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The Results from a Canadian National Field Trial Comparing 1,8-Diazafluoren-9-one (DFO) with Ninhydrin and the Sequence DFO Followed by Ninhydrin.

Prepared by

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**The Results from a Canadian National Field Trial
Comparing 1,8-Diazafluoren-9-one (DFO) with Ninhydrin
and the Sequence DFO Followed by Ninhydrin.**

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Abstract

This paper describes the Canadian National Field Trials comparing 1,8-diazafluoren-9-one (DFO), ninhydrin and the sequence DFO followed by ninhydrin. The solutions were used to process casework exhibits in three RCMP and one OPP Forensic Identification Sections located across the country. The sequence DFO followed by ninhydrin recovered the highest number of latent fingerprints.

Introduction

Forensic Identification Sections (FIS) have been using ninhydrin to recover latent fingerprints since the 1950s [1]. Ninhydrin reacts with amino acids present in the sweat residue of a latent fingerprint to produce the familiar coloured reaction product, Ruhemann's Purple [2]. After the porous substrate has been immersed in the ninhydrin working solution, it is heated in an oven at 80 °C and 65% relative humidity [3]. These conditions speed up the reaction between ninhydrin and the amino acids resulting in visible fingerprints usually within 20 minutes. Ruhemann's Purple is not a fluorescent compound but it can be further treated with a solution of zinc chloride which renders it fluorescent [4]. Over the years ninhydrin has proven to be a very reliable, effective reagent for porous surfaces that is easy to apply and relatively inexpensive. The reaction pathway of ninhydrin with amino acids has been

thoroughly studied over the years and each intermediate product in the reaction mechanism has been identified [5-6].

DFO use by forensic identification specialists began in the 1990s [7]. DFO also reacts with amino acids, but unlike ninhydrin, a fluorescent product is formed that is often only visible under blue/ green light [3]. Immediate examination under white light can mislead forensic identification specialists into thinking that no reaction has occurred. Porous exhibits once treated with DFO must be heated in a dry oven since humidity is detrimental to the reaction [8]. For this reason, and the fact that DFO has proven to be more sensitive to amino acids than ninhydrin, it is recommended that DFO be used in a sequence prior to ninhydrin [3]. In contrast to ninhydrin, the reaction mechanism and analogues of DFO have not received much attention in the ten years since its introduction into the forensic community [9].

1,2-Indanedione made its debut as another reagent for developing fingerprints on porous surfaces in 1997 [10]. It has similarities to both ninhydrin and DFO; it produces a pink-coloured product more in keeping with ninhydrin, and this product is also fluorescent which makes it resemble DFO [11]. The development conditions for 1,2-indanedione vary according to climate and paper acidity [12-14]. In Israel, for example, it is necessary to increase humidity in the oven during development [13]. In the UK it is necessary to add acetic acid to the formulation since UK paper stock tends to be less acidic than the Israeli stock [14]. Studies into the performance of 1,2-indanedione are

ongoing but the results to date are mixed [12, 14-15]. The chemical structures of all three amino acid reagents are shown in Figure 1.

