. The last type are not at all specific for blood; however, because of the high proportion of protein and its products present in blood, and the fact that they do not rely on the effectiveness of cell lysis (as do the haem-specific type) the techniques that interact with proteinous material are the most sensitive available to the forensic investigator [23].
- 2.4 Many researchers measure the sensitivity of their techniques by diluting blood with water [23,26,48,49,50]. This method favours techniques that utilise the haem as all the red cells would be lysed because of osmotic pressure during dilution, something that will not happen when these techniques are used operationally. Dilution with a buffer at the same osmotic pressure as blood serum would give a clearer indication of ultimate technique sensitivity.
- 2.5 There is also one other major advantage of the protein staining techniques, in that they generally incorporate a stage that either denatures or fixes proteins to the surface; as most proteins, including haemoglobin, are water soluble, the blood-contaminated fingerprint is not then diffused during treatment.
- 2.6 There are two types of techniques that can be used to target proteins in blood; those that react with amines (e.g. ninhydrin), and those that stain proteinous material (e.g. acid dyes). It is this class of protein dyes that constitute the processes recommended by the Centre for Applied Science and Technology (CAST), i.e. acid black 1, acid violet 17 and acid yellow 7.

- 2.7 As stated above, the protein dyes used by HOSDB for the enhancement of fingerprints in blood are a group known as acid dyes. They are often characterised by the presence of one or more sulphonate ($-\text{SO}_3$) groups, usually the sodium (Na^+) salt. These groups function in two ways; firstly to provide solubility in water or alcohol, the favoured major solvents from which to apply these dyes, and secondly by virtue of their negative charge (anionic). If acidic conditions are used (acetic acid being the favoured option), the blood protein molecules acquire a positive charge (cationic) and this attracts the acid dye anions. Hydrogen bonding and other physical forces such as Van der Waals bonds may also play a part in the affinity of acid dyes to protein molecules [51].
- 2.8 Protein stains are applied via a three-stage process.
- Firstly the marks are fixed using a solution of 5-sulphosalicylic acid in water; this precipitates the basic proteins and thus prevents diffusion of the marks and any associated loss of detail. This fixing stage gives the protein dyes another advantage over the presumptive tests for fingerprint development because as well as being more sensitive, it is often found that the fingerprint ridges are more sharply defined and the detail is clearer.
 - The marks are then treated with an acidic protein stain that dyes the precipitated basic proteins in the manner described above to give a coloured product.
 - A washing stage is required post-staining. On non-porous surfaces this just removes excess dye, however on porous surfaces this also acts as a de-stainer, removing dye that has been absorbed by the background surface. The wash solution has to be carefully constructed so that it dissolves the dye, does not either diffuse or wash away the dyed fingerprint and retains the intensity of colour of the dye in the fingerprint. For this reason the same solvent mix as that used for the dyeing process, or some small variation of it, is generally most effective in this application [11].
- 2.9 Fluorescence examination can also assist in the subsequent visualisation of marks developed using the acid dyes. The use of acid black 1 or acid violet 17 can further intensify the contrast between the fingerprint and the background by increasing the light absorption properties of the blood, and this may aid visualisation of developed marks during fluorescence examination.
- 2.10 Acid yellow 7 stains blood with a fluorescent species that can be excited by blue (420–485nm) light. The resultant fluorescence from the stained mark can be less pronounced on heavy deposits of blood as the haem group retains its ability to absorb both the excitation light and that emitted as fluorescence.
- 2.11 It has also been observed that acid violet 17 has weak fluorescence in the deep red and near infra-red (IR) regions of the spectrum when excited with green/yellow and yellow wavelengths, and this fluorescence could also be utilised to view developed marks.

3. CAST processes

- 3.1 CAST recommends the use of a number of fingerprint development and blood enhancement processes for use on fingerprints in blood, the ultimate process selection being dependent on the characteristics of the surface the blood is present on [18]. Three acid dyes (acid black 1 [naphthlene black, naphthol blue black, CI 20470], acid violet 17 [Coomassie brilliant violet R150, CI 42650] and acid yellow 7 [brilliant sulphoflavine, CI 56205]) are recommended only for use on blood. DFO and ninhydrin will also develop marks in blood, but are also the most sensitive techniques for the development of latent fingerprints on porous surfaces [19,22,23].
- 3.2 A holistic approach has been adopted for the acid dyes: the formulations for fixing, staining and de-staining have been very carefully constructed so that the blood is fixed effectively, then it is kept from diffusing during the staining and de-staining stages, and finally the strong coloration from the dye is retained during de-staining [19].
- 3.3 The most effective formulation for the three recommended acid dyes is as follows [23]:
- fixing solution – 23g 5-sulphosalicylic acid dihydrate dissolved in 1 litre water;
 - staining solution – 1g acid dye (acid black 1, acid violet 17 or acid yellow 7) dissolved in 700mL distilled water, 250mL ethanol and 50mL acetic acid;
 - washing solution – 700mL water, 250 mL ethanol and 50 mL acetic acid.
- 3.4 If acid dye formulations are applied directly to fingerprints in blood without a fixing stage, the blood will dissolve and the ridges will either diffuse or be completely washed away. A number of different fixing agents have been investigated, but the most effective are 5-sulphosalicylic acid and methanol. Which fixing agent is used will depend upon the major solvent used in the dyeing process; in the current (post-2004) formulations where water is the main solvent, a solution of 5-sulphosalicylic acid is most effective. However, in the previously recommended formulations where the main dyeing solvent was methanol, methanol was found to be the best fixing agent [19]. These fixing agents act in different ways; 5-sulphosalicylic acid precipitates basic proteins and methanol dehydrates the blood. All-in-one formulations that stain and fix are generally not stable for more than a day or two and are not as effective as a two-stage process, both in fixing and dyeing.
- 3.5 Acid black 1 (also commonly known as amido black) is a protein stain that dyes the proteins present in blood to give a blue/black colour. It can be absorbed by some porous surfaces so an area away from the mark to be enhanced needs to be tested first to ensure that there is no background staining.

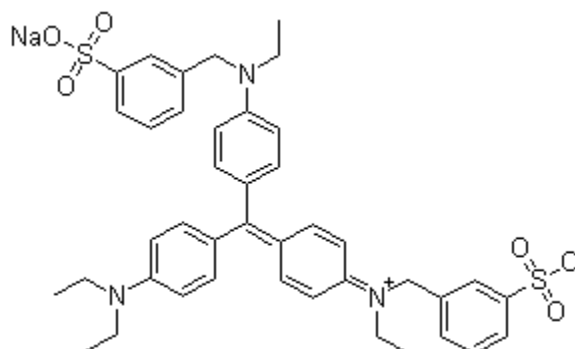


Structure of acid black 1.

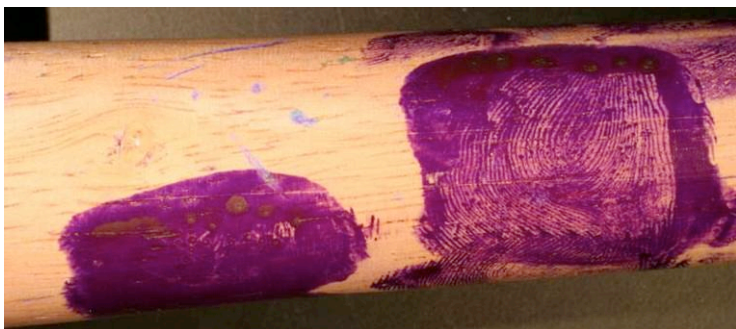


Fingerprints in blood on paper enhanced using acid black 1.

- 3.6 Acid violet 17 is a protein dye that stains the proteins present in blood to give a bright violet product. It can also be absorbed by some porous surfaces, therefore an area of the substrate away from the target enhancement area should be tested to assess background staining.

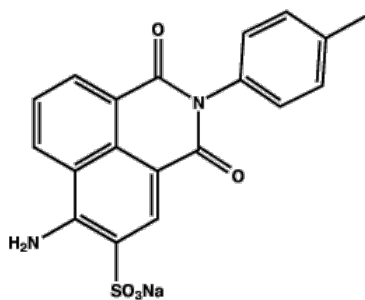


Structure of acid violet 17.



Fingerprints in blood on a wooden handle enhanced using acid violet 17.

- 3.7 Acid yellow 7 stains the proteins present in blood to give a pale yellow product that fluoresces bright yellow when viewed under blue/green 385–509nm illumination. The haem group acts as an energy sink that improves the enhancement of lighter marks. Acid yellow 7 is recommended in the *Manual of Fingerprint Development Techniques* [18] for use on dark non-porous surfaces only because it can not easily be removed from the background of porous surfaces.



Structure of acid yellow 7.



Fingerprints in blood on a dark glass bottle enhanced using acid yellow 7.

- 3.8 It was found that concentrations of these dyes of less than 0.1w/v resulted in less effective staining [19] and therefore the dye concentration used in the

formulation above is selected to minimise dye content yet retain staining effectiveness.

- 3.9 The presence of a short chain alcohol in the dyeing solution helps to prevent the blood from diffusing during the dyeing stage [19]. Ethanol is preferred as this offers lower toxicity and flammability than methanol. The use of water as the major solvent gives the solution a flash point of around 30°C enabling this formulation, containing water, ethanol and acetic acid, to be used at scenes of crime with a few simple precautions [18].

4. Critical issues

- 4.1 The entire scope of blood evidence (blood pattern analysis, footwear enhancement, DNA recovery) should be taken into account before deciding on a treatment for fingerprint evidence alone. In some cases the correct sequence of application will be essential in order to maximise evidential opportunities and the use of protein stains may affect other forms of evidence.
- 4.2 The protein stains should not be used as the sole means of determining whether a mark is in blood, because they give positive reactions with a number of other protein-containing substances (e.g. egg white). Other presumptive tests should be used to confirm the presence of blood (preferably using an area that does not contain ridge detail) before proceeding to enhancement with protein stains.
- 4.3 The fixing stage is essential for the process to be effective. If a fixative is not used, the blood marks will diffuse as the dye solution is applied to them, possibly destroying the ridge detail.
- 4.4 The current (post-2004) solutions are flammable, with a flash point of 30°C. The solutions should not be used in situations where the flash point is likely to be exceeded or where sources of ignition are present.

5. Application

- 5.1 Suitable surfaces: The three protein dyes recommended are suitable for use on all non-porous surfaces where blood contamination is suspected to be present. Acid black 1 and acid violet 17 are also suitable for use on porous surfaces contaminated with blood, whereas acid yellow 7 is not recommended for porous surfaces because it is more difficult to wash the dye out of the background, making fingerprints more difficult to see.
- 5.2 Currently (2011) it is considered that combinations of fluorescence examination, two amino acid reagents and three acid dyes are the most effective means of enhancing fingerprints in blood [23]. The most appropriate and effective techniques to use, either individually or in a sequence, depend on the porosity of the surface to be treated. This applies to both latent fingerprint development and enhancement of blood-contaminated fingerprints.

- 5.3 Fluorescence examination of the surface should always be carried out before any other technique to see if any marks are revealed as dark absorbing ridges against a fluorescing background. High-intensity light sources with outputs between 350–450nm are most effective.
- 5.4 When the blood-contaminated or latent fingerprints are on porous surfaces the most effective sequence of techniques is DFO, ninhydrin, either acid black 1 or acid violet 17, after carrying out a spot test to see which is most suitable, and then finally physical developer [23].
- 5.5 When the blood-contaminated or latent fingerprints are on non-porous surfaces the most effective sequence of techniques is vacuum metal deposition (VMD), powders, acid yellow 7, acid violet 17 then finally either powder suspensions or solvent black 3 (Sudan Black). Superglue may be used instead of VMD or powders but this will inhibit the dyeing process for blood by sealing the surface and preventing the dye reaching the blood [23].
- 5.6 The three recommended acid dyes, acid black 1, acid violet 17 and acid yellow 7, should all be applied to blood that has been fixed for at least five minutes with a solution of 5-sulphosalicylic acid. Dyeing of fixed blood is most effective if immersed in the dyeing solution for at least three minutes for acid black 1 and acid violet 17 whereas acid yellow 7 requires at least 5 minutes. Areas heavily contaminated with blood need longer dyeing times. If it is not possible to immerse the bloodied fingerprints then the dyeing solution should be applied above the area of interest and allowed to flow down over it, keeping the area damp for the specified time. A well may be constructed around the area of interest on horizontal surfaces, which may be flooded and drained as appropriate, or tissues soaked in dye may be applied to the surface [52]. Ethanol-containing staining or de-staining solutions should never be sprayed because this lowers the flash point by at least 100°C making it impossible to work without creating a flammable atmosphere.
- 5.7 Areas of interest will then need to be washed or de-stained to remove excess dye. The most effective solution for doing this is the same solvent composition as the dye solution, washing as required to remove dye or de-stain the background.
- 5.8 High-intensity light sources capable of delivering output wavelengths between 420–485nm must be used to excite fluorescence from blood dyed with acid yellow 7. The fluorescence emitted is between 480–550nm. The use of shorter wavelengths between 350–450nm to excite background fluorescence after acid black 1 or acid violet 17 treatment may be beneficial.
- 5.9 Work carried out by CAST has demonstrated that positive DNA identification may be made after fluorescence examination and any single chemical treatment provided simple guidelines are followed. If more than one fingerprint development technique is used in sequence then the chances of successfully carrying out DNA identification are much reduced [18].

6. Alternative formulations and processes

6.1 There are a great number of blood reagents, only some of which have been mentioned above, and there can be many different formulations of each of those reagents to consider. Some of these will be described in more detail in Chapter 5.1. The water-based formulation of the acid dyes are probably the most practical alternative formulations because they can be used at all times, although methanol-based solutions might prove beneficial under some specialised circumstances.

	Water-based method	Methanol-based method
Fixing solution	20g 5-Sulphosalicylic acid 1,000mL Distilled water	Methanol (99%+)
Staining solution	2g Acid dye 20g Citric acid or 5% v/v acetic acid 1,000mL Distilled water	2g Acid dye 900mL Methanol 100mL Acetic acid
De-staining solution 1	Distilled water (5% v/v acetic acid helps to retain coloration)	900mL Methanol 100mL Acetic acid
De-staining solution 2	Distilled water (5% v/v acetic acid helps to retain coloration)	950mL Distilled water 50mL Acetic acid

Methanol-based and water-based acid dye and de-staining formulations.

6.2 Originally these formulations were developed for use with acid black 1, but both can be used equally well with acid violet 17 and acid yellow 7.

6.3 Advantages of methanol-based and water-based acid dye formulations

6.3.1 The water-based formula does not use flammable or toxic solvents and can therefore be used safely regardless of the temperature at the scene of a crime. It can also be used in a laboratory if extraction is not available. It is an easy process to use and cheap to carry out.

6.3.2 The methanol-based formula is very effective, cheap and an easy method to use for enhancing fingerprints in blood. It gives good ridge definition, little background staining and produces dark blue-black fingerprints.

6.4 Disadvantages of methanol-based and water-based acid dye formulations

6.4.1 The water-based formula does not always produce optimum results as it may give diffuse fingerprint ridges and weaker coloration with less contrast, especially on porous surfaces. More coloration may be retained by the inclusion of 5% v/v acetic acid in the de-staining solutions. Also on porous surfaces, the contrast between the fingerprint and the background can sometimes be poorer than that achieved when using the methanol-based formulation because of relatively high background staining and the lower colour intensity of the developed ridges.

6.4.2 The methanol-based solutions are toxic by ingestion and skin absorption. Methanol is also a highly flammable solvent. Although this formulation can be used safely in a laboratory, its use at scenes of crime is not recommended due to potential ignition or the possibility of absorption of methanol through the skin. Leaching of blood from heavy deposits also occurs with this formulation unless long fixing times (> 10 minutes) are used. The methanol-based formulation may also soften or destroy some surfaces including paints, varnishes and some plastics, damaging or obliterating ridge detail.

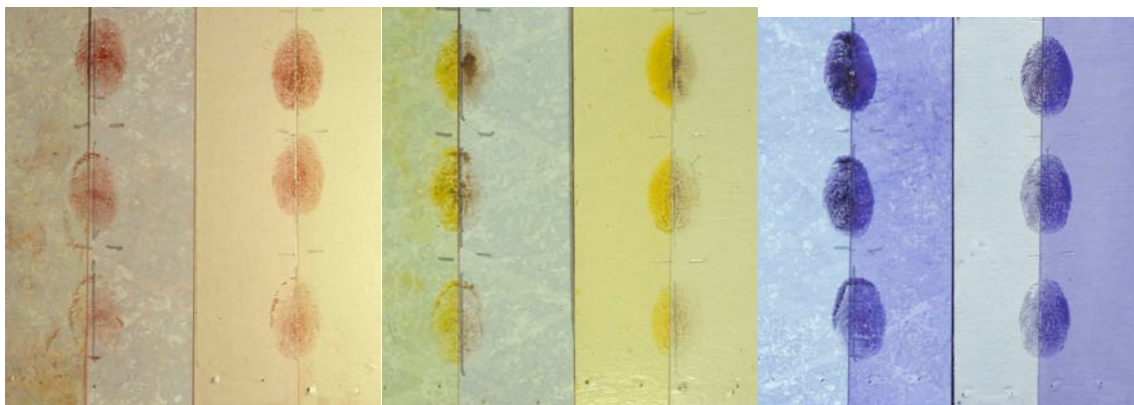
6.5 Rejected dyes and techniques

6.5.1 The CAST blood enhancement project investigated many dyes and reactive techniques that proved less effective, and considered many others that were not ultimately studied because of health and safety concerns. The dyes and techniques that were investigated in practical experiments are listed below in categories.

6.5.2 Protein dyes [22] : Acid blue 74 (indigo carmine), acid blue 83 (Coomassie brilliant blue R250), acid blue 90 (Coomassie brilliant blue G250), acid blue 92 (Coomassie blue R), acid blue 147 (xylene cyanol FF), acid red 1 (amido naphthol red G), acid red 71 (Crocein scarlet 7B), acid red 87 (eosin y), acid red 88 (roccellin), acid red 112 (Ponceau S), acid violet 19 (fuchsin acid, Hungarian Red), acid yellow 23 (tartrazine), benzoxanthene yellow (Höchst 2495), brilliant sulphaflavine, Crowles double-stain (*acid blue 83 and acid red 71*), direct yellow 12 (chrysophenine), MBD (7-[p-methoxybenylamino] -4-nitro-2,1,3-benzoxadiazole).

6.5.3 Haem-specific reactive techniques [23]: Azino-di-benzthiazoline sulphonic acid (ABTS); diaminobenzidine (DAB) or tetraamino-biphenyl (TAB); guaiacol; leucocrystal violet (LCV); leucomalachite green (LMG); luminol; organic acid (formic or acetic) and hydrogen peroxide (hæmatoporphyrin); fluorescein.

6.5.4 Amine and protein reactive techniques [23]: ATTO-TAG™ CBQCA; ATTO-TAG™ FQ; fluorescamine; Lucifer Yellow vinyl sulphone (VS) ; SYPRO® Ruby Protein Blot Stain.

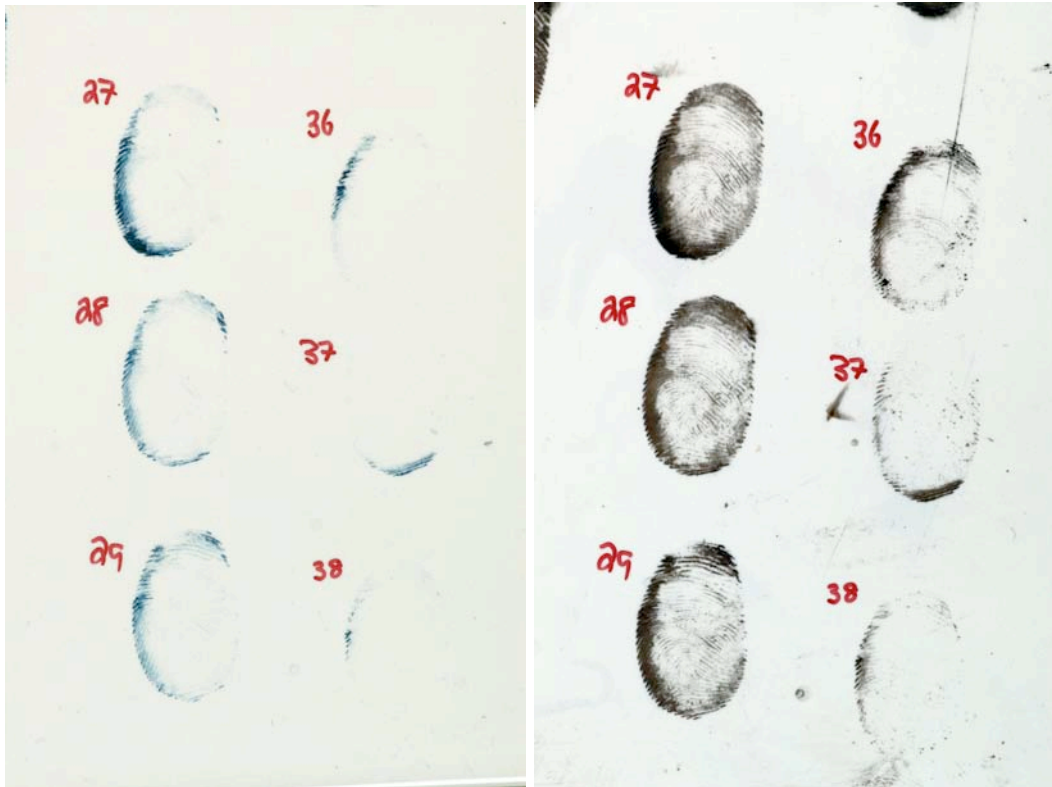


Examples of split depletion experiments carried out on wallpaper and painted wall surfaces using a range of alternative protein stains.