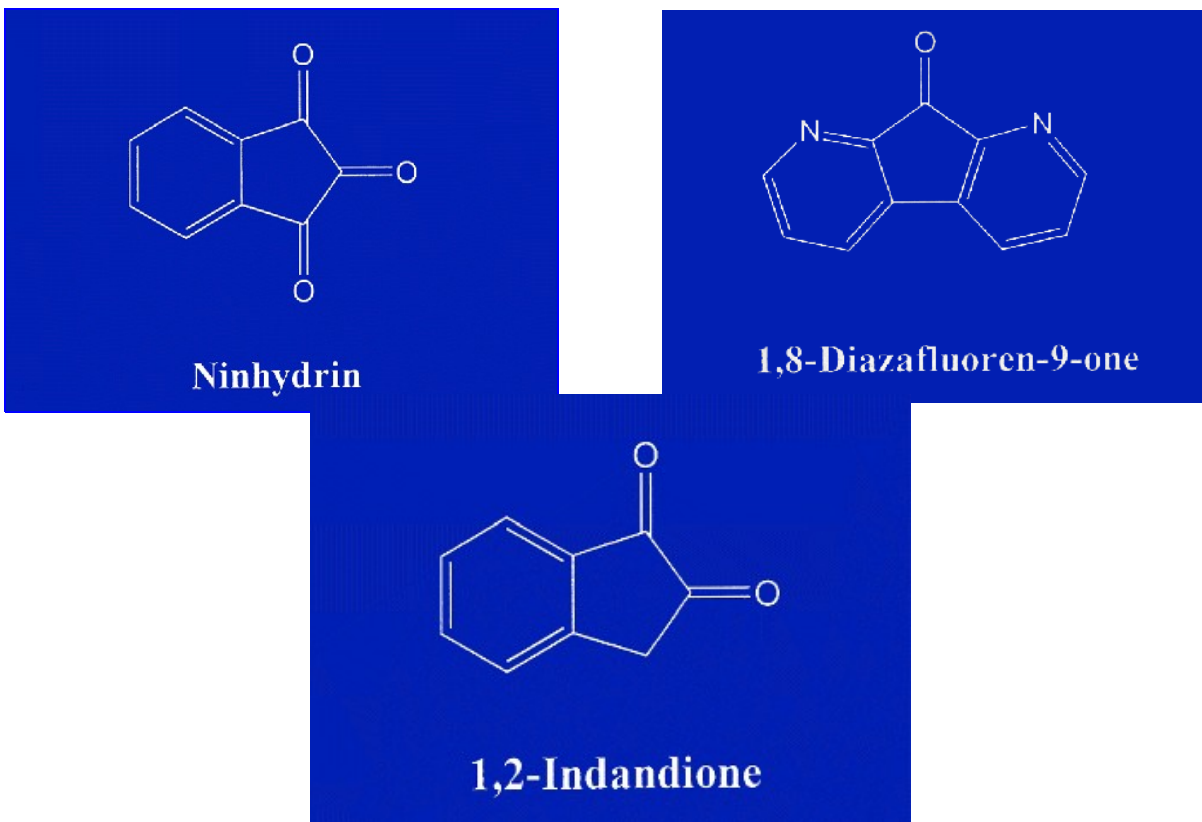


Figure 1: Chemical structures of ninhydrin, DFO and 1,2-indandione.

Original formulations of DFO involved 1,1,2-trichlorotrifluoroethane (more commonly known as Freon 113 or CFC113). CFC113 was the perfect solvent for the treatment of porous surfaces since it is non-flammable, non-toxic, highly volatile, does not cause ink running and produces stable solutions. Environmental concerns with CFCs and their detrimental effects on the ozone layer, however, resulted in the banning of CFC113 in most industrialized countries under the Montreal Protocol signed in 1987 [16].

In the 1990s fingerprint research groups attempted to find replacement solvents for many popular fingerprint reagents that utilized CFC113, including DFO [16]. Heptane [17], ethers [18], hydrofluoroethers (HFEs) and hydrofluorocarbons (HFCs) [19] were all used to prepare solutions of ninhydrin and DFO. HFEs and HFCs were judged to be particularly good candidates because they have similar physical properties to CFCs, i.e. non-flammable, non-toxic, and highly volatile [16]. In 1997 a new ninhydrin formula was introduced in which CFC113 had been replaced by HFE 7100 [20]. In 1998, a new formulation of DFO was published that simply substituted the CFC113 in the original formulation with HFE 7100 [21]. After several years of laboratory research and an extensive national field trial, researchers at the Police Scientific Development Branch (PSDB) eventually recommended a new formulation for DFO involving the solvent HFE 7100 and a second hydrofluoroether, HFE 71DE [22]. HFE 71DE is an azeotropic mixture containing 50% HFE 7100 and 50% 1,2-*trans*-dichloroethylene. Interestingly 1,2-*trans*-dichloroethylene and CFC113 have two chlorine atoms with similar spacial relationships.

The re-formulation of DFO turned out to be more complicated than first expected. The solvent appeared to play a role in the DFO reaction that was not observed for ninhydrin. It was apparent that gaining an understanding of the DFO and amino acid reaction could provide insight into these differences. A research study into the reaction mechanism of DFO with the amino acid, 1-

alanine has since been published [9]. This study showed that DFO reacts with amino acids in a similar manner to ninhydrin to form a reaction product (see Figure 2) similar to Ruhemann's Purple. One interesting observation from this research was that DFO reacted with alcohols such as methyl alcohol to form unstable intermediates known as hemiketals. Interestingly, 20% hemiketal was formed with methyl alcohol, 10% formed with ethyl alcohol and no reaction was observed with butyl alcohol. Researchers at the PSDB observed a drastic reduction in performance when ethyl alcohol was used instead of methyl alcohol, and no reaction at all with butyl alcohol [23]. Therefore the formation of these hemiketals appears to be important for the reaction between DFO and amino acids.