7. Post-treatments

7.1 Fluorescence examination is the most notable post-treatment process and this has been discussed fully above in sections 2.9 and 2.10.

7.2 However, it appears from more recent studies on footwear marks that powder suspensions may have an affinity for blood and can be used as an enhancement technique after the protein dyes [52]. It should be noted that the current (post-2009) application methods will cause potentially disastrous over-development on heavy blood deposits, but on faint fingerprints on non-porous surfaces there may be significant enhancement. Powder suspensions are not specific for blood and cannot be used to determine that any additional ridge detail is in blood.



Finger marks in blood in a depletion series on a ceramic tile showing deposited marks numbers 27,28,29,36,37,38,39, a) enhanced using acid black 1 and b) subsequently treated using iron oxide-based powder suspension.

8. Validation and operational experience

8.1 The validation of blood dyes is carried out both in terms of the number of graded marks, and also in terms of sensitivity to diluted blood. The first test will give an indication of how far down a depletion series the blood reagent will work (i.e. how many multiple contacts from a single finger contaminated with blood at normal concentration can be detected) and the second will indicate how sensitive the technique is to dilute traces of blood (as may be experienced where efforts have been made to clean a crime scene). Because blood is being targeted as a contaminant, the results obtained for fingerprints will be applicable to development of other types of blood evidence, such as footwear marks (and vice versa). There will be some exceptions to this, e.g. luminol is recommended as a footwear development process for carpets, a surface for which there is no recommended fingerprint development process, but would not be recommended as a primary technique for development of fingerprints because the requirement for spray application without fixing may diffuse fingerprint ridges and destroy evidence.

8.2 Laboratory trials

8.2.1 During the late 1990s and early 2000s, HOSDB conducted a series of experiments to optimise the Acid Black 1 formulation and to identify alternative blood enhancement agents with potentially improved performance [19,22,23]. Experiments to assess the effectiveness of protein dyes were carried out by

using series of 6 split depleted blood-contaminated fingerprints on 9 or 15 surface types, depending on whether or not the technique was appropriate for both porous and non-porous surfaces. However, it became obvious that this experiment was not sufficient to resolve the differences in sensitivity of some fluorescent dyes on non-porous surfaces, so the number of depletions was increased to 18.

- 8.2.2 Additionally, in the literature it is common to compare the sensitivity of blood enhancement techniques by diluting blood with distilled water. Accordingly it was decided to assess techniques in this manner so a series of 12 dilutions from 1/100 to 1/100,000 were used along with a distilled water control. These tests were carried out on photocopy paper and glass using 5µL of solution for each spot.
- 8.2.3 Of the 17 protein stains investigated, 2 absorbing (acid violet 17 and acid violet 19) and 2 fluorescent (brilliant sulphaflavine and benzoxanthene yellow) dyes were identified for further study. Ultimately the original fluorescent dyes became unavailable and Acid Yellow 7 (brilliant sulphoflavine) was identified as a suitable substitute. Further comparisons showed that acid violet 19 was less effective than both acid black 1 and acid violet 17. The lighter coloration of marks stained with Acid Violet 19 produced ridge detail with less contrast with the background than the other two dyes.
- 8.2.4 On porous surfaces acid violet 17 proved to be more effective than both the water- and methanol-based formulations of acid black 1, and was very similar in performance to the newly developed water/ethanol/acetic acid (WEAA) formulation of acid black 1.
- 8.2.5 Experiments on a further 24 porous surfaces failed to show conclusively whether one of these dyes was more effective than the other. However, there were some surfaces where one dye performed better than the other. It proved impossible to define before treatment whether the acid violet 17 or the acid black 1 would give greatest contrast.
- 8.2.6 Some of the results of comparing and grading fingerprints developed using acid black 1, acid violet 17 and acid yellow 7 across eight different non-porous surfaces are shown below.

Grade	24 Hours after deposition		2 Weeks after deposition	
	Acid black 1	Acid violet 17	Acid black 1	Acid violet 17
0	0	0	0	0
1	0	0	0	0
2	42	46	46	49
3	35	26	31	20
4	167	160	166	174

Grade	24 Hours after deposition		2 Weeks after deposition	
	Acid black 1	Acid yellow 7	Acid black 1	Acid yellow 7
0	0	0	0	0
1	0	0	0	0
2	50	46	51	70
3	39	40	33	38
4	185	188	191	167

Examples of comparative grading exercises carried out between acid black 1, acid violet 17 and acid yellow 7 on non-porous surfaces including glass, ceramic tile, polymers and metals

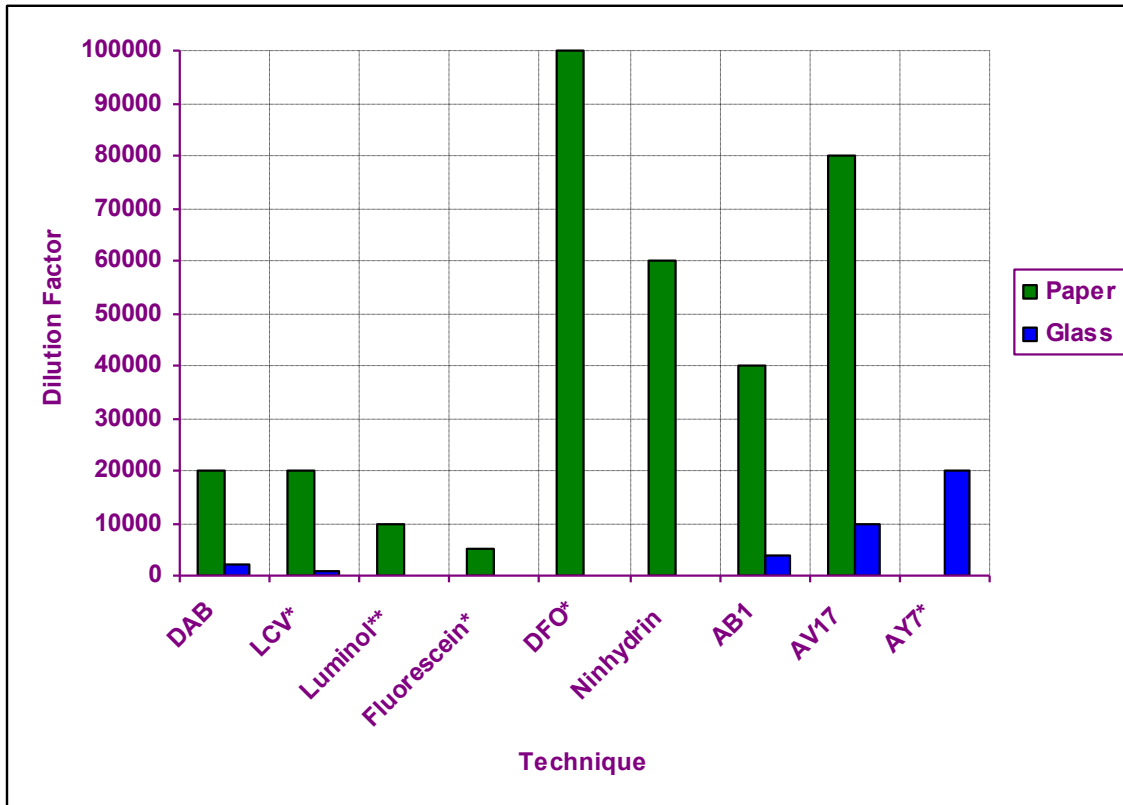
8.2.7 It can be seen that the performance of each of the recommended acid dyes is closely equivalent and the three dyes can be used interchangeably according to which dye will give the best contrast on the particular surface.

8.2.8 The comparative performance of the recommended protein dyes with other types of blood enhancement techniques and with alternative formulations of the same dyes on split depleted fingerprints are shown in the table below.

Technique	Type (H = haem, A = amine, P = protein)	Subjective performance assessment ***** = excellent, * = poor		
		Porous	Semi-porous	Non-porous
DAB	H	**	**	*
LCV	H	**	**	-
Acid Violet 19 + organic acid/peroxide	H	**	**	*
Fluorescein	A	*	*	-
DFO	A	*****	**	-
Ninhydrin	A	****	**	-
SYPRO ruby protein blot stain	A	***	**	**
Acid Black 1 (methanol)	P	***	***	****
Acid Black 1 (water)	P	***	***	**
Acid Black 1 (water/ethanol/acetic acid)	P	****	***	***
Acid Violet 17 (water/ethanol/acetic acid)	P	****	***	***
Acid Yellow 7 (water/ethanol/acetic acid)	P	-	-	*****

Summary table showing subjective overview of the comparative effectiveness of several regularly used blood enhancement agents.

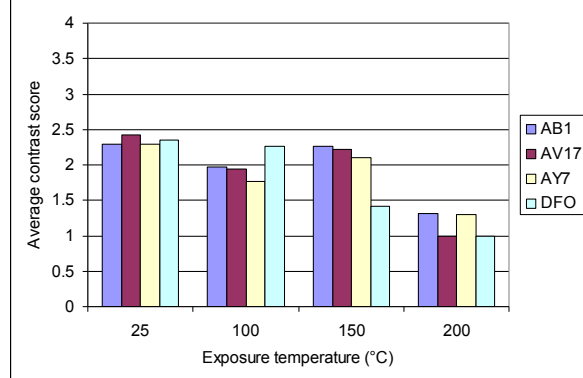
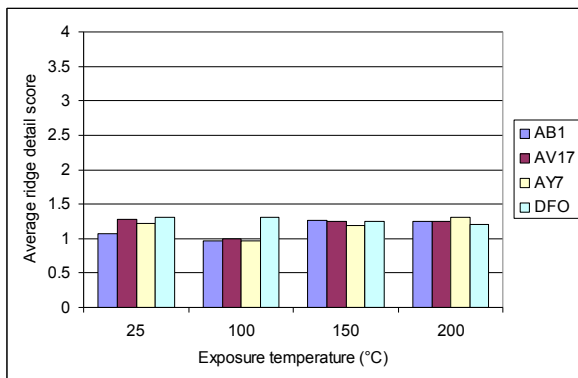
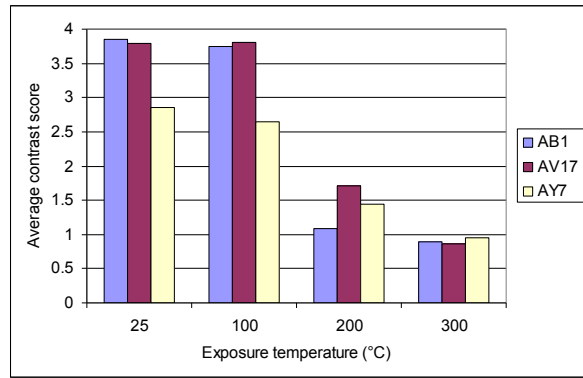
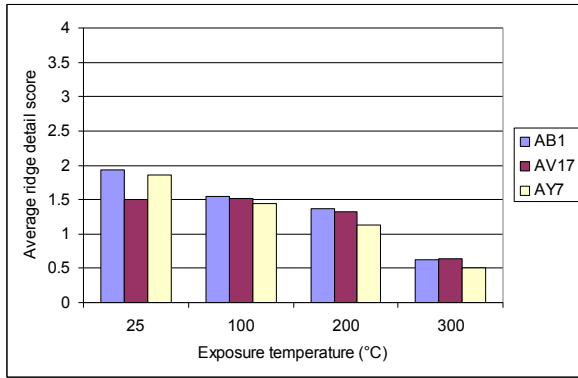
8.2.9 The graph below illustrates the sensitivity of each dye at developing the diluted blood spots on photocopy paper and glass. The sensitivity achieved with diluted blood is not always consistent with the results of experiments with depleted fingerprints, so it is believed that comparative dye performance cannot be measured using dilution series alone. The results below do not take into account the contrast between the stained spots and the background. If spots could be seen then they were counted, even if the contrast between them and the background was very poor.



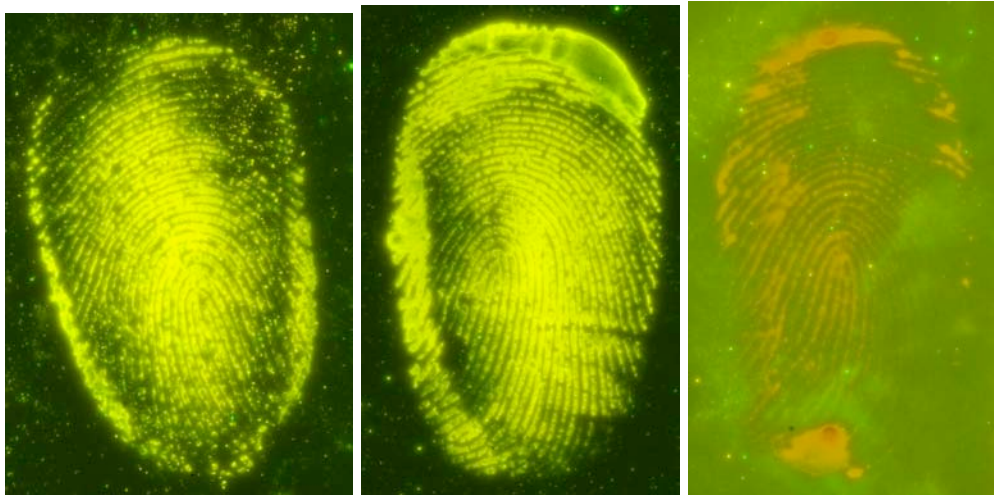
Graph to show the relative performance of various blood enhancement agents in the spot dilution sensitivity test. * = visualised by fluorescence, ** = visualised by chemiluminescence.

8.2.10 It should be noted that the graph above shows the sensitivity of luminol to be relatively poor. This may be because the viewing conditions used were not optimised. Subsequent research to investigate enhancement of footwear marks in blood has shown that dark adaption and optimised viewing conditions are essential and that the sensitivity of luminol may be far greater than is represented here.

8.2.11 Other laboratory trials that have been carried out using the acid dyes include an assessment of the technique's effectiveness on marks in blood that had been exposed to elevated temperatures [53]. In these studies marks were deposited on a range of surfaces and exposed to temperatures in the range 100–300°C for periods between one and eight hours. Marks were graded in terms of both quality and contrast, because it was observed that the contrast of the developed mark decreased as exposure time and temperature decreased.



Recorded results of fingerprint quality and contrast for marks enhanced using blood dyes on a ceramic tile (top row) and white card (bottom row), after exposure to different temperatures.



a)

b)

c)

Series of images for acid violet 17 (top row) and acid yellow 7 (bottom row) showing how quality of developed mark and contrast degrade with increased exposure temperature and exposure time, a) control, b) 8 hours at 100°C and c) 8 hours at 200°C

8.2.12 The results of this study demonstrated that the acid dyes were capable of developing marks exposed to 200°C for eight hours, albeit with reduced effectiveness. Once again, there was little significant difference between the performance of the three recommended dyes. A further important observation from the study was that the haem specific reagent leucocrystal violet had stopped enhancing marks after exposure to temperatures of 150°C, further supporting the recommendation of the acid dyes for operational use in scenarios such as arson scenes.

8.3 Pseudo-operational trials and operational experience

8.3.1 Pseudo-operational trials have not been conducted on the acid dye formulations because this is not practical with articles contaminated with blood. Because the contaminant is known, unlike 'real' fingerprints that are variable in composition, the performance in operational use will be the same as that in laboratory tests. Since the introduction of the new formulation of acid black 1 and the new dyes acid violet 17 and acid yellow 7 in 2005, feedback from operational work has been favourable. Feedback has been especially good for acid yellow 7, which has resulted in, for the first time, the capability of enhancing blood-contaminated fingerprints on dark non-fluorescing surfaces. The new dye has been successfully used to develop marks on exhibits including a black Maglite torch and a dark wood banister, surfaces for which no previous treatment would have been effective. The dyes have also been used for the successful enhancement of footwear marks on large areas of non-porous flooring.

9. References

1. **Teichmann, L.** (1853) 'Ueber die Krystallisation des Orpnischen Be-standtheile des Blutes', *Z. Ration. Med.*, vol. 3, p 375.
2. **Takayama, M.** (1912) 'A Method for Identifying Blood by Hemochromogen Crystallization', *Kokka Igakkai Zasshi*, vol. 306, p 463.
3. **Van Deen, J.** (1862) 'Tinctura Guajaci und ein Ozontrager, als Reagens auf schrgeringe Blutmenge', namentlich in medico-forensischen, *Fallen Arch*, Hollend, *Beitr, Natur-Heilk* vol. 3 (2), p 228.
4. **Schönbein, C.F.** (1863) 'Ueber das Verhalten des Blutes zum Sauerstoff', *Verh Naturforsch Ges Basel*, vol. 3, p 516.
5. **Adler, O. and Adler, R.** (1904) 'Über das Verhalten gewisscr organischer Verindungen gegenüber Blut mit besonderer Berücksichtipng des Nachweises von Blut', *Z. Physiol. Chem.*, vol. 41, p 59.
6. **Medinger, P.** (1933) 'Zum Nachweis minimalster Blutspuren', *Dtsch. Z. Gesamte Gerichtl. Med.*, vol. 20, p 74.
7. **Kastle, J. H. and Shedd O. M.** (1901) 'Phenolphthalin as a reagent for the oxidizing ferments', *Am. Chem. J.*, vol. 26, p 526.
8. **Meyer, E.** (1903) 'Beiträge zur Leukocytenfrage', *Muench Med. Wochenshr*, vol. 50 (35), p 1489.
9. **Kastle, J. H. and Amos H. L.** (1906) 'Variations in the Peroxidase Activity of the Blood in Health and Disease', *US Hygien. Lab. Bull.*, No. 31. USA, Washington DC: Public Health and Marine Hospital Service, US Government Printing Office.
10. **Ruttan, R. F. and Hardisty, R. H. M.** (1912) 'A new reagent for detecting occult blood', *Can. Med. Assoc. J.*, vol. 41 (2), p 995.