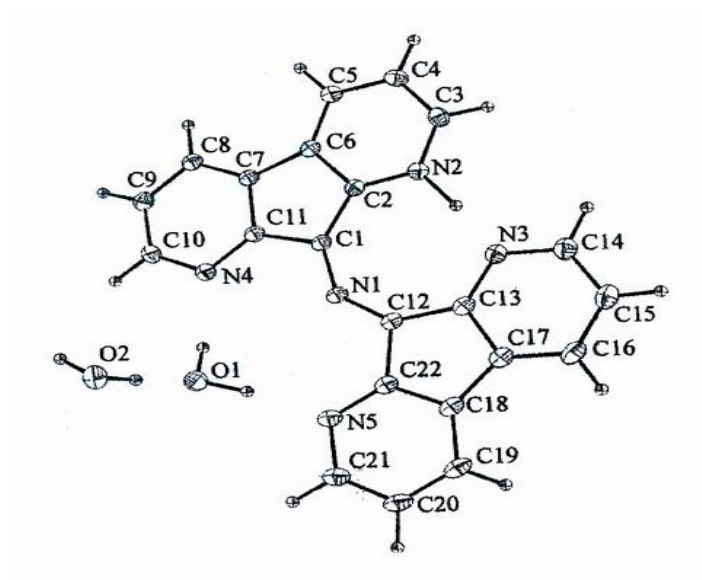


Figure 2: Chemical structure of the reaction product from DFO and the amino acid, *l*-alanine.

In a separate study it was shown that 1,2-indanedione reacts with methyl alcohol to form 100% hemiketal [24]. This hemiketal is presumed to be more stable than the DFO version since its formation prevents reaction with amino acids completely. Therefore it was recommended that alcohols not be used to formulate 1,2-indanedione formulations [24].

A previous year-long Canadian field trial was published and showed that DFO formulated with HFE 7100 and HFE 71DE found more identifiable latent fingerprints than the formulation involving only HFE 7100 [15]. Both formulations of DFO significantly outperformed 1,2-indanedione, which was not recommended for field use.

The differences observed between the Israeli and UK research groups regarding 1,2-indanedione formulations and development conditions highlight the need to perform regional field trials in order to be confident that the solutions recommended in Canada are truly the best for that region of the country. This was the rationale behind the first field trial and it is the rationale behind the current one. The sequential use of DFO followed by ninhydrin exists in RCMP Policy but, under Canadian conditions, do the results justify the extra time and costs involved? Perhaps the more important question is how many fingerprints are missed if a forensic identification specialist were to use only one of these procedures?

Experimental

DFO, Ninhydrin and HFE 7100 were purchased from Forensi-Tech (Ottawa, Ontario). Acetic acid, ethyl acetate and methyl alcohol were purchased from Fisher Scientific. Absolute ethyl alcohol (product number A016EAAN) was purchased from Commercial Alcohol (1-800-256-3149) and HFE 71 DE was purchased from VWR Canlab. All chemicals and solvents were used without further purification.

Preparation of Solutions:

DFO: 1.0g of DFO was placed in a clean, dry 1 litre beaker and 120 mL of methanol was added, followed by 80 mL of glacial acetic acid. The beaker was covered and the mixture stirred using a magnetic stirrer until all the DFO particles had dissolved (approximately five minutes). This clear yellow solution was poured into a 4 litre bottle with a screw-top lid. In a clean, dry 4 litre brown bottle, 1.2 litres of HFE 71DE was mixed with 2.8 litres of HFE 7100. The solvent mixture was added to the clear yellow solution and stirred for thirty minutes using a magnetic stirrer.

Ninhydrin: 25.0g of ninhydrin were added to a clean, dry 1 litre glass beaker. Absolute ethyl alcohol (225 mL) was added, followed by 10 mL of ethyl acetate and 25 mL glacial acetic acid. The solution was stirred using a magnetic stirrer until the ninhydrin was completely dissolved (approximately one minute). 208 mL of the solution was poured into a 5-litre glass flask and 4

litres of HFE 7100 were added. The solution was covered and stirred for thirty minutes using a magnetic stirrer and poured into a clean 4 litre brown bottle.

National Field Trials

Three RCMP and one OPP Forensic Identification Sections (FIS) participated in the National Field Trial. The DFO and ninhydrin solutions were prepared by one of the authors (DW) in Ottawa to ensure consistency and shipped on an as needed basis to the different locations. The locations included Moncton, New Brunswick; Ottawa, Ontario; Orillia, Ontario, and Edmonton, Alberta. A west coast location was not possible for this field trial due to re-organization of the RCMP's FIS in the lower mainland. Local city departments were contacted but felt that the volume of porous exhibits was too unpredictable to provide useful information. The forensic identification specialists were instructed to treat incoming cases with one of the following three protocols on a rotating basis:

Protocol #1: DFO only;

Protocol #2: Ninhydrin only;

Protocol #3: DFO followed by ninhydrin.