11. **Specht, W.** (1937) 'Die Chemiluminescenz des Hämins, ein Hilfsmittel zur Auffindung und Erkennung forensisch wichtiger Blutspunn', *Angew. Chem.*, vol. 50, p 155.
12. **Gershenfeld, L.** (1939) 'Orthotolidine and Orthotoluidine Tests for Occult Blood', *Am. J. Pharm.*, vol. 111, p 17.
13. **Abderhalden, E. and Schmidt, H.** (1911) 'Utilization of Triketohydrindene Hydrate for the Detection of Proteins and Their Cleavage Products', *Zeit. Physiol. Chem.*, vol. 72, p 37.
14. **Oden, S** (1954) 'Detection of Fingerprints by the Ninhydrin Reaction', *Nature*, vol. 173, p 449.
15. **Godsell, J.** (1963) 'Fingerprint Techniques', *J. Forens. Sci. Soc.*, vol. 3 (2), p 79.
16. **Faragher, A. and Summerscales, L.** (1981) *Fingerprint Enhancement Using the Amido Black Technique after Chemical Fixation*, Forensic Science Service UK, Technical Note 240. London: Home Office.
17. **Hussain, J. I. and Pounds, C. A.** (1989) *The Enhancement of Fingerprints in Blood Part II: A Modified Amido Black Staining Technique*, Forensic Science Service UK, HO CRE Report 649, June. London: Home Office.
18. **Bowman, V. (ed) Bowman, V. (ed)** (1998 (2004 revision)) *Manual of Fingerprint Development Techniques*, 2nd edition. ISBN 1 85893 972 0. London: Home Office.
19. **Sears, V. G. and Prizeman, T. M.** (2000) 'The Enhancement of Fingerprints in Blood – Part 1: The Optimization of Amido Black', *J. Forens. Ident.*, vol. 50 (5), p 470.
20. **Norkus, P. and Noppinger, K.** (1986) 'New Reagents for the Enhancement of Fingerprints in Blood,' *Ident. News*, April, p 5.
21. **Barnett, K. G., Bone, R. G., Hall, P. W. and Ide, R. H.** (1988) *The Use of Water Soluble Protein Dye for the Enhancement of Footwear Impressions in Blood on Non-Porous surfaces Part 1*, Forensic Science Service UK, Technical Note No. 629, July. London: Home Office.
22. **Sears, V. G., Butcher, C. P. G. and Prizeman, T. M.** (2001) 'The Enhancement of Fingerprints in Blood – Part 2: Protein Dyes,' *J. Forens. Ident.*, vol. 51(1), p 28.
23. **Sears, V., Butcher, C. and Fitzgerald, L.** (2005) 'Enhancement of Fingerprints in Blood – Part 3: Reactive Techniques, Acid Yellow 7 and Process Sequences,' *J. Forens. Ident.*, vol. 55 (6), p 741.
24. **Garner, D. D., Cano, K. M., Peimer, R. S. and Yeshion, T. E.** (1976) 'An Evaluation of Tetramethylbenzidine as a Presumptive Test for Blood,' *J. Forens. Sci.*, Oct, vol. 21 (4), p 816.
25. **Hussain, J. I. and Pounds, C. A.** (1989) *The Enhancement of Marks Made in Blood with 3,3',4,4'-Tetraaminobiphenyl*, Forensic Science Service UK, CRSE Report 653. London: Home Office.
26. **Cheeseman, R. and DiMeo, L. A.** (1995) 'Fluorescein as a Field-worthy Latent Bloodstain Detection System,' *J. Forens. Ident.*, vol. 45 (6), p 631.

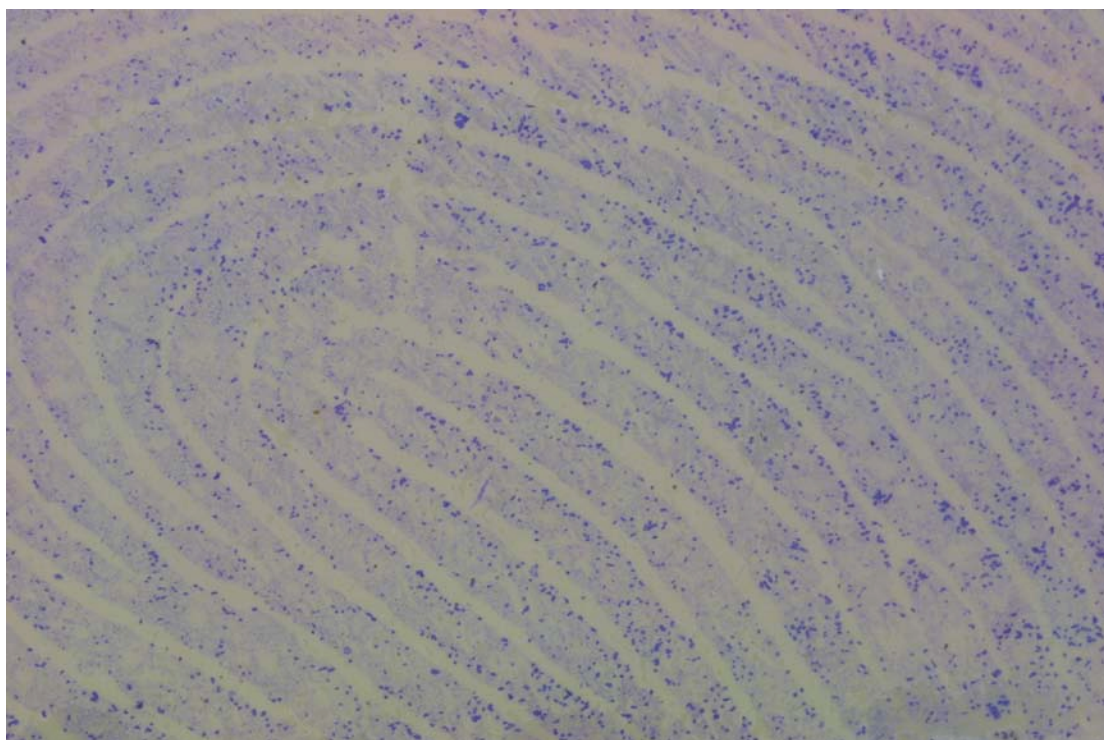
27. **Bodziak, W. J.** (1996) 'Use of Leuco-Crystal Violet to Enhance Shoeprints in Blood,' *Forens. Sci. Int.*, vol. 82, p 45.
28. **Crown, D. A.** 1969 'The Development of Latent Fingerprints with Ninhydrin,' *J. Crim. Law Crim. Police Sci.*, vol. 60 (2), p 258.
29. **Morris, J. R. and Goode, G. C.** (1974) 'NFN an improved Ninhydrin Reagent for the Detection of Latent Fingerprints', *Police Res. Bull.*, (24), p 45.
30. **Watling, W. J. and Smith, K. O.** (1993) 'Heptane an Alternative to the Freon Ninhydrin Mixture,' *J. Forens. Ident.*, vol. 43 (2), p 131.
31. **Hewlett, D. F., Sears, V. G. and Suzuki, S.** (1997) 'Replacements for CFC113 in the ninhydrin process part 2,' *J. Forens. Ident.*, vol. 47 (3), p 300.
32. **Morris J. R.** (1978) *Extensions to the NFN (Ninhydrin) Reagent for the Development of Latent Fingerprints*, SSCD Memorandum, CRP Work Item 41A, February. Aldermaston: Atomic Weapons Research Establishment.
33. **Everse, K. E. and Menzel, E.R.** (1986) 'Sensitivity Enhancement of Ninhydrin – Treated Latent Fingerprints by Enzymes and Metal Salts,' *J. Forens. Sci.*, vol. 31, p 446.
34. **Stoilovic, M., Kobus, H. J., Margot, P. A. and Warrenner, R. N.** (1986) 'Improved Enhancement of Ninhydrin Developed Fingerprints by Cadmium Complexation using Low Temperature Photoluminescence Techniques,' *J. Forens. Sci.*, vol. 31, p 432.
35. **Almog, J., Hirshfield, A. and Klug, J. T.** (1982) 'Reagents for the Chemical Development of Latent Fingerprints: Synthesis and Properties of some Ninhydrin Analogues,' *J. Forens. Sci.*, vol. 27 (4), p 912.
36. **Almog, J. and Hirshfield, A.** (1988) '5-methoxyninhydrin: A Reagent for the Chemical Development of Latent Fingerprints that is Compatible with the Copper Vapour Laser,' *J. Forens. Sci.*, vol. 33 (4), p 1027.
37. **Grigg, R., Mongkolaussavaratana, T., Pounds, C. A. and Sivagnanam, S.** (1990) '1,8-Diazafluorenone and Related Compounds. A new Reagent for the Detection of α -Amino Acids and Latent Fingerprints,' *Tetrahedron Letters*, vol. 31 (49), p 7215.
38. **Ramotowski, R., Cantu, A. A., Joullie, J. and Petrovskaia, O.** (1997) '1,2 Indandiones: A Preliminary Evaluation of a New Class of Amino Acid Visualizing Compounds,' *Fingerprint Whorld*, vol. 23, p 131.
39. **Hochmeister, M. N., Budowle, B., Sparkes, R., Rudin, O., Gehrig, C., Thali, M., Schmidt, L., Cordier, A. and Dirnhofer, R.** (1999) 'Validation Studies of an Immunochromatographic 1-Step Test for the Forensic Identification of Human Blood,' *J. Forens. Sci.* vol. 44 (3), p 597.
40. **Johnston, E., Ames, C. E., Dagnell, K. E., Foster, J. and Daniel, B. E.** (2008) 'Comparison of Presumptive Blood Test Kits including Hexagon OBTI,' *J. Forens. Sci.*, vol. 53 (3), p 687.
41. **Hoppe, F.** (1862) 'Uebcr das Verhalten des Blutfarbstoffes in Spectrum des Sonnenlichtes,' *Arch. Pathol. Anat. Physiol. Klin. Med.*, vol. 23 (4), p 446.
42. **Stokes, G. G.** (1864) 'On the reduction and oxidation of the colouring matter of the blood,' *Proceedings of the Royal Society*, London, vol. 13, p 355.

43. **Soret, J. L.** (1883) 'Analyse Spectrale: Sur le Spectre d'Absorption du Sang Dans la Partie violette et Ultra-Violette,' *Comptes Rendus de l'Académie des Sciences*, vol. 97, p 1269.
44. **Sorby, H.C.** (1865) 'On the Application of Spectrum Analysis to Microscopical Investigations, and Especially to the Detection of Bloodstains,' *Q. J. Sci.*, 2, p 198.
45. **Hardwick, S. A., Kent, T. and Sears, V. G.** (1990) *Fingerprint Detection by Fluorescence Examination – A Guide to Operational Implementation*, ISBN 0 86252 554 3. London: Home Office.
46. **Kotowski, T. M. and Grieve, M. C.** (1986) 'The Use of Microspectrophotometry to Characterize Microscopic Amounts of Blood,' *J. Forens. Sci.*, vol. 31 (3), p 1079.
47. **Stoilovic, M.** (1991) 'Detection of Semen and Blood Stains Using Polilight as a Light Source', *Forens. Sci. Int.*, vol. 51, p 289.
48. **Olsen, R. D.** (1985) 'Sensitivity Comparison of Blood Enhancement Techniques,' *Ident. News*, vol. 35 (8), p 10.
49. **Cheeseman, R.** (1999) 'Direct Sensitivity Comparison of the Fluorescein and Luminol Bloodstain Enhancement Techniques,' *J. Forens. Ident.*, vol. 49 (3), p 261.
50. **Theeuwens, A. B. E., van Barneveld, S., Drok, J. W., Keereweer, I., Limborgh, J. C. M., Naber, W. M. and Velders, T.** (1998) 'Enhancement of Footwear Impressions in Blood,' *Forens. Sci. Int.*, vol. 95, p 133.
51. **Christie, R. M., Mather, R. R. and Wardman, R. H.** (2000) *The Chemistry of Colour Application*, ISBN 0-632-04782-8, pp 19–20. Oxford: Blackwell Science Ltd.
52. **Bandey, H.** (2008) *Fingerprint and Footwear Forensics Newsletter*, HOSDB Publication No. 24/08, May. London: Home Office.
53. **Moore, J. E, Bleay, S. M., Deans, J. J. and Nic Daeid, N.** (2008) 'Recovery of fingerprints from arson scenes: Part 2 Fingerprints in blood', *J. Forens. Ident.*, vol. 58 (1), p 54–82

3.2 Basic violet 3 (Gentian Violet)

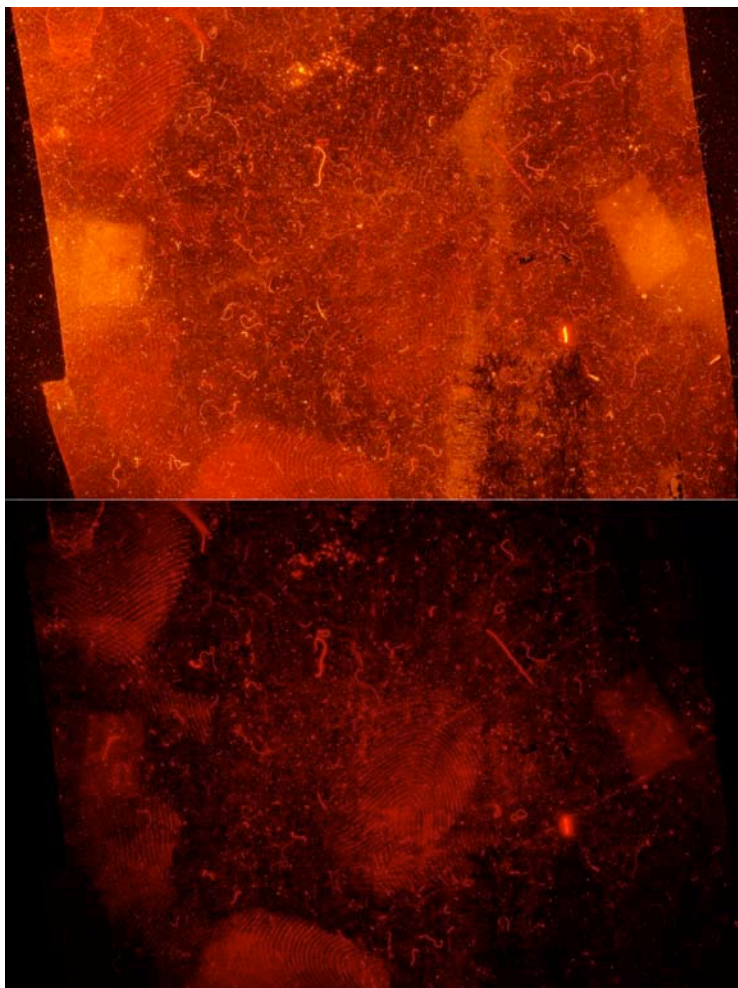
1. History

- 1.1 The history of basic violet 3 begins with the discovery of the first synthetic dye, 'Mauve', by W. H. Perkin in 1856. In the following years a series of aniline dyes were synthesised for the dyeing of textiles, including methyl violet (basic violet 1) by Lauth in 1861 [1]. A range of closely related compounds were subsequently synthesised including basic violet 3 (also known by several alternative names including gentian violet and crystal violet).
- 1.2 The applications of these dyes were not confined to the textile industry and microbiologists began to explore the potential of synthetic dyes for the staining of biological sections. The German biologist Paul Ehrlich used aniline water and gentian violet to stain bacteria cells, the gentian violet targeting the lipids in the cell walls to give a purple stain. In 1884 the Danish physician Hans Christian Joachim Gram further developed this staining process for selectively staining bacteria and providing information about the structure of the cell walls. The test is still known as Gram staining to this date. Basic violet 3 has since been used for a variety of medical applications, including treatments for ringworm and scabies, where the ability of the dye to inhibit bacterial action is beneficial.
- 1.3 Aniline dyes (of which basic violet 3 is one) have been proposed as fingerprint reagents since the early part of the 20th century. In 1917 Bock [2] patented a process for recording latent fingerprints by brushing the fingerprint with a powder of aniline dye and then fixing the mark by heating. In 1920 Mitchell was reporting the use of aniline dyestuffs in powder form as a means of detecting fingerprints [3], with the observation that basic dyestuffs were preferable.
- 1.4 As research work into the constituents of fingerprints progressed in the 1960s, reagents were proposed that targeted particular components of fingerprint residues. Basic violet 3 was proposed as a technique for the selective staining of epithelial cells and fatty components of fingerprint residues. Epithelial cells are most likely to be present on the adhesive side of tapes, where a layer of dead cells may be pulled off the fingerprint ridges when the tape is touched. The use of basic violet 3 in this application was reported by the Italian Police in the late 1960s and it their recommended phenolic formulation was adopted by PSDB and some forces in the UK during the late 1970s [4,5].



Photograph of adhesive side of tape sample treated with basic violet 3, showing violet staining of lipids in ridges and of epithelial cells in particular.

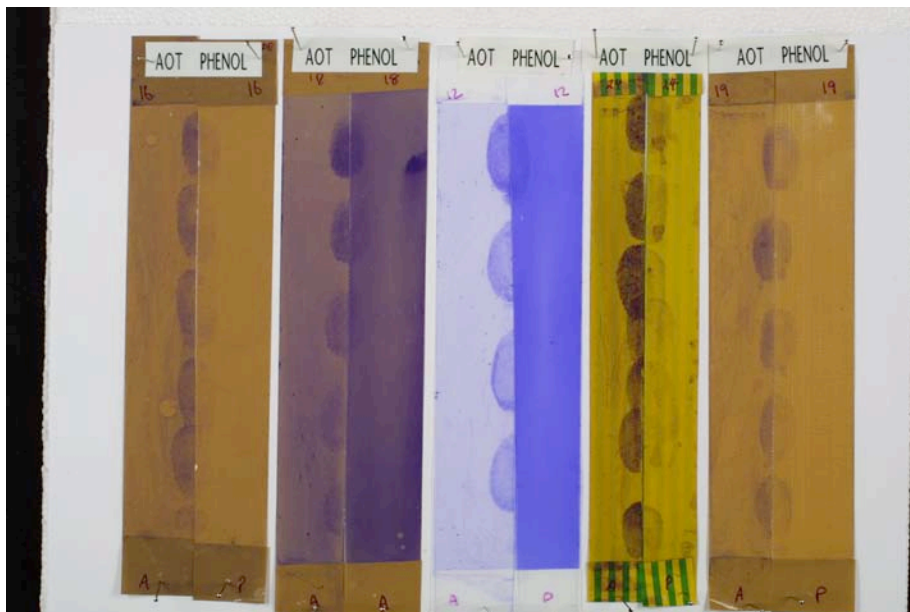
- 1.5 Basic violet 3 continued to be used worldwide for development of latent fingerprints on adhesive surfaces [6] but although good results were obtained for a wide range of tapes the detection of marks on black tapes remained problematic, the only technique available for visualisation being photography under oblique lighting. This was overcome by the development of the transfer process by the Police Scientific Development Branch (PSDB) [7,8] and others [9]. This process involved the sandwiching of the tape between sheets of photographic paper, resulting in the transfer of the purple stain from the developed fingerprint to the surface of the white paper.
- 1.6 It has been found that marks developed using basic violet 3 on adhesive tapes are also fluorescent, and can be visualised using green/yellow light to excite the fluorescence and a deep red viewing filter [10]. It was found that the fluorescence had a peak at 720nm in the deep red region of the spectrum and extended to a small degree into the near infra-red region [11]. More recently, yellow (577nm) lasers have become commercially available and studies have shown that this gives excellent results when used to image fluorescent marks developed using basic violet 3 [12].



Photograph of basic violet 3 fluorescence in fingerprints developed on adhesive tape, imaged using a 5W 532nm green laser (top) and a 5W 577nm yellow laser (bottom)

- 1.7 Basic violet 3 can also be used for detection of fingerprints on a wide range of non-porous surfaces and can be especially useful where contamination may be present on the surface.
- 1.8 The work carried out by CAST on basic violet 3 includes the development of the transfer process for black tapes in the late 1970s. More recently, concerns about the toxicity of phenol have prompted in-depth studies into the development of an effective phenol-free formulation for basic violet 3 [13] and a comparative study between basic violet 3 and a possible alternative dye, basic violet 2 [14]. These studies have culminated in the recent issue of a revised formulation of basic violet 3 based on Aerosol OT™ (AOT), also known by its chemical name of dioctyl-sulfosuccinate, sodium salt [15], which in laboratory trials has consistently out-performed the phenol formulation in terms of number, quality and contrast of marks developed, and has exhibited a reduced amount of background staining. However, recent reclassification of chemicals has resulted in basic violet 3 itself being classed as a suspect

carcinogen and both phenol and AOT-based formulations must be used under controlled conditions.

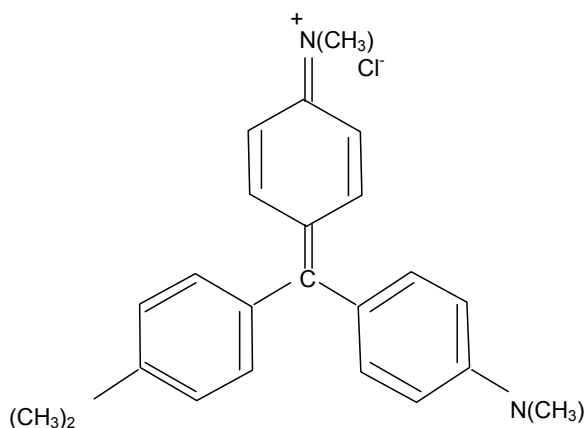


Photograph of different adhesive tapes, showing difference in fingerprint development between phenol and Aerosol OT-based basic violet 3 formulations.