Over time, cases were distributed in such a way as to ensure an even grouping of substrate types (i.e. cardboard, currency, paper, etc.) between the three protocols as well as a similar number of exhibits. After DFO treatment the exhibits were examined under blue and green light using KV 550 nm and

Wratten No. 29 viewing filters, respectively. The number of exhibits that developed fingerprints was recorded along with the total number of fingerprints found on each exhibit. After ninhydrin treatment the exhibits were examined under white light and the number of exhibits with fingerprints as well as the number of fingerprints were recorded. The field trial ran from February 2003 to January 2004 to ensure that all seasonal meteorological conditions were considered.

Results

The number of exhibits that were examined by each protocol, over the course of the year-long study for Moncton FIS is shown in Figure 3a.

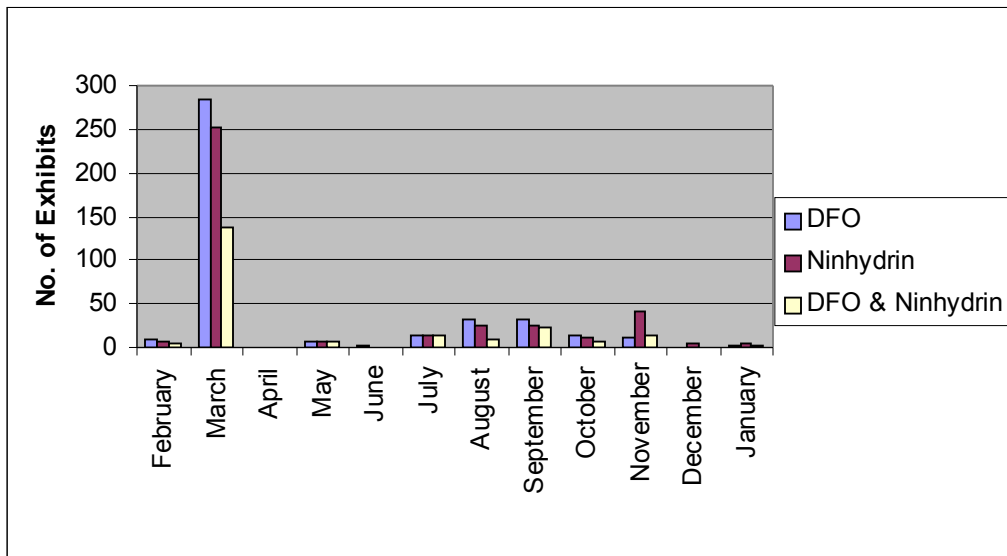


Figure 3a: Number of exhibits processed monthly by DFO, ninhydrin and DFO followed by ninhydrin for Moncton FIS (2003 - 2004).

The number of identifiable and non-identifiable latent fingerprints (combined data will subsequently be referred to as number of fingermarks) that were recovered from these exhibits over the course of the study for Moncton FIS is shown in Figure 3b.