2. Theory

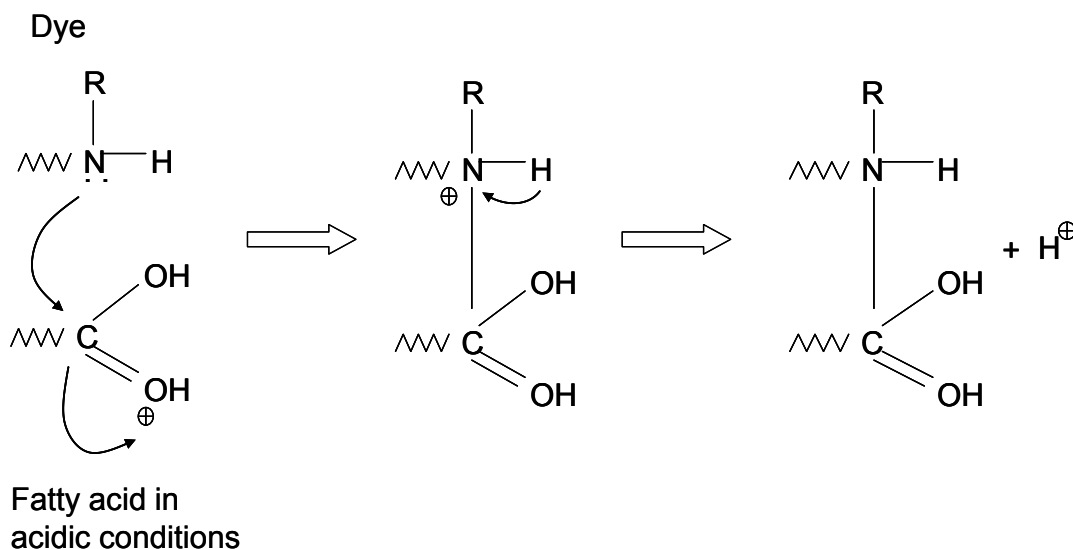
2.1 The exact mechanism by which basic violet 3 selectively dyes fingerprint deposits is not known, nor has it been determined which individual fingerprint constituents are targeted by the dye. However, there are two mechanisms that have been proposed for the interaction of the basic violet 3 dye molecule with the lipids in the fingerprint.

2.2 The basic violet 3 molecule is shown below:



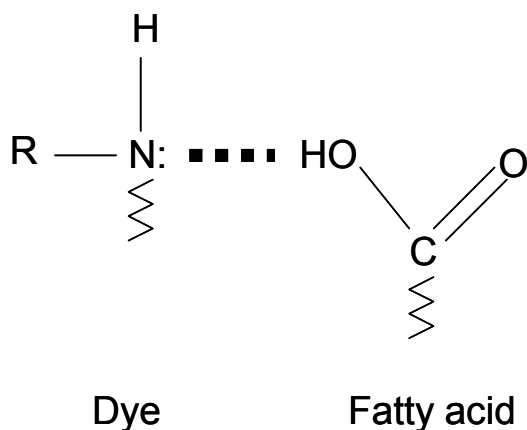
The basic violet 3 molecule.

- 2.3 Gurr [16] proposes that the basic groups such as amines (NH₂, or in this case NH-R) of neutral dyes such as basic violet 3 could form a chemical union with the acidic group of the lipids being stained. It is thought that the staining action occurs via a reaction between the amine group of the dye and the acidic group of a lipid component (such as a fatty acid). The possible reaction is shown below.



Possible reaction mechanism for staining action of basic violet 3.

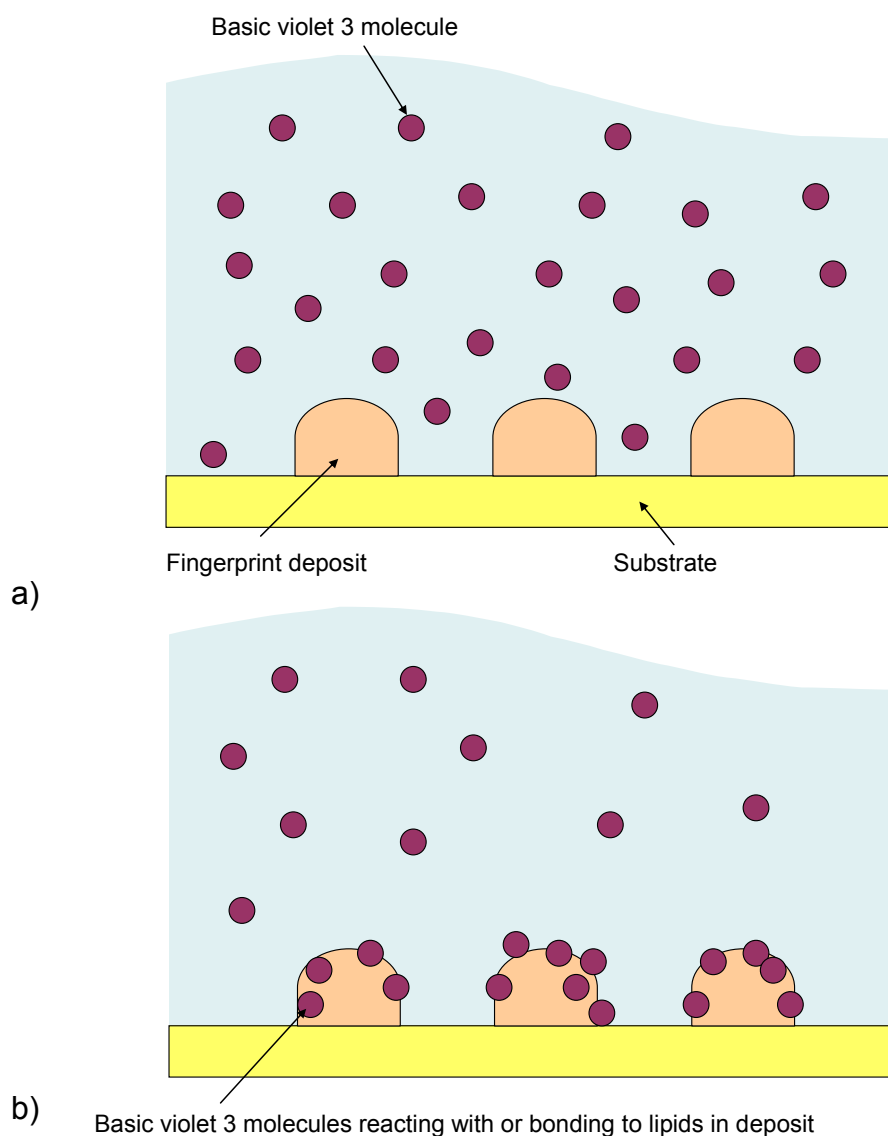
- 2.4 Another proposal is that the dye could link to fatty acids by the formation of a hydrogen bond between the nitrogen in basic violet 3 and the hydroxyl group in the fatty acids, as shown below.

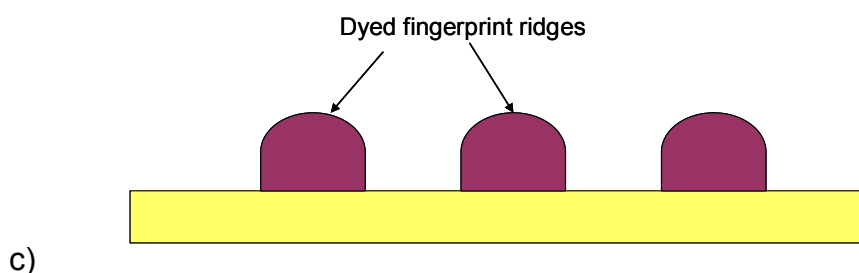


Alternative reaction mechanism for staining action of basic violet 3.

- 2.5 Both the mechanisms are applicable to the phenolic water solution used in the basic violet 3 formulation currently (as at 2011) recommended in the *Manual of Fingerprint Development Techniques* [17].

- 2.6 It is also known that the basic violet 3 molecule is fluorescent, but when basic violet 3 is used as a development reagent on non-porous surfaces fluorescence is not observed in most cases. However, on adhesive tapes fluorescence is observed, and weak marks that are not visible under conventional lighting may be revealed by these means. The fluorescence observed on adhesive surfaces is attributed to the fact that for fluorescence to occur the structure of the compound must be rigid [18]. It is thought that the adhesive promotes fluorescence by binding with the dye molecule and making it more rigid. This theory has been investigated by spraying non-fluorescent marks developed using basic violet 3 with spray adhesive. In these studies a significant increase in fluorescence was observed [13]. It is thought that additional marks are revealed by fluorescence examination because the more strongly developed, visible marks 'self quench', i.e. the dye absorbs the fluorescence from the fingerprint, whereas for the weakly coloured marks that are not visible by eye the fluorescence is not re-absorbed and the marks are detected.
- 2.7 The fingerprint development process using basic violet 3 is shown schematically in the series of figures below.





c)

Schematic illustration of the basic violet 3 development process a) basic violet 3 molecules in solution b) basic violet 3 molecules preferentially binding to skin cells and lipids in fingerprint ridges and c) dried mark leaving dyed fingerprint ridges.

3. CAST processes

- 3.1 There are two formulations recommended for use by CAST, one based on phenol and the other based on AOT.
- 3.2 The phenol formulation is produced by first mixing a stock solution comprising 5g basic violet 3 and 10g of phenol dissolved into 50mL of 96% ethanol.
- 3.3 A working solution is produced by measuring 1mL of stock solution and progressively adding distilled water until the gold film formed on the surface of the solution disappears.
- 3.4 The role of basic violet 3 in the formulation is to selectively stain the fingerprint deposits. The quantity used is sufficient to produce a supersaturated solution of basic violet 3, thus promoting the transfer of the dye into the lipids in the fingerprint.
- 3.5 The role of phenol in the formulation is not fully understood. The presence of phenol has been found to promote the staining ability of basic violet 3 and appears to make it more specific to fingerprint constituents. Several theories have been proposed [13], including:
 - the pH change due to the addition of the mildly acidic phenol aids staining;
 - phenol aids the wetting of the lipids;
 - phenol increases the solubility of the dye, forming a supersaturated solution;
 - phenol replaces the dye anion forming a phenolate, which acts as a dye carrier and aids penetration of the fats;
 - phenol disaggregates dye molecules, increasing their diffusion rates.
- 3.6 Experiments have been carried out to investigate some of these theories and while these did not provide conclusive evidence it is thought more

likely that phenol acts by affecting the solution properties, either making it supersaturated or by changing its surface tension and increasing staining.

- 3.7 The ethanol component of the formulation provides a common solvent for both phenol and basic violet 3.
- 3.8 The AOT formulation of basic violet 3 is produced by first producing a stock solution by dissolving 5g of basic violet 3 in 50mL of absolute ethanol. A separate 1% w/v AOT solution is then produced by dissolving AOT in distilled water, stirring for at least 12 hours to allow the AOT to dissolve. The working solution is produced by placing 1mL of concentrated stock solution into a clean, dry beaker, then adding 25mL of AOT solution.
- 3.9 Similarly to phenol, the role of AOT in the formulation is not fully understood. AOT is an unusual detergent, being preferentially soluble in non-polar solvents and forming reverse micelles. One theory is that basic violet 3 molecules could become contained within the reverse micelles, which are in turn preferentially soluble in the fingerprint lipids compared with the polar water/ethanol solution [13].

4. Critical issues

- 4.1 Basic violet 3 is classified as being carcinogenic and phenol (a major constituent in one of the formulations) is mutagenic. Although the solution can be used safely in a laboratory environment if the procedures outlined in the *Manual of Fingerprint Development Techniques* [17] are followed, it should not be used in the uncontrolled environment of a crime scene.
- 4.2 If a gold film forms on the surface of the basic violet 3 working solution it should be discarded because this may give a high background staining on the surface being treated.
- 4.3 In general strongly stained fingerprints either do not fluoresce or fluoresce weakly, this is believed to be due to quenching effects. Fluorescence is therefore most valuable for detection of weakly stained fingerprints. However, this means that on dark tapes strongly dyed fingerprints may be missed unless a transfer technique is used.

5. Application

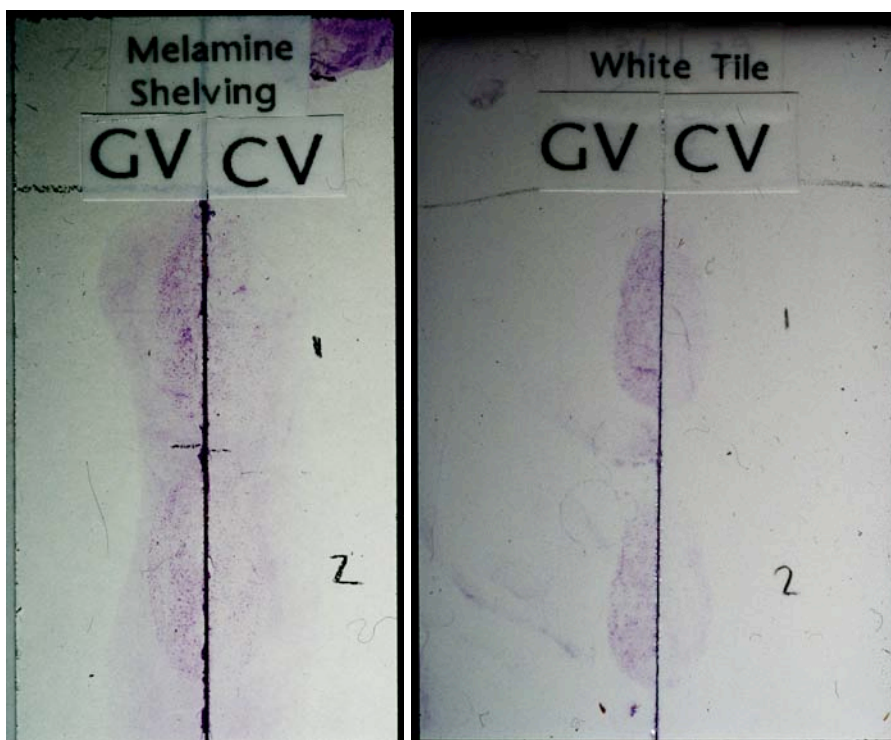
- 5.1 Suitable surfaces: Basic violet 3 is suitable for use on the adhesive side of adhesive tapes and on surfaces contaminated with fats. It is also suitable for use on all non-porous surfaces as the final process in a sequential treatment. Its use should be restricted to small articles because of issues with carcinogenicity of the solution.

- 5.2 The principal application for basic violet 3 is in the development of fingerprints on the adhesive side of adhesive tapes, where it can be used as a single treatment or in sequence to develop additional marks after powder suspensions or superglue [19].
- 5.3 The AOT-based basic violet 3 formulation can be used on tapes of any colour and also on tapes with both acrylic and rubber-based adhesive. However, if used as a single treatment, laboratory trials indicate that it is less effective than powder suspensions and superglue and it is more appropriate for use as part of a sequential processing regime.
- 5.4 Basic violet 3 is also recommended as a treatment for contaminated surfaces, where its specificity as a lipid dye may be capable of selectively dyeing the fingerprint ridges without background staining of the contaminant. This is only recommended for small articles because of the toxicity issues associated with the phenol-based formulation. Solvent black 3 can be considered as an alternative treatment for contaminated surfaces and although laboratory trials indicate that solvent black 3 may be more effective than basic violet 3 on latent prints, the most effective treatment on contaminated surfaces has not been conclusively identified.
- 5.5 Basic violet 3, used in the form of the Forensic Science Service (FSS) crystal violet formulation, see below, has also been proposed as a treatment for soot-covered articles retrieved from arson scenes, where the phenol in the formulation was believed to assist in lifting surface soot and developing the fingerprint. More recent experiments indicate that other chemical treatments and soot removal techniques may be more effective in this application [20].
- 5.6 Most recently, studies on plastic packaging materials show that basic violet 3 will develop additional marks if used as the final stage in a sequential treatment regime, and it is now recommended for these purposes on plastic packaging and non-porous surfaces.

6. Alternative formulations and processes

- 6.1 An alternative composition based on basic violet 3 is used in the UK by the FSS [21]. This formulation (known as the FSS crystal violet formulation) consists of the following:
 - 50g of basic violet 3 dissolved into 2.5 litres of ethanol (min. 95% assay) to form a stock solution;
 - 200ml of stock solution added to 4.8 litres of water to form a working solution.
- 6.2 This formulation was tested against the phenolic formulation in the *Manual of Fingerprint Development Techniques* [17] on a range of substrates, including clear, black and white polythene sheet, laminate,

ceramic tiles, melamine and white hardboard using split depletion series. In this comparison [22] it was found that the formulation in the manual produced stronger staining and more ridge detail than the FSS crystal violet formulation.



Images showing relative effectiveness of Home Office Centre for Applied Science and Technology basic violet 3 formulation (GV) and Forensic Science Service crystal violet formulation (CV) on non-porous surfaces.

6.3 CAST has also conducted an extensive evaluation of alternatives to phenol in the formulation, including disinfectants and antibacterial agents, substances with similar chemical structures, properties or functional groups, detergents and surfactants, and substances used as phenol replacements in other formulations [13]. These are summarised in the table below.

Disinfectants / antibacterials	Similar structure, properties, functional groups	Detergents / surfactants	Other phenol replacements
Hexachlorophene	Cyclohexanol	Aerosol OT (AOT)	Ammonium hydroxide
Benzalkonium Chloride	Phenylalanine	Aerosol 22	Sodium chloride
Cetrimide	Asparagine	1-pentane sulfonic acid	Ammonium oxalate
Chlorhexidine	Arginine	1-hexane sulfonic acid	Pyridoxamine.2HCl

Chlorhexidine diacetate monohydrate	L-Ascorbic acid	1-heptane sulfonic acid	Pyridoxine.HCl
Chlorhexidine digluconate	Salicylic acid	1-octane sulfonic acid	Pyridoxal.HCl
2,4,6-Trichlorophenol	Sulfosalicylic acid	1-decane sulfonic acid	
	Oxalic acid	Cholic acid	
		Deoxycholic acid	
		Aurocholic acid	
		Dehydrocholic acid	
		Alginate acid	
		Caprylic acid	
		N-Lauryl Sarcosine	
		LOC High Suds	
		Arylan PWS	

Alternatives to phenol investigated for basic violet 3 formulations.

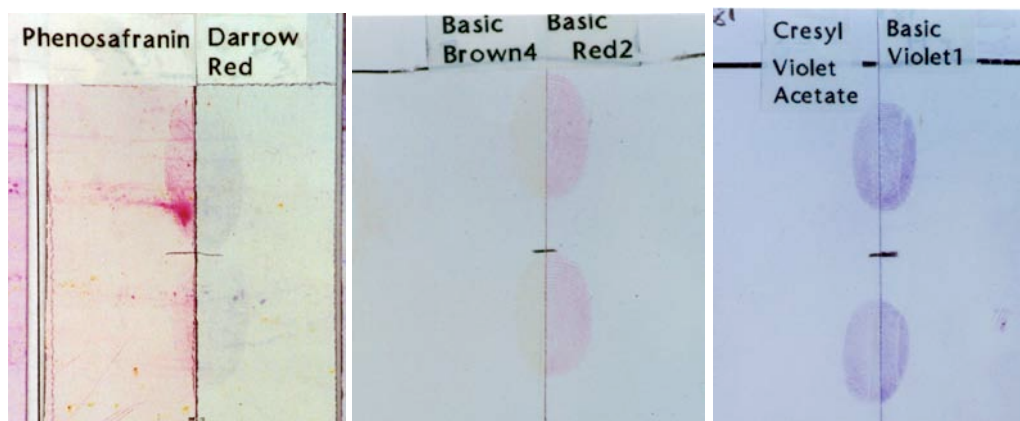
- 6.4 Of these, only AOT gave performance equivalent to or better than the phenol-based formulation and hence was the only compound considered in further, more focused studies. These subsequent studies [14,23] confirmed the observation that the AOT formulation consistently outperformed the phenol formulation in laboratory trials and this formulation was ultimately recommended for operational use on adhesive tapes after a brief operational trial.
- 6.5 CAST has also assessed a wide range of alternative lipid dyes, some water soluble, some ethanol soluble and some soluble in both solvents. In addition, some other dyes containing NH₂ groups were also evaluated because this characteristic appeared to be important in the staining of fingerprints. The full list of dyes evaluated is given in the table below.

Common name	Colour Index name	Colour Index number
2,7-Dichlorofluorescein	–	–
Basic fuschin	Basic red 9	42500
Bismark brown R	Basic brown 4	21010
Cresyl violet acetate	–	–
Darrow red	–	–
Indophenol blue	–	49700
Lucifer yellow CH	–	–
Methyl violet	Basic violet 1	42535
Methylene blue	Basic blue 9	52015
Neutral red	Basic red 5	50040
New fuschin	Basic violet 2	42520
Nigrosin	Solvent black 5	50415
Nile blue Chloride	Basic blue 12	51180

Nile red	–	–
Oil blue N	Solvent blue 14	61555
Oil red O	Solvent red 27	26125
Phenosafranin	–	50200
Primulin	Direct yellow 59	49000
Pyronine B	–	45010
Rose engal	Acid red 94	45440
Safranin O	Basic red 2	50240
Sudan green 4B	Solvent green 3	61565
Sudan III	Solvent red 23	26100
Sudan orange G	Solvent orange 1	11920
Solvent violet R	Disperse violet 1/solvent violet 11	61100
Thiazol yellow G	Direct yellow 9	19540
Thionin	–	52000
–	Acid black 48	65005

Dyes investigated as possible alternatives to Basic Violet 3.