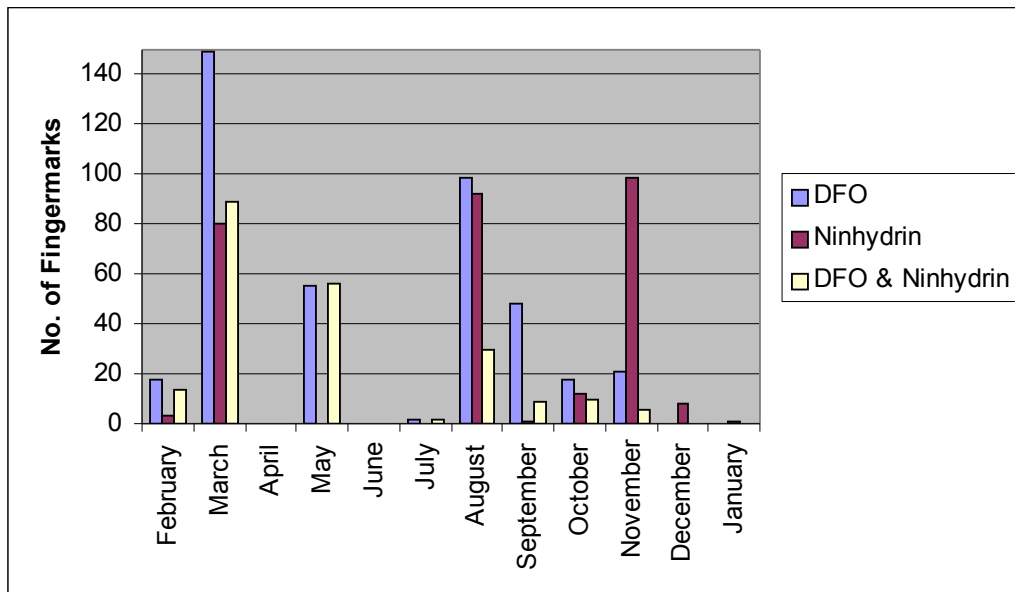


Figure 3b: Number of fingerprints recovered monthly by DFO, ninhydrin and DFO followed by ninhydrin for Moncton FIS (2003-2004).

A comparison between figure 3a and 3b indicates that there were four occasions where the large number of fingerprints recovered does not correspond to a large number of exhibits. These inconsistencies can be explained by the recovery of an unusually large number of fingerprints from a small number of exhibits (see Table 1, at end of text). The May results for protocol #1 (DFO only) indicate 8 identifiable and 39 non-identifiable fingerprints were recovered from three exhibits. Dividing the number of fingerprints by the number of exhibits provides a crude measure of performance by indicating the number of fingerprints per exhibit. The ratio, for identifiable and non-identifiable fingerprints per exhibit for this data, typically ranged between 0 and 5. In Table 1, the text shown in bold for the non-identifiable fingerprint, clearly falls outside of this range.

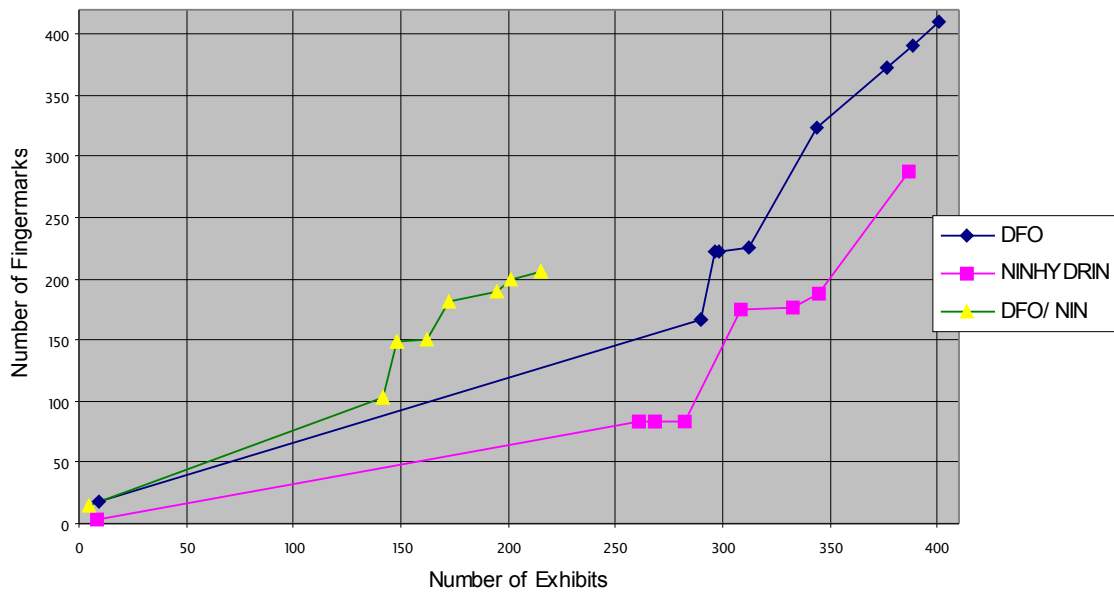


Figure 4a: Regional performance for Moncton FIS (uncorrected data).

The plot of the number of fingerprints against the number of exhibits is shown in Figure 4a for Moncton FIS. The data in Table 1 are indicated by steeper gradients in the plots. Only one case produced an unusually high

number of fingermarks as indicated in bold in Table 1. As can be seen from Figure 4b removing this data point from the data set does not significantly alter the results.