6.6 Of this selection of dyes, basic red 5, direct yellow 59, phenosafranin, basic red 2, cresyl violet acetate, basic violet 2 and basic violet 1 were considered worthy of further investigation. Optimised formulations based on basic violet 2 were ultimately developed, but in comparative trials with an experimental formulation of basic violet 3, the basic violet 2 formulation was found to be less effective [14].



Examples of some of the split depletion experiments conducted using alternative lipid dyes.

7. Post-treatments

7.1 The principal post-treatment used for fingerprints treated with basic violet 3 is the transfer process [7], used for fingerprints on the adhesive side of dark tapes where the violet colour of the dye cannot be seen. In this process the tape is placed in contact with the glossy surface of photographic paper and pressed. Dye is transferred to the surface of the

white paper and the violet dye can be easily visualised. Another advantage of this process is that the fluorescence of the marks is generally increased because the concentration of the dye transferred is less than that present in the original developed fingerprint, reducing the self-quenching effect of the dye.

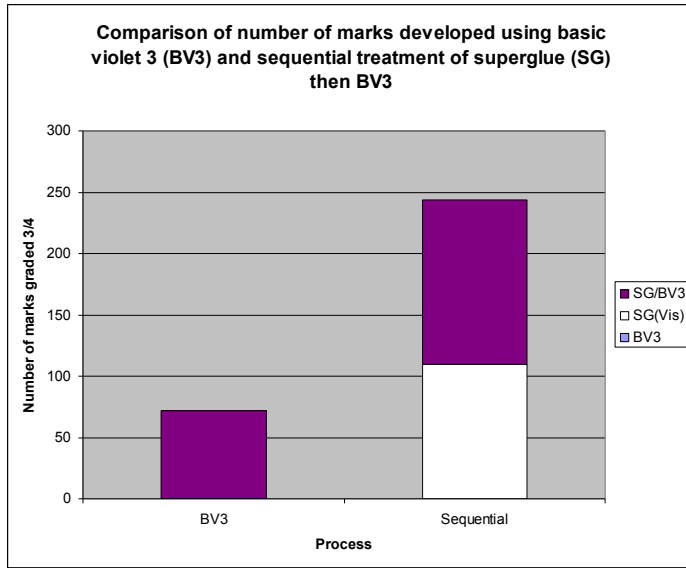
8. Validation and operational experience

8.1 Laboratory trials

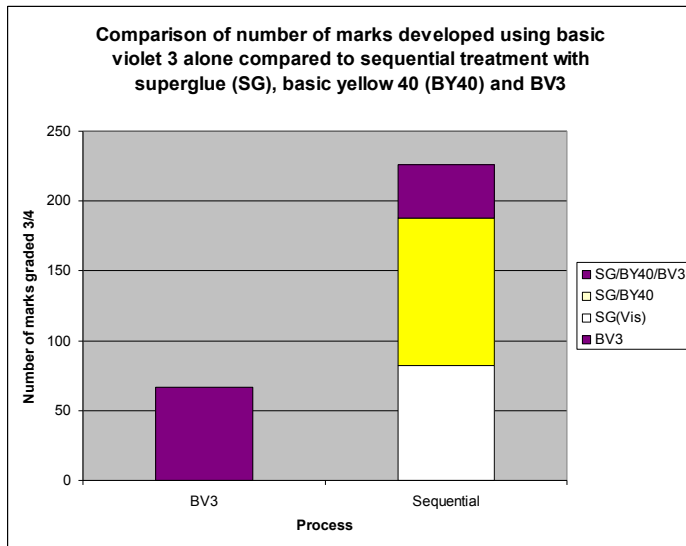
8.1.1 Extensive laboratory trials have indicated the following.

- Basic violet 3 alone is not the most effective treatment for any type of adhesive tape.
- Basic violet 3 can be effectively used as a final sequential treatment for adhesive tapes after either superglue or powder suspensions.
- The AOT-based basic violet 3 formulation appears to develop better quality fingerprints with better contrast, causes less background staining and has fewer health and safety issues associated with it than the phenol-based formulation.
- Basic violet 3 can also be used on contaminated, non-porous surfaces but it has not been conclusively shown whether basic violet 3 or solvent black 3 (or indeed iodine or powder suspensions) are the optimum treatment in these circumstances. Basic violet 3, solvent black 3 and iodine all stain fats, whereas powder suspensions will not stain fats but will develop latent fingerprints laid on contaminated surfaces.

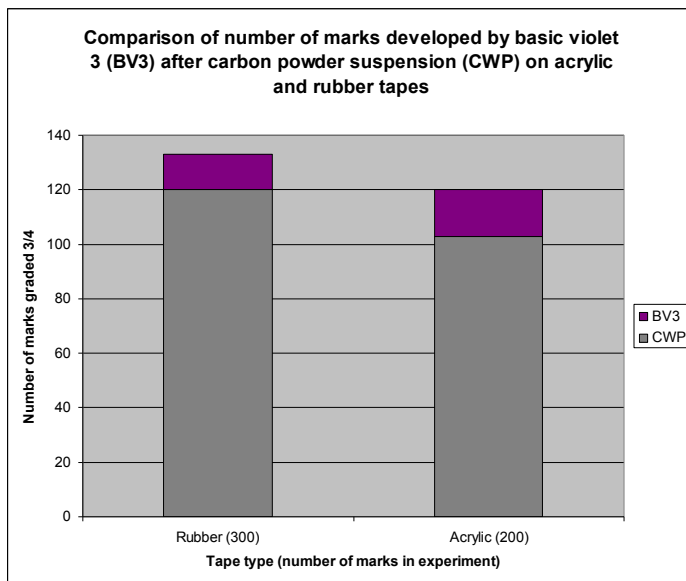
8.1.2 The experiments conducted to support the statements above are summarised below. During studies on optimum treatments for adhesive tapes, basic violet 3 was compared with superglue as a single treatment, and as a secondary treatment after superglue. It was also investigated as a secondary treatment after carbon-based powder suspensions. In total, over 1,000 marks were graded during this study. The results are shown graphically below.



a)



b)

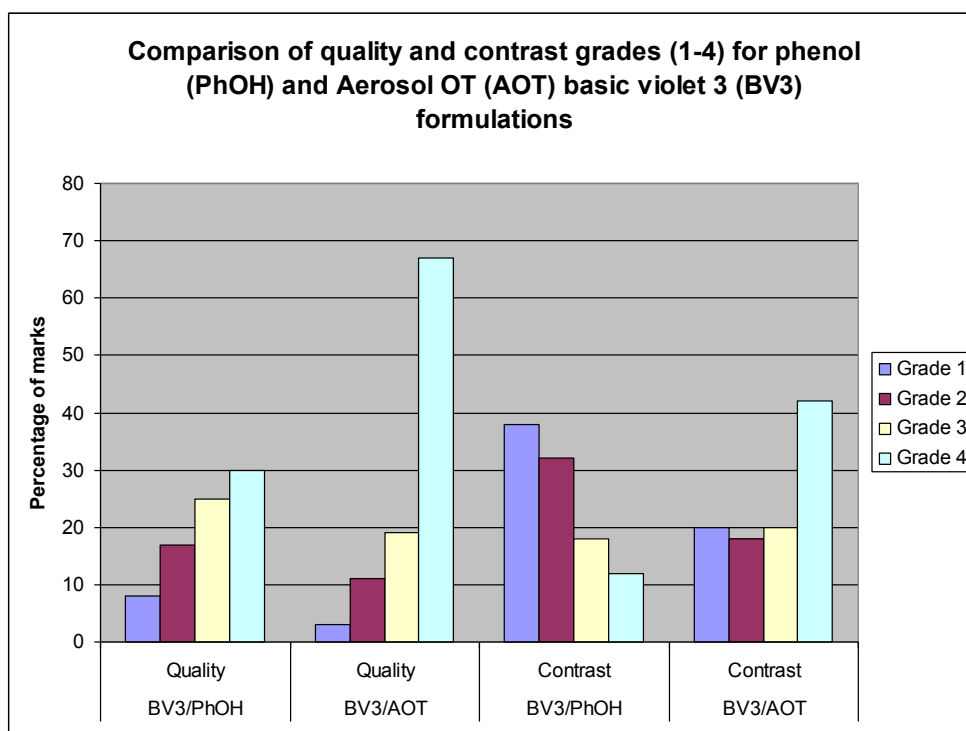


c)

Graphs showing additional marks developed using basic violet 3 (BV3) in sequence on adhesive tapes a) comparison of BV3 alone with superglue (SG) followed by BV3 b) comparison of BV3 alone with superglue followed by basic yellow 40 (BY40), followed by BV3 and c) BV3 after carbon powder suspension (CWP) on both acrylic and rubber-based adhesives.

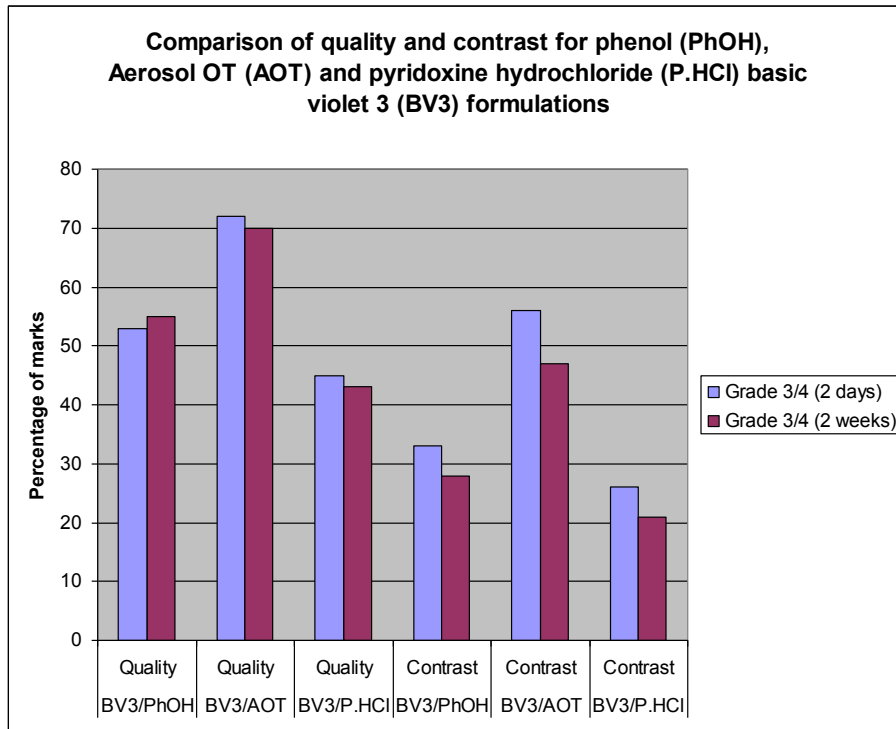
8.1.3 It can be seen that basic violet 3 is not as effective as superglue, but does develop additional marks after both superglue and powder suspensions on adhesive tapes.

8.1.4 Research into phenol replacements evaluated a range of surfactants, of which AOT gave performance equivalent to, or better than, the phenol-based formulation and hence was the only compound considered in further, more focused studies on adhesive tapes.

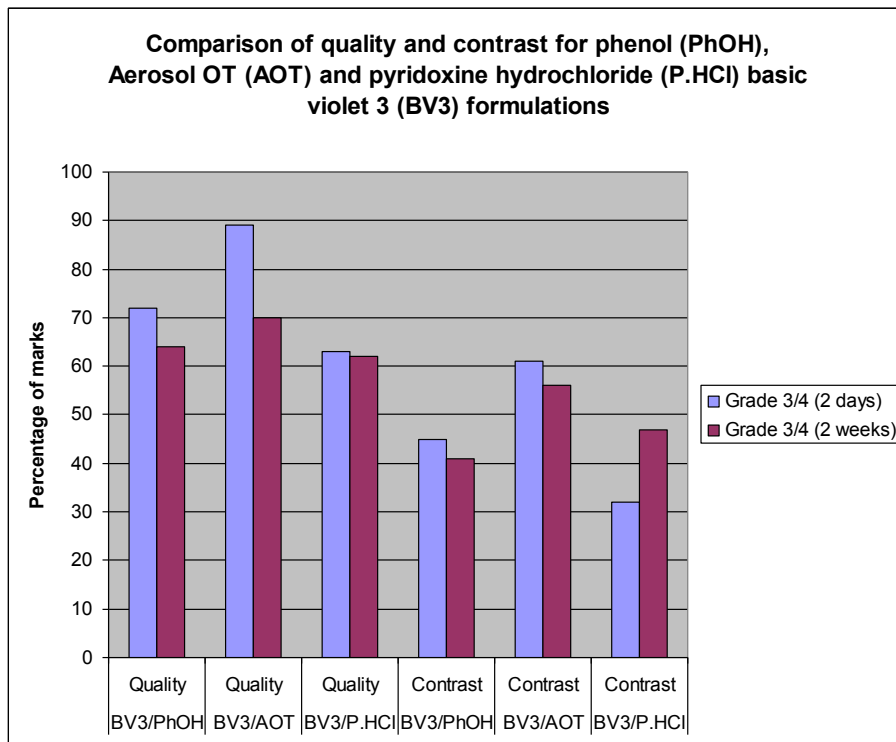


Graph comparing effectiveness of phenol (PhOH) and Aerosol OT (AOT)-based basic violet 3 (BV3) formulations, results based on grading of 1,920 half prints.

8.1.5 These subsequent studies [14,23] incorporated further phenol alternatives and confirmed the observation that the AOT formulation consistently out-performed the phenol and pyridoxine hydrochloride formulations in laboratory trials and this formulation was ultimately recommended for operational use on adhesive tapes after a brief operational trial.

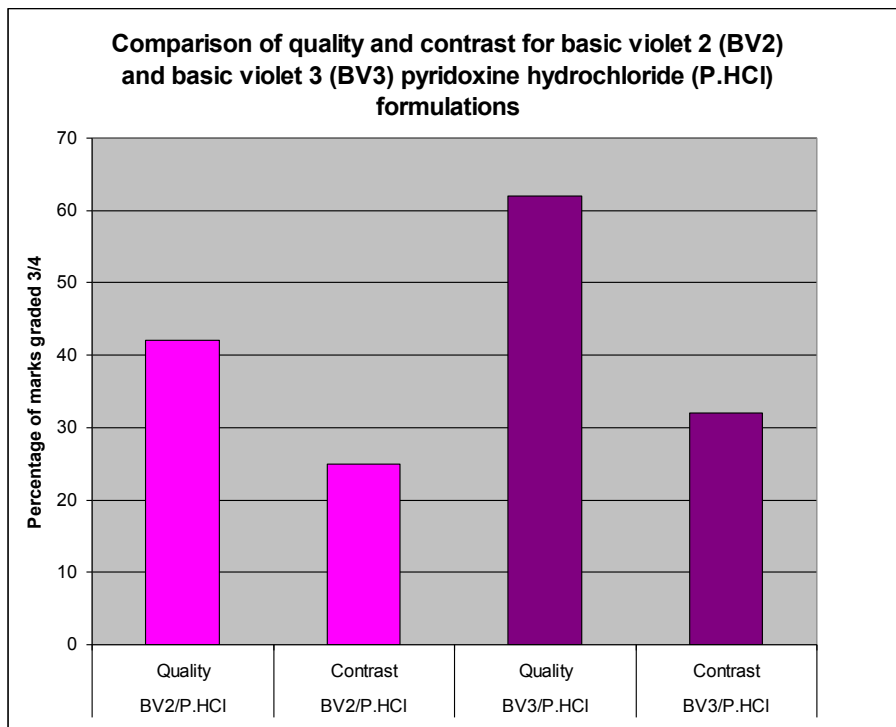


Three-way comparison of phenol, Aerosol OT and pyridoxine hydrochloride basic violet 3 formulations on tapes peeled off plastic bags, based on 4,494 half prints.



Three-way comparison of phenol, AOT and pyridoxine hydrochloride basic violet 3 formulations on tapes removed from plastic bags using freezer spray, based on 1,798 half prints

8.1.6 Of all of the alternative lipid dyes evaluated, the most promising was basic violet 2. Comparative studies were conducted between basic violet 2 and basic violet 3, the results indicating that basic violet 2 was inferior in performance based on the colour of marks developed and hence no further research was carried out on this dye.

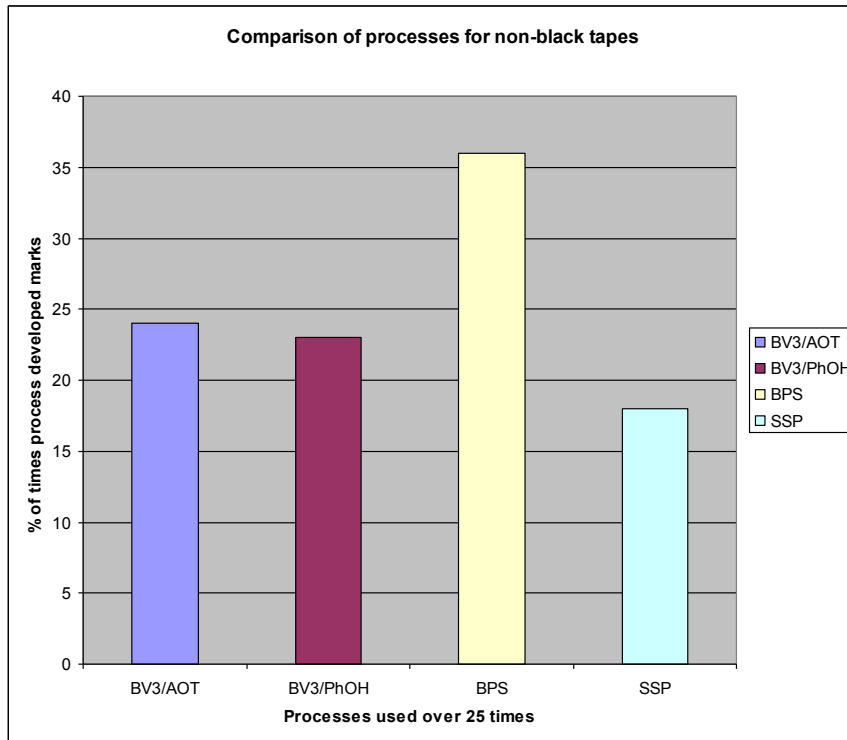


Comparison of pyridoxine hydrochloride-based basic violet 2 and basic violet 3 formulations (pyridoxine hydrochloride-based basic violet 3 subsequently found to be less effective than phenol and AOT-based basic violet 3). Results obtained from grading 300 half fingerprints.

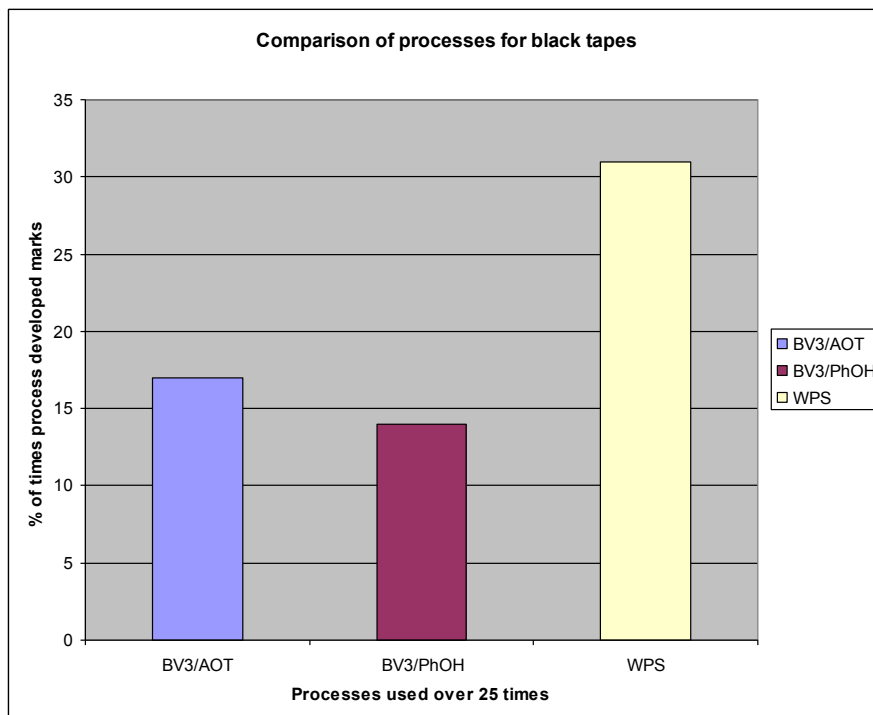
8.1.7 Comparative studies were also carried out between basic violet 3 and solvent black 3 on a range of non-porous surfaces. These are reported in Chapter 3.9 and show that there is no clear difference between the two processes in this application.