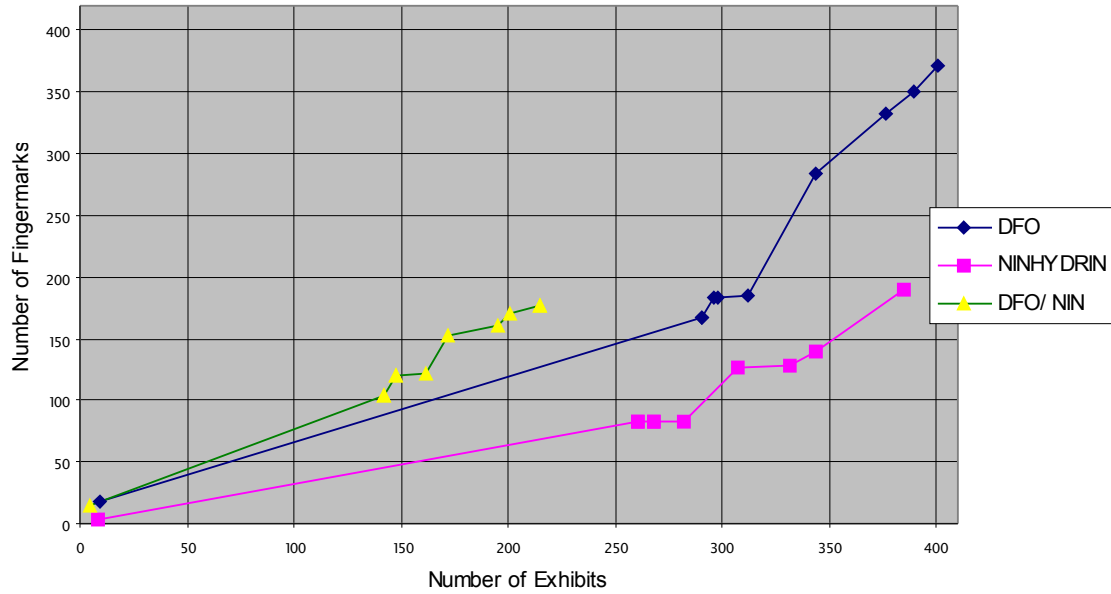


Figure 4b: Regional performance for Moncton FIS (corrected data).

The number of exhibits that were processed using all three protocols in the OPP Orillia FIS is shown in Figure 5a and the number of fingerprints

recovered is illustrated in Figure 5b.

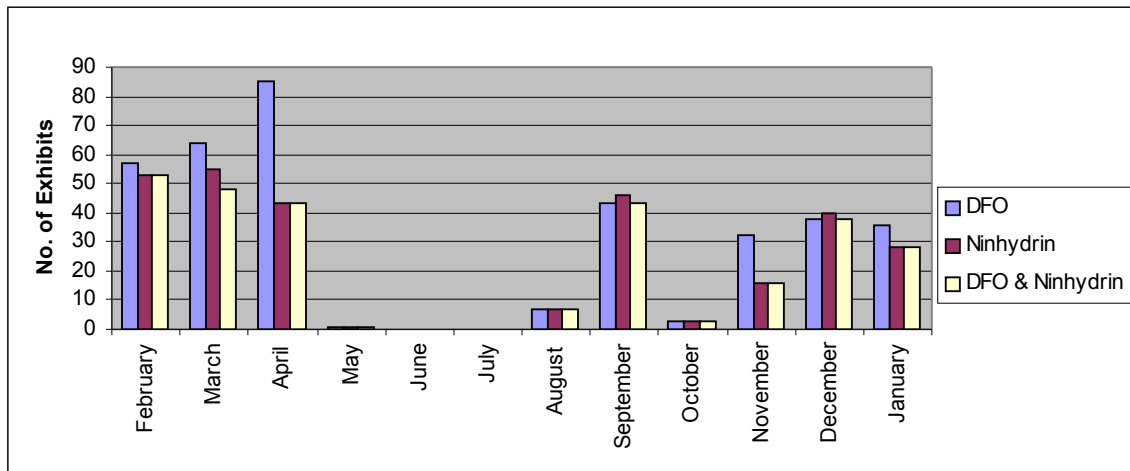


Figure 5a: Number of exhibits processed monthly by DFO, ninhydrin and DFO followed by ninhydrin for Orillia FIS (2003-2004).