8.2 Pseudo-operational trials and operational experience

8.2.1 An operational trial was conducted, comparing the effectiveness of both phenol- and AOT-based basic violet 3 formulations with CAST-formulated powder suspensions and a commercial powder suspension. Results were obtained on both black tapes (where a white powder suspension was used) and light tapes (where black powder suspensions were used). These were conducted with police forces traditionally receiving large numbers of tape exhibits over a period of 18 months. However, tapes are not common exhibits and it took a considerable time to generate sufficient data for a reasonable comparison to be made.



a)



b)

Results of operational trial on adhesive tapes, comparing Aerosol OT and phenol-based basic violet 3 formulations with a) Home Office Centre for Applied Science and Technology black powder suspension and Sticky-Side Powder on non-black tapes and b) Home Office Centre for Applied Science and Technology white powder suspension on black tapes.

8.2.2 The trial effectively confirmed the results of the laboratory trials in that the AOT-based formulation was more effective than the phenol-based formulation on adhesive tapes and that powder suspensions were more effective than basic violet 3 as a single treatment. As a consequence, formulations for AOT-basic violet 3 and black and white powder suspensions were issued by HOSDB in 2006 [15].

8.2.3 A pseudo-operational trial was recently conducted on plastic wrapping materials, which incorporated basic violet 3 as the final process in a sequential treatment scheme. The results of this trial are more fully reported in Chapter 3.7 Powder suspensions, and demonstrate that basic violet 3 develops up to 10% additional marks in both visual and fluorescence modes after sequences involving other treatments. As a consequence, basic violet 3 has been incorporated as the final stage in the processing treatments for non-porous surfaces and plastic packaging materials.

9. References

1. **Lauth, C.** (1867) 'On the new aniline dye, "Violet de Paris"', *Lab.*, vol. 1, pp 138–139.
2. **Bock, A. C. O.** (1917) US Patents 1,497,971 and 1,497,972, June.
3. **Mitchell, C. A.** (1920) *Anal.*, vol. 45, pp 122–129.
4. **Knowles, A. M. and Thomas, G. L.** (1974) *Development of Latent Fingerprints by Police Forces in the United Kingdom; Results of a survey carried out by PSDB in 1973*, HO PSDB Report (unnumbered). London: Home Office.
5. **Haylock, S. E.** (1979) 'Carbolic Gentian Violet Solution', *Fingerprint Whorld*, vol. 4 (15), 1979, pp 82–83.
6. **Arima, T.** (1981) 'Development of Latent Fingerprints on Sticky Surfaces by Dye Staining or Fluorescent Brightening', *Ident. News*, vol. 31, pp 9–10.
7. **Kent, T.** (1980) *A Modified Gentian Violet Development Technique for Fingerprints on Black Adhesive Tape*, HO PSDB Technical Memorandum 1/80. London: Home Office.
8. **Reed, F. A.** (1980) 'Fingermark Recovery – An Outline of some Current Reagents and a Look to the Future', *Police Res. Bull.*, 35/36, pp 32–38.
9. **Wilson, B. L. and McCloud, V. D.** (1982) 'Development of Latent Prints on Black Plastic Tape using Crystal Violet Dye and Photographic Paper', *Ident. News*, vol. 32, pp 3–4.

10. **Hardwick, S. A., Kent, T. and Sears, V. G.** (1990) *Fingerprint Detection by Fluorescence Examination, A Guide to Operational Implementation*, HO PSDB, Publication No. 3/90. ISBN 0 86252 554 3 London: Home Office.
11. **Bramble, S. K.** (2000) 'Deep Red to Near Infra-Red (NIR) Fluorescence of Gentian Violet-treated Latent Prints', *J. Forens. Ident.*, vol. 50 (1), p 33.
12. **HOSDB** (2008) *Fingerprint and Footwear Forensics Newsletter*, HOSDB Publication No. 26/08, May. London: Home Office.
13. **Taylor, M.** (2001) *Reformulation of Gentian Violet for the Development of Fingerprints on Adhesive Tapes*, PSDB Student Placement Report.
14. **Miller, E. I.** (2003) *Fingerprint Development on Adhesive Tapes*, PSDB Student Placement Report.
15. **HOSDB** (2006) *Additional Fingerprint Development Techniques for Adhesive Tapes*, HOSDB Newsletter, Publication No. 23/06, March. London: Home Office.
16. **Gurr, E.** (1965) *The Rational Use of Dyes in Biology and General Staining Methods*. London: Leonard Hill.
17. **Bowman, V. (ed)** (1998 (revised 2002, 2004, 2009)) *Manual of Fingerprint Development Techniques*, 2nd edition. ISBN 1 85893 972 0. London: Home Office.
18. **Taylor, R. J.** (1975) *A Unilever Educational Booklet: Fluorescence*. London: Unilever,
19. **Cuikzsa, T.** (2007) *Enhancement of Latent Fingerprints on Adhesive Tapes with Powder Suspensions and other Processes*, HOSDB Student Placement Report.
20. **Bleay, S. M., Bradshaw, G., Moore, J. E.** (2006) *HOSDB Fingerprint Development and Imaging Newsletter: Special Edition, Arson*, April, HOSDB Publication No. 26/06. London: Home Office.
21. **Forensic Science Service** (1995) 'Preparation 10: Crystal Violet – stock and working solutions'. In *Preparations Manual*, pp 1–2, Forensic Science Service. London: Home Office.
22. **Sutcliffe, L.** (2000) *Lipid Reagents – the detection and development of fingerprints by the staining of lipid components*, PSDB Work Placement Report, 1999–2000
23. **Alderwick, E.** (2002) *The Development of Fingerprints on Adhesive Tapes*, PSDB Student Placement Report.

3.3 1,8-Diazafluoren-9-one (DFO)

1. History

- 1.1 1,8-Diazafluoren-9-one (DFO) was first synthesised by Druey and Schmidt in the CIBA laboratories in Switzerland in 1950 [1]. The potential of the chemical for the labelling of amino acids and detection of fingerprints was not recognised until the late 1980s, when the Central Research Establishment (CRE) of the then Home Office Forensic Science Service (FSS) placed a contract with Queens University, Belfast to investigate ninhydrin analogues. During the course of this research DFO was identified as a highly promising alternative to ninhydrin, producing marks of a reddish colour when viewed under normal light. The most significant feature of the product formed by the reaction between the DFO reagent and fingerprint residues was that it was inherently fluorescent and eliminated the need for toning with metal salts, the process used to make ninhydrin marks fluorescent.
- 1.2 Before open publication of information on the effectiveness of DFO, quantities were sent to selected fingerprint research laboratories worldwide for evaluation. Operational trials were also conducted at two UK police forces, Surrey [2] and the Metropolitan Police [3]. A comparative assessment of DFO with ninhydrin and 5-methoxyninhydrin was carried out in both Israel [4] and New Zealand [5]. The Israeli study looked at results obtained on a series of paper samples and banknotes and found DFO to out-perform both forms of ninhydrin. The New Zealand researchers investigated sequential treatment and found that DFO could be used before ninhydrin, and did not affect the subsequent use of physical developer. However, ninhydrin used after DFO was far less effective and did not produce any additional marks. The fluorescence of DFO was also superior to that of both ninhydrin forms after toning with zinc chloride. Another observation made by the New Zealand group was that DFO also enhanced blood, and could be used in sequential treatments before amido black (acid black 1).
- 1.3 With all researchers reporting significant improvements in the number of marks developed using DFO over the numbers found with ninhydrin, the first information on the new reagent was published in open literature in 1990 [6,7,8]. The initial formulation issued was based on the chlorofluorocarbon (CFC) 1,1,2-trifluoroethane (CFC113) solvent, with small quantities of methanol and acetic acid, and required the exhibits to be dipped twice in the solution, allowing them to dry each time before finally heating in a dry oven at 100°C for 10 minutes to develop the marks. Excitation and emission spectra for DFO were also presented, with the UK laboratories initially using an argon ion laser to promote fluorescence. However, it was also found that a high intensity light source (i.e. the high intensity filtered light sources then becoming available) could also be used to produce fluorescence [9,10].

- 1.4 Fundamental research into DFO continued, with studies carried out into the reaction products formed between DFO and amino acids [11]. Assessments of the relative sensitivity of DFO and different ninhydrin analogues were also carried out [12], looking at their relative detection limits for serine. This study indicated that DFO was similarly sensitive to ninhydrin in colorimetric mode and as sensitive as 5-methoxyninhydrin toned with zinc chloride (the best of the ninhydrin analogues) in fluorescence mode.
- 1.5 The issue of the first DFO formulation and the subsequent commercial availability of the reagent prompted further investigations worldwide, with assessments being carried out of alternative solvents to CFC113 including petroleum ether [13] and a petroleum ether/xylene mixture [14]. Sequential treatments were also reassessed, with Masters *et al.* [14] studying a range of different paper types and finding that the DFO-ninhydrin sequence was far superior to ninhydrin-DFO. Corson [13] also investigated sequential treatment and indicated that occasionally DFO could develop additional marks after ninhydrin, but did not state which sequence was best. Masters *et al.* [14] also studied a range of different light sources and filter combinations for excitation and viewing of the fluorescent fingerprints. A red camera/viewing filter was recommended to reduce the background fluorescence that was sometimes observed on coloured papers and from some writing inks.
- 1.6 The Home Office Scientific Research and Development Branch (HO SRDB) studies into DFO also began in 1990, initially looking at the components of the formulation and the dipping and heating stages. In a split depletion comparison carried out over seven different paper types using five donors, it was found that there was no benefit in dipping the article twice. The purpose of double-dipping was stated to be to increase the uptake of DFO by the fingerprint, but the HO SRDB study showed no difference in either the visible appearance or the intensity of fluorescence between single-dipped and double-dipped articles. Single-dipped articles, in particular cheques, showed less evidence of background staining and therefore single dipping was recommended. Heating experiments were also conducted, monitoring the change in fluorescence with increasing exposure time in an oven using a luminance meter. At 100°C, optimum fluorescence was reached after 20–30 minutes, whereas at 50°C development took several hours [15]. Temperatures in excess of 100°C were not considered because of potential charring to the paper, although development rates were increased; Australian researchers suggested that development occurred in approximately 20-30 seconds at 160°C [16]. A dry oven was found to be more effective than a heat gun in delivering the optimum heating conditions. It was considered important that the oven used in processing had a laminar air flow across each shelf as opposed to being a convection oven, because paper articles were loaded on cardboard in the same way as used for the processing of ninhydrin treated articles. Further studies also investigated alternative solvent systems and reductions in the amount of DFO in the formulation. It was found that the quantity of DFO could be reduced from

0.5g to 0.25g without any detriment to the intensity of fluorescence produced. This also overcame issues with instability of the working solution, where DFO precipitated rapidly, sometimes before processing had commenced. Operational trials were conducted between the original and revised formulation and processing conditions, with the revised formulation giving marginally better performance. A summary of these studies was published in 1993 [15].

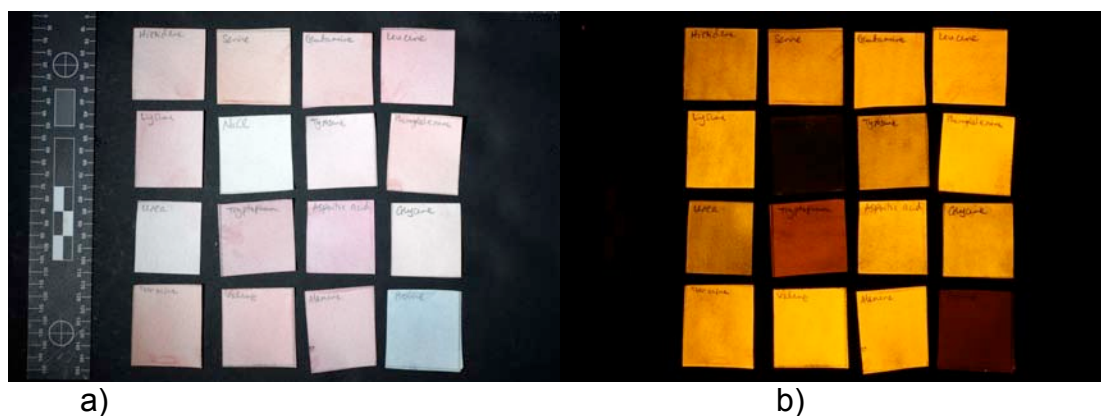
- 1.7 Other extensive studies of DFO, its reactions and optimum viewing conditions were conducted by Cantu *et al.* [17] and Stoilovic [16]. Cantu *et al.* compared the effectiveness of DFO with a range of ninhydrin analogues on the amino acid glycine and concluded that DFO was the only compound acting as a fluorescer without secondary treatment, with the intensity of fluorescence exceeding that of any of the zinc or cadmium complexes formed with ninhydrin. Cantu *et al.* also demonstrated that the presence of acetic acid in the formulation was essential for fluorescence to occur. Formic acid will also produce a good reaction, but when used in combination with methanol the two constituents react rapidly to produce the unwanted methyl formate. Stoilovic also investigated changes to the formulation, adding chloroform and reducing the methanol and acetic acid components in order to reduce inks running when treating documents. He also conducted a sensitivity study and concluded that DFO was equivalent in sensitivity to ninhydrin toned with zinc chloride. Samples were treated by heating with an ironing press at 160°C, which was thought to give superior results to oven heating (although the oven used in this case was a convection, rather than a laminar flow oven). The optimum excitation and viewing conditions were also investigated using a filtered high intensity light source (Polilight).
- 1.8 The introduction of the Montreal Protocol on Substances that Deplete the Ozone Layer in 1987 and the subsequent prohibition on the use of ozone-depleting solvents, including CFC113, meant that from the mid-1990s efforts were directed towards an 'ozone-friendly' DFO formulation. In 1995 Lennard [18] proposed petroleum ether, which was in wide scale use in the US as a solvent for ninhydrin, as a replacement for CFC113, but it was also desirable to identify a solvent replacement without the associated issues of high flammability. During the period 1994–1997, PSDB evaluated a range of candidate replacement solvents including hydrochlorofluorocarbons (HCFCs), hydrofluorocarbons (HFCs) and hydrofluoroethers (HFEs). Of these HFC4310 and 1-methoxynonafluorobutane (HFE7100) [19] both showed promise, but required other additives to produce the same level of reaction as CFC113. HFE7100 had also been supplied to French researchers for evaluation, and they too developed a DFO formulation based on this solvent [20]. HOSDB carried out an operational trial of the most promising new DFO formulations, comparing them with the existing CFC113 formulation and an optimised 1,2 indandione formulation based on HFE7100. In this trial, conducted on 650 articles in an operational police laboratory, the HFE7100-based DFO formulation gave the best

results [21] and was therefore recommended for operational use in the UK. A fuller description of the alternative formulations investigated by PSDB was later published by the researchers [22].

- 1.9 Further fundamental research was carried out on the DFO system. Wilkinson studied the reaction mechanism between DFO and amino acids [23] and the synthesis of DFO analogues [24]. Conn *et al.* [25] investigated whether metal salt treatment of DFO would give any further benefits in fluorescence but concluded that, in contrast to ninhydrin, there was little effect on the fluorescent product.
- 1.10 The impact of DFO on other types of forensic evidence was also studied. The emergence of DNA and its importance as an identification tool prompted studies into the effect of DFO treatment of blood on the subsequent recovery of DNA profiles [26]. The authors concluded that DFO had no detrimental effect on DNA. PSDB and the FSS also showed that DFO treatment had little impact on the recovery of DNA from latent fingerprint residues [27]. Strzelczyk [28] considered the effects of DFO treatment on subsequent document examination, comparing the PSDB HFE7100 formulation with the CFC113 formulation. The HFE7100 formulation was found to be less detrimental to handwriting evidence.
- 1.11 A survey of fingerprint development processes for porous surfaces conducted in 2004 [29] showed that DFO had become the second most widely used reagent for this surface worldwide, with 86% of those responding to the survey saying that they used it in their laboratory.
- 1.12 More recently, the development of formulations of 1,2 indandione incorporating zinc salts have resulted in claims that 1,2 indandione-zinc is actually more effective than DFO. As a consequence several groups of researchers have carried out further comparative work [30-32]. To date the results of these have given conflicting results with most favouring 1,2 indandione-zinc but some favouring DFO. It is clear that further research is required to establish whether DFO should remain the primary chemical treatment in sequential processing regimes for porous surfaces. This further work should take into consideration the overall effectiveness of sequential treatment routines, as well as the effectiveness of individual techniques.

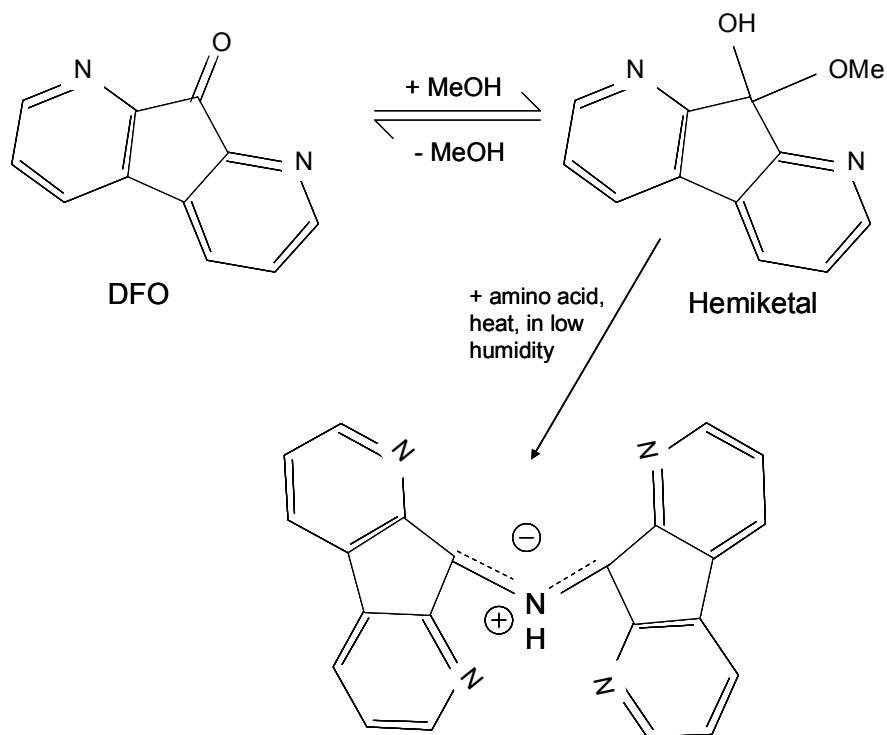
2. Theory

- 2.1 The reaction mechanism for DFO has been studied by both Grigg *et al.* [11] and Wilkinson [23, 24]. Grigg *et al.* isolated the red reaction product between DFO and various α -amino acids and found it to be closely related to the protonated Ruhemann's purple structure developed with ninhydrin.