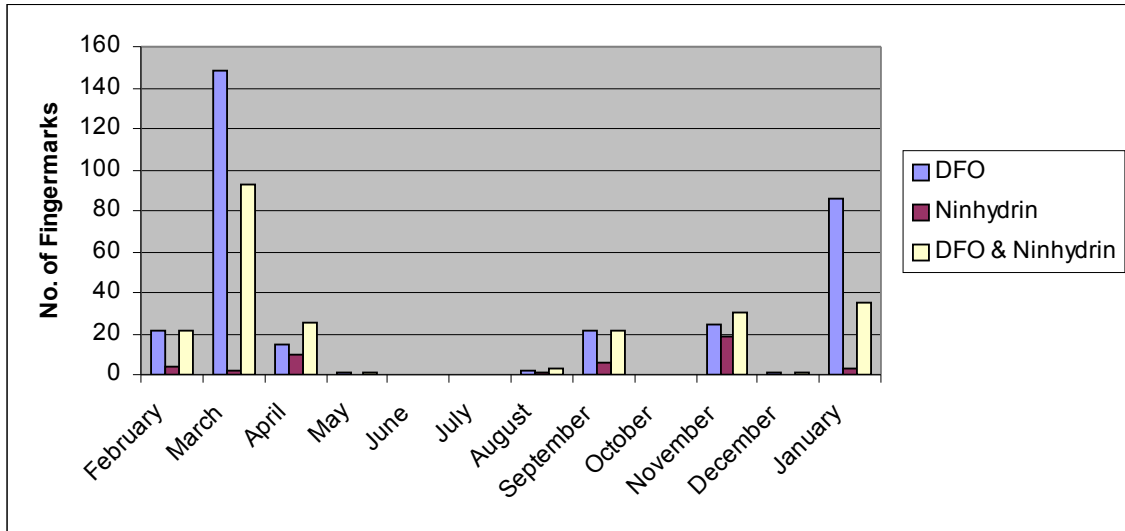


Figure 5b: Number of fingermarks recovered monthly by DFO, ninhydrin and DFO followed by ninhydrin for Orillia FIS (2003-2004).

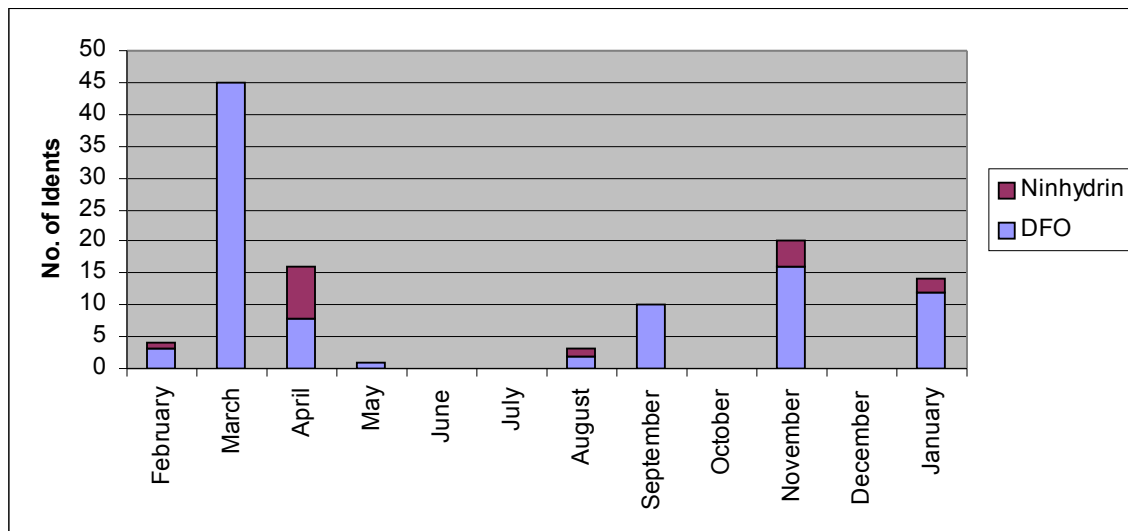


Figure 5c: Number of identifiable fingerprints recovered with DFO and then with ninhydrin for Orillia FIS (2003-2004).

The number of additional fingermarks that were recovered when exhibits were treated with ninhydrin after DFO for Orillia is shown in Figure 5c.

The plot of the number of exhibits against the number of fingermarks recovered for Orillia is shown in Figure 6.