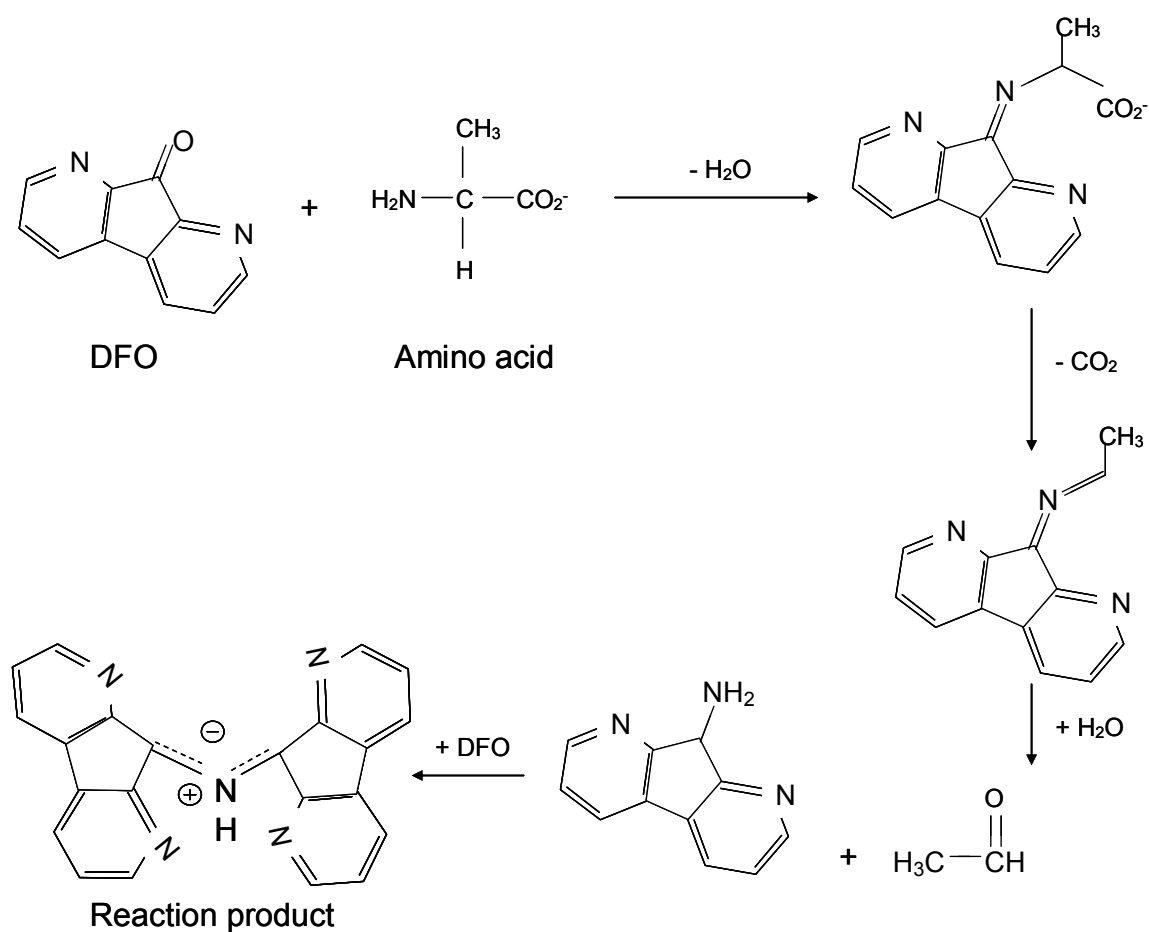


Reaction products formed between 1,8-diazafluoren-9-one (DFO) and 0.1M solutions of amino acids and other fingerprint constituents a) visible and b) fluorescence.

2.2 The analytical studies carried out by Wilkinson used a range of techniques including nuclear magnetic resonance spectroscopy (NMR) and gas chromatography – mass spectrometry (GC-MS) to isolate and identify reaction products. A reaction mechanism was proposed, which is illustrated below.



Proposed mechanism for formation of hemiketal.



Proposed reaction path of 1,8-diazafluoren-9-one (DFO) with amino acids [24]

- 2.3 Wilkinson [24] proposed that the DFO reaction follows a very similar path to that of ninhydrin with amino acids. DFO reacts with the methanol in the solvent mixture to form a hemiketal, which has a higher reactivity with amino acids than the DFO molecule. The nitrogen atom of the amino acid is able to attack the hemiketal at the electron deficient carbon in the polarised carbonyl, with the loss of water. This forms an aromatic imine, which retains the alkyl fragment of the amino acid and undergoes decarboxylation to form a further intermediate product. Hydrolysis then occurs at the nitrogen-carbon double bond, which forms an aromatic amine and acetaldehyde. The aromatic amine finally reacts with another DFO molecule to form the red, fluorescent reaction product identified in this and previous studies [11, 24]. X-ray crystallography carried out on the reaction product between DFO and L-alanine [23] indicated that the structure of the reaction product consisted of two DFO molecules linked by a bridging nitrogen atom, and was therefore in close agreement with Grigg *et al.*'s original predictions [11]. In the crystalline product analysed, molecules of the reaction product were shown to be linked by hydrogen-bonded bridges with water molecules.
- 2.4 The reaction between DFO and amino acids is not thought to proceed to completion, which accounts for the observation that ninhydrin will develop additional marks when used after DFO. Alternatively (or additionally), there may not be sufficient DFO to completely react in a 2:1 ratio with all amino acids present.

3. CAST processes

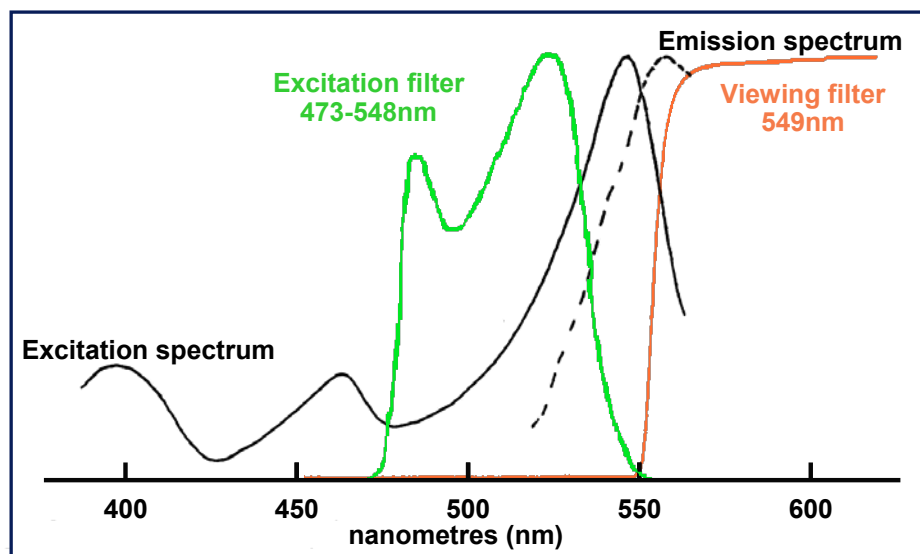
- 3.1 The process currently (as of 2011) recommended by CAST is to add 30mL of methanol and 20mL of acetic acid to 0.25g of DFO, stirring to produce a yellow solution. To this is then added 275mL of HFE71DE followed by 725mL of HFE7100, stirring together to produce a working solution.
- 3.2 Working solution is poured into a shallow tray, and articles to be treated drawn slowly through the solution with forceps, then removed and allowed to dry on a sheet of tissue. Alternatively, DFO solution may be applied with a soft brush.
- 3.3 Once dry, articles are heated in a non-humidified oven at 100°C for 20 minutes, followed by examination in white light (where developed marks may be detected due to their pale pink colour) and subsequent fluorescence examination.
- 3.4 The role of DFO in the formulation is to react with amino acids present in fingerprint residues to give a fluorescent reaction product. The CAST formulation makes the assertion that the primary purpose of DFO is to produce a fluorescent product, and therefore the presence of any coloured reaction product is of secondary importance. The formulation

uses 0.25g of DFO per litre, found to give the maximum intensity of fluorescence. Any increase in DFO content will make the coloured product more intense (although still far less visible than the purple of ninhydrin) but does not enhance fluorescence. Quantities of > 0.2g DFO are essential for the reaction to occur, and quantities of > 0.75g cannot be dissolved.

- 3.5 Methanol is an essential component of the DFO formulation, its presence allowing DFO to form hemiketals, which in turn have greater reactivity with amino acids. Longer chain alcohols are not as effective, using ethanol, propan-1-ol or propan-2-ol reduces the yield and fluorescence of developed fingerprints and *t*-butanol inhibits the reaction completely. Studies have shown that 30% of DFO reacts with methanol, whereas only 10% reacts with ethanol. The formulation uses the minimum amount of methanol possible due to its toxic nature.
- 3.6 Acetic acid is added to acidify the solution. If acidification is not carried out, virtually no fingerprints are developed. Propanoic acid can be used in place of acetic acid but has no benefit, whereas formic acid rapidly esterifies with the methanol component of the formulation, producing water as an unwanted by-product. The presence of water causes phase separation of the solution, reducing the amount of DFO in the non-polar phase available for fingerprint development, although a small amount of water is essential for the reaction to take place. Dried solutions are brown in colour and do not produce fluorescent marks if used to treat fingerprints.
- 3.7 HFE7100 is used as the principal carrier solvent for DFO. However, during reformulation work it was found that it could not be used as a straight replacement for CFC113 because CFC113 appeared to catalyse the reaction between DFO and amino acids in some way, whereas HFE7100 did not. If HFE7100 was used on its own, the developed fingerprints appeared noticeably less fluorescent and fewer in number. The addition of *trans*-1,2-dichloroethylene as a co-solvent (i.e. in the HFE71DE component of the formulation) is essential for the development of greater quantities of brighter fluorescent fingerprints.
- 3.8 CAST recommends only a single dip in the DFO working solution. Early studies indicated that double dipping had no benefit in terms of number or intensity of marks developed, and may lead to increased background staining.
- 3.9 The heating temperature of 100°C is selected to give a combination of a reasonably short development time combined with a low risk of damage to exhibits, such as paper charring and melting of plastic windows in envelopes. It is also compatible with the upper temperature limit of the ninhydrin oven, enabling a single piece of equipment to be used for both processes. Early studies using a luminance meter showed that optimum fluorescence was obtained after 20 minutes for a significant majority of

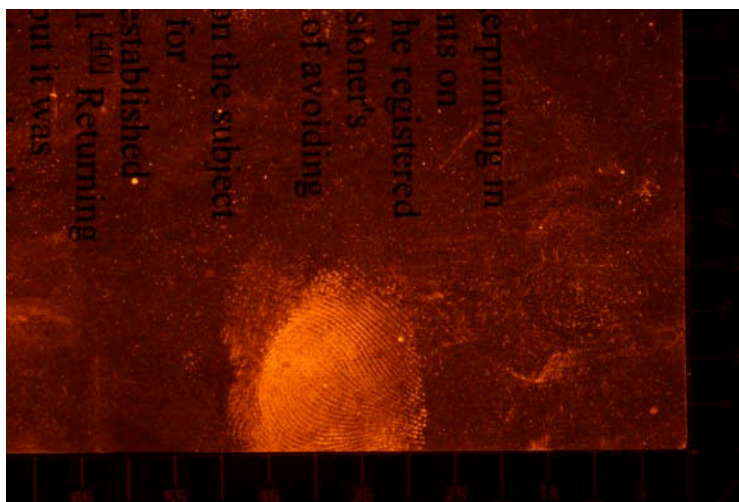
exhibits and this was therefore recommended in place of the original 10-minute period.

- 3.10 CAST recommends the viewing of marks developed using DFO using excitation in the green region of the spectrum (the 473–548 excitation band of the Quaser series of light sources) and viewing fluorescence through an orange, Schott glass OG570 (549nm long-pass) filter. This gives the optimum match with the excitation and emission spectra for DFO, with the illumination waveband overlapping the DFO excitation and the viewing filter transmitting close to the optimum emission wavelength.



Emission and excitation for 1,8-diazafluoren-9-one, overlaid with the Quaser excitation waveband used and the corresponding transmission of the viewing/camera filters recommended.

- 3.11 In some circumstances, such as coloured papers, background fluorescence from the paper or ink may make the developed marks more difficult to visualise and in these situations the narrower green excitation waveband of the Quaser (491–548) should be used instead, in combination with a 593 (Schott RG610) filter to cut background fluorescence. More recently, green neodymium:yttrium aluminium garnet (Nd:YAG) lasers with output at 532nm have become more widely available. This output is further towards the optimum excitation wavelengths for DFO, and being single wavelength will cause far less background fluorescence. Therefore, 532nm lasers in combination with 549 (Schott OG570) long-pass filters are recommended for optimum viewing of fluorescent marks developed using DFO.



Fingerprints developed using 1,8-diazafluoren-9-one, illuminated with green (532nm) light and viewed using a 549 long-pass (Schott glass OG570) filter.

- 3.12 The broad excitation and emission spectra of DFO means that for surfaces where background fluorescence is appreciable when illuminated with light in the green region of the spectrum, better results may be obtained using yellow illumination sources (such as the new 577nm laser) in conjunction with 593 long-pass filters. DFO will still fluoresce under these conditions whereas the background fluorescence may be considerably reduced. This is particularly relevant for many types of brown and coloured paper.

4. Critical issues

- 4.1 The presence of methanol and *trans*-1,2-dichloroethylene in the formulation is essential for the optimum operational effectiveness. Formulations that substitute or omit these constituents will develop less highly fluorescent marks and fewer marks overall.
- 4.2 Heating of DFO-treated exhibits should be carried out in a dry oven with even heating via laminar airflow across each shelf; high levels of humidity equivalent to those used for ninhydrin are not beneficial for the reaction.
- 4.3 Appropriate excitation wavelengths and viewing filters must be selected when visualising developed marks. These are detailed in paragraphs 3.10–3.12 above. Light sources with higher output powers (e.g. lasers) will detect more marks.
- 4.4 If any separation of the working solution into oily droplets is observed, the solution should be discarded and not used for processing.

5. Application

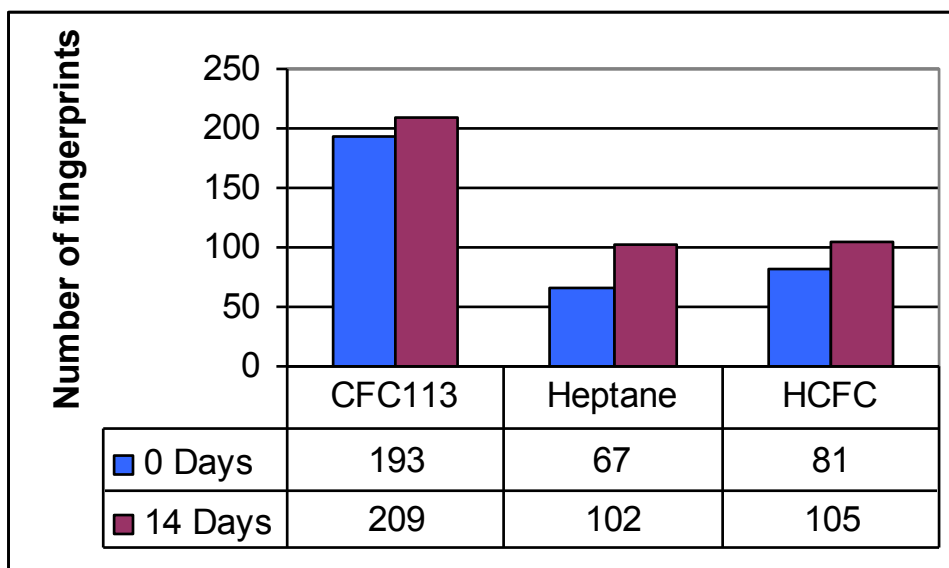
- 5.1 Suitable surfaces: DFO is suitable for use on all porous surfaces, including paper, cardboard, raw wood and matt painted walls.
- 5.2 The principal application of DFO is in the development of fingerprints on porous items, in particular paper. It has been found to be the single most effective treatment for this surface and can be used as the first process in a sequential processing routine consisting of DFO – ninhydrin – physical developer. The use of DFO does not destroy marks that could have been developed by ninhydrin or physical developer and both processes can reveal further marks that have not been developed by DFO.
- 5.3 DFO is not as widely used as ninhydrin because it requires access to a forensic light source and appropriate viewing filters to see many of the marks developed. Consequently, ninhydrin is the method of choice for many laboratories processing volume crime exhibits because the marks are visible under normal lighting conditions and can be easily captured. However, ninhydrin is a less effective process (DFO typically develops 1.6 times more marks) and potential marks will be missed if it is used as a sole treatment.
- 5.4 DFO is also an effective blood dye, reacting strongly with the protein constituents in the blood to produce highly fluorescent marks. Heavy deposits of blood will reabsorb the fluorescence making this process less effective in these areas. It can therefore be used to enhance marks in blood on porous surfaces, but is not specific to the 'haem' component of blood and cannot be used to determine whether a mark is blood or not. The application of DFO has been shown not to affect subsequent recovery of DNA from marks deposited in blood [26].
- 5.5 DFO is applied in the laboratory by solution dipping, passing the exhibit through a shallow tray containing the DFO working solution, allowing it to dry then heating it in an oven at 100°C for 20 minutes. Neither the exhibit nor the oven are humidified in any way. For larger items, such as boxes, DFO can be applied as a solution using a soft brush, again allowing the exhibit to dry before placing it in an oven.
- 5.6 DFO cannot be effectively used at scenes of crime. Although the solution can be applied using a brush, the conditions of temperature required to develop fingerprints in a reasonable time are not compatible with working at scenes. It is possible to apply heat locally using equipment such as a heat gun, but this is less effective than oven treatment and will still require long periods of heat application to develop marks, depending on the particular system used. Some heat guns are capable of heating to several hundred degrees centigrade and must therefore be used with caution.

6. Alternative formulations and processes

- 6.1 Since 1990 and the introduction of DFO, several different formulations have been investigated. Many of these were prompted by the search for alternative solvents after the banning of CFCs. A summary of some significant alternative formulations proposed is given below.
- 6.2 Bratton and Juhala [33] proposed a variation of the DFO formulation and process called 'DFO-Dry', which involved impregnating sheets of filter paper with a solution of DFO, allowing them to dry, then sandwiching paper exhibits between the impregnated sheets and applying heat from a steam iron filled with 5% acetic acid solution. Samples were then placed in a dry press at 110°C for 10 minutes to complete development. The formulation used to impregnate the filter papers sheets was:
- 200mL methanol, 200mL ethyl acetate, 40mL acetic acid, 1g DFO.
- 6.3 Marks developed in this way were equal in intensity to those developed using a solution dipping process using the same formulation diluted with petroleum ether. The principal advantages of the dry process were that there was no ink run, no background staining and no background fluorescence.
- 6.4 Petroleum ether was also proposed as a replacement solvent for CFC113 [14,18] but CAST would not recommend the use of this, or any other, highly flammable solvent in a laboratory because of the fire and explosion risks. It was found during testing by CAST that the formulation proposed by Masters *et al.*[14], containing propan-2-ol, xylene and acetone in addition to petroleum ether, developed brightly fluorescent fingerprints but caused significant damage to writing inks and was unstable when stored.
- 6.5 CAST carried out extensive studies into the identification of replacement solvents for CFC113, using a range of different solvent types including hydrocarbons, HCFCs and HFCs [22]. During these studies, initial evaluations were carried out using split depletions. Any formulations showing promise were taken forward to more detailed trials involving the treatment of a batch of 75 fraudulently passed cheques, using each formulation and counting all developed fingerprints with more than eight minutiae visible.
- 6.6 The best performing hydrocarbon and HCFC formulations are given below, together with their performance relative to the CFC113 formulation of batches of 75 cheques.

	CFC113	Hydrocarbon	HCFC
DFO	0.25g	0.25g	0.25g
Methanol	30mL		25mL
Acetic acid	20mL	20mL	20mL
Ethanol		100mL	

Ethyl acetate		50mL	
Methyl acetate			5mL
Heptane		850mL	
CFC113	1 litre		
HCFC141b			1 litre

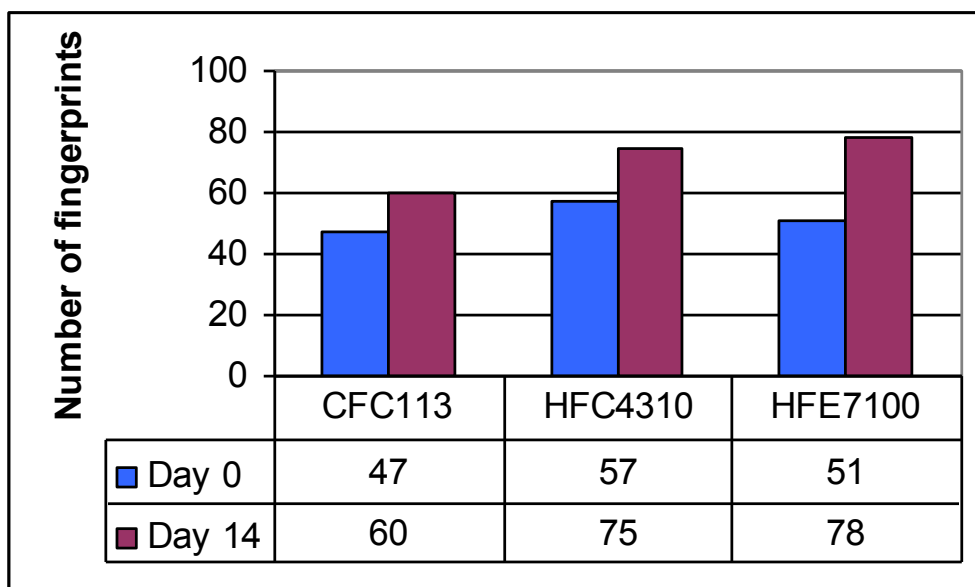


Formulations based on hydrocarbons (heptane) and hydrochlorofluorocarbons (HCFCs) and their performance relative to the 1,1,2-trifluorotrchloroethane (CFC113) formulation.

- 6.7 Despite promising results from laboratory split depletion tests, neither of these formulations performed well when compared with the CFC113 formulation in a realistic trial.
- 6.8 Another non-CFC formulation evaluated was provided by the Bundeskriminalamt (BKA), Weisbaden, Germany, and consisted of:
 0.5g DFO, 40mL methanol, 20mL acetic acid, 1 litre *t*-butyl methyl ether.
- 6.9 This gave more fluorescent prints than the heptane formulation, but caused significant ink running. The solvent posed an explosion risk, and did not perform as well as the CFC formulation in comparative trials on batches of cheques.
- 6.10 The final class of solvents evaluated were HFCs, the most suitable of those evaluated being HFE7100 and HFC4310mee. The formulations were trialed against CFC113 and the results are shown below.

	CFC113	HFC4310mee	HFE7100
DFO	0.25g	0.25g	0.25g
Methanol	30mL	30mL	30mL
Acetic acid	20mL	20mL	20mL

<i>trans</i> -1,2 dichloro-ethylene		100mL	150mL
HFC4310mee		1 litre	
HFE7100			850mL
CFC113	1 litre		



Formulations based on hydrofluorocarbons and hydrofluoroethers and their performance relative to the CFC113 formulation.

6.11 Both formulations appeared to give superior performance to the CFC113 system and were taken to a full operational trial alongside it [21]. From this trial, the HFE7100-based formulation (with minor modifications) was ultimately recommended for operational use and is described in more detail in the CAST processes section above.

6.12 There are several DFO formulations in operational use worldwide. A survey of these has recently been conducted by Wallace-Kunkel *et al.* [29], the most commonly used being summarised in the table below.

% usage	DFO (g)	Methanol (mL)	Ethyl acetate (mL)	Acetic acid (mL)	Dichloromethane (mL)	Petroleum ether (mL)	HFE7100 (mL)	HFE71DE (mL)
18	0.25	40		20			940	
14	0.5	40		20			940	
11	0.5	100	100	20		780		
7*	0.25	30		20			725	275
4	0.2	50		20	50	880		

* currently (2011) recommended HOSDB formulation.

Compositions representative of 1,8-diazafluoren-9-one formulations used worldwide.

6.13 The two most commonly used formulations use HFE7100, but do not incorporate *trans*-1,2 dichloroethylene. CAST has found that

formulations without this component are less effective and would therefore recommend its inclusion.

- 6.14 Formulations based on petroleum ether are not recommended by CAST because of the fire and explosion hazards associated with the solvent, and CAST would seek to minimise use of dichloromethane where possible due to health and safety concerns.
- 6.15 A modified formulation has been proposed by CAST for the treatment of thermal receipts [27]. When thermal receipts are treated with DFO they blacken due to reaction between acetic acid and the thermal ink layer, blackening also occurring due to the heat in the oven used to develop marks. To counteract this, CAST carried out trials and devised a formulation with the amount of methanol increased to 60mL. This dissolves away the thermal ink layer and significantly reduces subsequent blackening. The thermal paper is retained in the dip bath until all black deposit is removed from the surface of the paper, then placed into the oven. In practice, this did reduce the problems associated with blackening of thermal receipts but as ink compositions changed it did not prove possible to remove easily all of the ink layer in this way. Pre-dipping the receipt in ethanol until all text disappears and then allowing it to dry prior to dipping in a solution of the standard formulation has proved more effective [34].

7. Post-treatments

- 7.1 There are no post-treatments used with DFO other than the examination of the developed mark using fluorescence, which is described above. Toning using metal salts is ineffective and does not increase the fluorescence of the mark.

8. Validation and operational experience

8.1 Laboratory trials

- 8.1.1 Although laboratory trials were conducted during the initial development of DFO formulations in the early 1990s, most of these results no longer survive. It has been found from experience that planted prints rarely give operationally representative results in such trials, typically performing worse than seen on casework. This is possibly because perpetrators of crimes may be under increased stress and sweat more, giving more eccrine prints than seen in the laboratory. As a consequence, development of revised formulations at HOSDB is usually carried out using small-scale comparative tests until best performing formulations are identified, after which testing proceeds to pseudo-operational trials using realistic items such as bundles of cheques.

8.1.2 One exception to this is the recent comparison between DFO and 1,2 indandione/zinc, carried out using split depletions on a range of different substrate types. This study showed closely equivalent performance between DFO and the 1,2 indandione/zinc formulation studied, and is more fully reported in Chapter 5.7, 1,2 Indandione.

8.2 Pseudo-operational trials and operational experience

8.2.1 Several pseudo-operational trials were conducted on alternative DFO formulations during research into a replacement solvent for CFC113. The results of these have been summarised in the section on 'Alternative formulations' above. The outcome of these studies was that the formulation based on HFE7100 solvent was selected for comparative trials with the CFC113-based DFO formulation.

8.2.2 There have also been several pseudo-operational and operational trials conducted to establish the relative effectiveness of the DFO and ninhydrin techniques and also to establish the best sequence of treatment. Before publication of the initial reports on DFO, operational trials were conducted at Surrey Police and the Metropolitan Police Serious Crimes Unit.

8.2.3 The trial at Surrey [2] involved treatment of the exhibits using DFO followed by laser examination, then ninhydrin treatment. An assessment was made of the number and quality of the marks developed using each process. The results of this trial were:

DFO > Ninhydrin 139 articles (69.8%);
Ninhydrin > DFO 13 articles (6.5%);
DFO = Ninhydrin 47 articles (23.6%).

8.2.4 The Metropolitan Police trial [3] involved a direct comparison of the effectiveness of DFO and ninhydrin when used as a single process on casework, and also looked at the impact of zinc chloride treatment on marks developed using ninhydrin. The results are summarised below:

DFO – 510 prints from 168 articles;
Ninhydrin – 1,135 prints from 1,356 articles;
Ninhydrin + zinc chloride – 1,249 prints from 1,356 articles.

8.2.5 Both these trials indicated significant benefits in the use of DFO, with more marks being developed than found using ninhydrin. DFO was found superior to ninhydrin even after zinc chloride toning had been used to make marks fluorescent.

8.2.6 HO SRDB also conducted pseudo-operational trials in 1990 [35], looking at the numbers of marks developed on batches of brown and white envelopes using DFO, ninhydrin and the DFO-ninhydrin sequence. Articles were examined visually and then using fluorescence examination

to enhance the DFO marks. The results of this exercise are tabulated below.

Visible examination				
	Ninhydrin		DFO	
	Brown	White	Brown	White
Articles	93	93	93	93
Fingerprints	18	24	6	16
Articles with fingerprints	11	14	6	11
% Articles with fingerprints	12	15	6	12

Fluorescence				
	Ninhydrin		DFO	
	Brown	White	Brown	White
Articles	93	93	93	93
Fingerprints	19	24	60	91
Articles with fingerprints	12	14	33	50
% Articles with fingerprints	13	15	35	54

Ninhydrin after DFO				
	New fingerprints		Overall	
	Brown	White	Brown	White
Articles	93	93	93	93
Fingerprints	9	10	15	26
Articles with fingerprints	7	8	12	16
% Articles with fingerprints	8	9	13	17

Results obtained from pseudo-operational trial on batches of envelopes.

8.2.7 Hardwick *et al.* [15] also carried out trials at PSDB in the early 1990s, comparing the original formulation issued by Pounds *et al.* [7] with revisions to the process suggested by PSDB, including reductions in the amount of DFO, single dipping and increasing the heat treatment time to 20 minutes. The study looked at 200 cheques, 100 from each of two banks, divided into two sets with 50 cheques from each bank. In this trial, both formulations developed just over 200 prints with >8 points ridge detail and so the reduction in DFO (and therefore in the cost of the formulation) was not felt to be detrimental to performance and was

recommended operationally. Subsequent treatment of these exhibits with ninhydrin developed an additional 10% of marks.

- 8.2.8 A direct comparison of the effectiveness of ninhydrin and the revised DFO formulation was also carried out. This study looked at 300 cheques, 100 from each of three banks, divided into batches containing 50 cheques from each bank. In this study DFO gave 60% more fingerprints than ninhydrin, in accordance with all previous studies.
- 8.2.9 All the studies above utilised DFO and ninhydrin formulations based on CFC113. As this solvent was being withdrawn from operational use, operational trials were conducted to compare the effectiveness of the replacement solvent formulations with CFC113, also to compare the effectiveness of DFO with 1,2 indandione, a new reagent being proposed as an alternative one-step fluorescent treatment for porous surfaces (see Chapter 5.7, 1,2 Indandione for further details).
- 8.2.10 Merrick *et al.* [21] carried out an operational trial at West Midlands Police in conjunction with PSDB. This was carried out over 7 weeks, examining over 650 articles at an average of 2.26 articles per case and counting fingerprints containing >8 points. The trial compared the CFC113 DFO formulation, the DFO formulations based on HFC4310mee and HFE7100 described in the section above, and a 1,2 indandione formulation based on HFE7100. The results are summarised in the tables below.

Formulation		Week						
		1	2	3	4	5	6	7
DFO (CFC)	Prints	86	91	109	132	156	201	214
	Cases	67						
DFO (HFC)	Prints	46	59	76	99	104	158	171
	Cases	66						
DFO (HFE)	Prints	93	97	130	144	174	213	218
	Cases	70						
IND (HFE)	Prints	70	89	92	105	116	149	164
	Cases	68						

Cumulative number of identifiable fingerprints developed with 1,8-diazafluoren-9-one and 1,2 indandione formulations, and total number of cases processed.

Formulation	Week						
	1	2	3	4	5	6	7
DFO (CFC)	69.6	61.3	61.5	59.2	57.9	61.5	59.7
DFO (HFC)	56.0	60.6	61.0	58.8	55.2	59.1	59.7
DFO (HFE)	78.3	71.0	69.2	65.3	63.2	66.2	62.5
IND (HFE)	52.4	51.6	46.5	49.0	49.1	55.4	54.2

Cumulative proportion of cases producing identifiable fingerprints.

8.2.11 The results showed that the HFE7100-based formulation gave equivalent, if not better, performance to the CFC113 formulation and this was therefore recommended for operational use by PSDB.

8.2.12 A similar trial was carried out by the Royal Canadian Mounted Police (RCMP) [36], assessing the HOSDB DFO formulation based on HFE7100, an alternative DFO formulation based on HFE7100 but without *trans*-1,2-dichloroethylene, and a 1,2 indandione formulation based on HFE7100.

8.2.13 Preliminary trials were conducted on 80 cheques, which indicated that the HOSDB formulation gave the best results. The study then proceeded to an operational field trial, the interim results of which are summarised below:

DFO (alternative HFE7100 formulation): 303 exhibits, 66 identifiable marks;

DFO (HOSDB HFE7100 formulation): 440 exhibits, 126 identifiable marks;

1,2 indandione (HFE7100-based): 165 exhibits, 7 identifiable marks.

8.2.14 The PSDB DFO formulation was therefore adopted by RCMP for operational work.

- 8.2.15 More recently there have been several papers reporting reformulations of 1,2 indandione to incorporate zinc salts as an integral constituent of the dip solution rather than as a post-treatment. Research has been conducted to compare the effectiveness of these revised formulations with DFO [30-32]. To some extent the results of these have been conflicting, with some researchers [30, 32] finding 1,2 indandione performing better, and others [31] finding DFO to give marginally better performance. Further refinements have since been made to the 1,2 indandione-zinc formulations and indications are that this reagent may now give improved performance over DFO under UK conditions. However, further validation work will be required to demonstrate this, and the overall impact of replacing DFO with 1,2 indandione on the total number of marks recovered during sequential processing will need to be assessed.
- 8.2.16 Another recent pseudo-operational trial that has been conducted by HOSDB has been the comparison of DFO, ninhydrin and 4-dimethylaminocinnamaldehyde (DMAC) for the development of marks on thermal receipts [37]. In this study DFO was found to significantly outperform the other two processes, yielding almost twice the number of marks. This study is more fully reported in Chapter 5.2, 4-Dimethylaminocinnamaldehyde (DMAC).

9. References

1. **Druey, J. and Schmidt, P.** (1950) 'Phenanthroline Quinones and Diazafluorenes', *Helvetica Chimica Acta*, vol. 33, pp 1080–1087.
2. **Lemon, J.** (1989) 'Fingerprint Operational Trial', Letter from Fingerprint Section to Chief Superintendent in charge, Surrey Police, regarding trial results on DFO, 9 March. England: Surrey Police Force.
3. **Brennan, J. S.** (1989) *1,8-Diazafluoren-9-one*, Metropolitan Police Serious Crime Unit operational trial report on DFO, April. London: Metropolitan Police.
4. **Frank, A., Grant, H. and Almog, J.** (1989) *The Comparison Between 1,8-Diazafluoren-9-one (DFO), Ninhydrin and 5-Methoxyninhydrin in the Detection of Latent Fingerprints*, Israeli Police Research Report DM/0604. Israel: Israeli Police.
5. **Burridge, P., Lavis, A. and Murphy, K. A.** (1990) *An Investigation of the new Fingerprint Reagent DFO*, Research Report, March. New Zealand: Department of Scientific and Industrial Research.
6. **Pounds, C. A.** (1990) *Ninhydrin Analogues and their use in the Fluorescent Detection of Latent Fingerprints*, HO CRE Report. London: Home Office.

7. **Pounds, C. A., Grigg, R. and Mongkolaussavaratana, T.** (1990) 'The Use of 1,8-diazafluoren-9-one (DFO) for the Fluorescent Detection of Latent Fingerprints on Paper. A Preliminary Investigation', *J. Forens. Sci. Soc.*, vol. 35 (1), pp 169–175.
8. **McComisky, P.** (1990) 'DFO – A Simple and Quick Method for the Development of Latent Fingerprints', *Fingerprint Whorld*, vol. 16 (62), pp 64–65.
9. **Anon.** (1990) 'DFO', *Minutiae*, Fall 1990, pp 2–3.
10. **Anon.** (1990) 'Forensic Light Sources and DFO', *Minutiae*, Winter, p 2.
11. **Grigg, R., Mongkolaussavaratana, T., Pounds, C. A. and Sivagnanam, S.** (1990) '1,8-Diazafluorenone and Related Compounds. A New Reagent for The Detection of α -Amino Acids and Latent Fingerprints', *Tetrahedron Letters*, vol. 31 (49), pp 7215–7218.
12. **Lennard, C.** (1991) *Evaluation of the Sensitivity of Amino Acid Specific Reagents*, Institut de Police Scientifique et de Criminologie Report. Switzerland.
13. **Corson, W. B., Lawson, J. E. and Kuhn, K. E.** (1991) 'Alternative Applications of DFO for Non-fluorescent Visualisation', *J. Forens. Ident.*, vol. 41 (6), p 437–445.
14. **Masters, N., Morgan, R. and Shipp, E.** (1991) 'DFO, its Usage and Results', *J. Forens. Ident.*, vol. 41 (1), pp 3–10.
15. **Hardwick, S., Kent, T., Sears, V. and Winfield, P.** (1993) 'Improvements to the Formulation of DFO and the Effects of Heat on the Reaction with Latent Fingerprints', *Fingerprint Whorld*, vol. 19 (73), pp 65–69.
16. **Stoilovic, M.** (1993) 'Improved Methods for DFO Development of Latent Prints', *Forens. Sci. Int.*, vol. 60, pp 141–153.
17. **Cantu, A. A., Leben, D. A., Joullie, M. M., Heffner, R. J. and Hark, R. R.** (1993) 'A Comparative Examination of Several Amino Acid Reagents for Visualising Amino Acid (Glycine) on Paper', *J. Forens. Ident.*, vol. 43 (1), pp 44–67.
18. **Lennard, C. and Mazella, W.** (1995) 'Evaluation of Freon-free Fingerprint Reagent Formulations', Proc. Meet., *Int. Assoc. Forens. Sci.*, vol. 4, pp 296–301.
19. **Rajtar, P. E.** (2000) '3M Novec™ Engineer Fluid HFE-7100', *Fingerprint Whorld*, vol. 26 (102), pp 143–152.

20. **Didierjean, C., Debart, M. – H. and Crispino, F.** (1998) 'New Formulation of DFO in HFE7100', *Fingerprint Whorld*, vol. 24 (94), pp 163–167.
21. **Merrick, S., Gardner, S., Sears, V. and Hewlett, D.** (2002) 'An Operational Trial of Ozone-friendly DFO and 1,2-Indandione Formulations for Latent Fingerprint Detection', *J. Forens. Ident.*, vol. 52 (5), pp 595–605.
22. **Sears, V. G. and Hewlett, D. F.** (2003) 'DFO Formulations in Non-ozone Depleting Solvents', *Ident. Canada*, vol. 26 (1), pp 4–12.
23. **Wilkinson, D.** (2000) 'A Study of the Reaction Mechanism of 1,8-Diazafluoren-9-one with the Amino Acid, L-Alanine', *Forens. Sci. Int.*, vol. 109 (2), pp 87–102.
24. **Wilkinson, D.** (2000) *Synthesis of 1,8-Diazafluoren-9-one (DFO) Analogues*, draft paper. Canada: Royal Canadian Mounted Police.
25. **Conn, C., Ramsey, G., Roux, C. and Lennard, C.** (2001) 'The Effect of Metal Salt Treatment on the Photoluminescence of DFO-treated fingerprints', *Forens. Sci. Int.*, vol. 116, pp 117–123.
26. **Fregau, C. J., Germain, O. and Fourney, R. M.** (2000) 'Fingerprint Enhancement Revisited and the Effects of Blood Enhancement Chemicals on Subsequent Profiler Plus Fluorescent Short Tandem Repeat DNA Analysis of Fresh and Aged Bloody Fingerprints', *J. Forens. Sci.*, vol. 45 (2), pp 354–380.
27. **PSDB** (2003) *Fingerprint Development and Imaging Update*, PSDB Publication No. 6/2003, April. London: Home Office.
28. **Strzelczyk K** (2000) *Ninhydrin Formulations on Handwriting Evidence*, Report by Document Evidence Ltd for PSDB.
29. **Wallace-Kunkel, C., Roux, C., Lennard, C. and Stoilovic, M.** (2004) 'The Detection and Enhancement of Latent Fingermarks on Porous Surfaces – A Survey', *J. Forens. Ident.*, vol. 54 (6) pp 687–705.
30. **Bickell, D. E. and Ramotoski, R. S.** (2008) 'Use of an Optimised 1,2-Indanedione Process for the Development of Latent Prints', *J. Forens. Sci.*, vol. 53 (5), pp 1–9.
31. **Sears, V., Batham, R. and Bleay, S.** (2009) 'The Effectiveness of 1,2-Indandione-Zinc Formulations and Comparison with HFE-Based 1,8-diazafluoren-9-one for Fingerprint Development', *J. Forens. Ident.*, vol. 59 (6), pp 654–678.
32. **Stoilovic, M., Lennard, C., Wallace-Kunkel, C. and Roux, C.** (2007) 'Evaluation of a 1,2-Indanedione Formulation Containing Zinc Chloride

- for Improved Fingermark Detection on Paper’, *J. Forens. Ident.*, vol. 57 (1), pp 4–18.
33. **Bratton, R. M. and Juhala, J. A.** (1995) ‘DFO-Dry’, *J. Forens. Ident.*, vol. 45 (2), pp 169–172.
34. **HOSDB** (2006) *Fingerprint and Footwear Forensics Newsletter*, HOSDB Publication No. 58/06, October. London: Home Office.
35. **Bola, S. S.** (1990) *An Investigation of Ninhydrin on Brown and Coloured Envelopes, and to Compare with DFO, a Possible Alternative, and Subsequent Ninhydrin Treatment*, PSDB Student Placement Report.
36. **Wilkinson, D., McKenzie, E., Leech, C., Mayowski, D., Bertrand, S. and Walker, T.** (2003) ‘The Results from a Canadian National Field Trial Comparing Two Formulations of 1,8-Diazafluoren-9-one (DFO) with 1,2-Indandione’, *Ident. Canada*, vol. 26 (2), pp 8–18.
37. **Lee, J. L., Bleay, S. M., Sears, V. G., Mehmet, S. and Croxton, R.** (2009) ‘Evaluation of the Dimethylcinnamaldehyde contact transfer process and its application to fingerprint development on thermal papers’, *J. Forens. Ident.*, vol. 59 (5), pp 544–569.