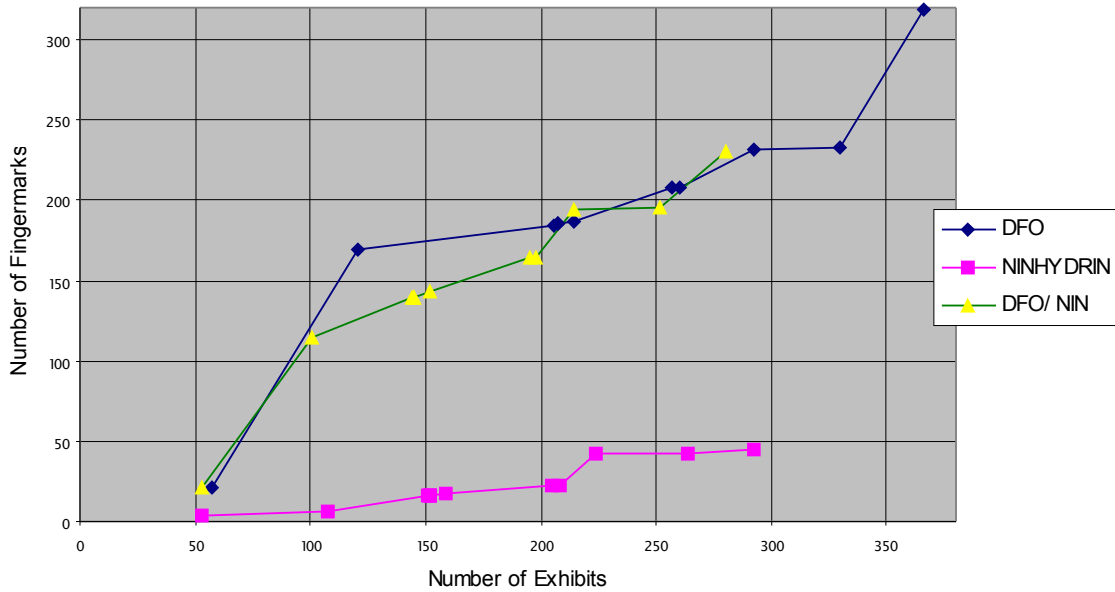


Figure 6:Regional performance of DFO, ninhydrin and DFO followed by ninhydrin for Orillia FIS.

The number of fingerprints that were obtained for each of the three protocols used in the National Field Trials is plotted against the total number of

exhibits examined for all of the sections involved in the field trial (Figure 7a). A similar plot showing the number of criminal identifications is shown in Figure 7b.

Table 2 (see end of text) shows the number of latent fingerprints recovered by the three protocols after 600 exhibits were processed by each.

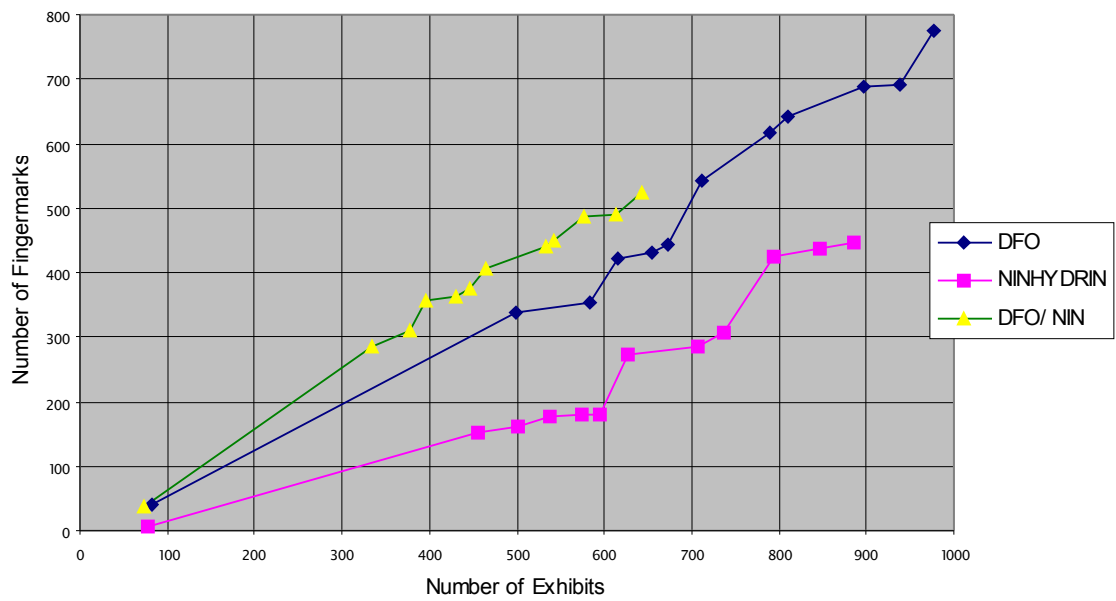


Figure 7a: National performance for DFO, ninhydrin and DFO followed by ninhydrin (identifiable and non-identifiable latents).

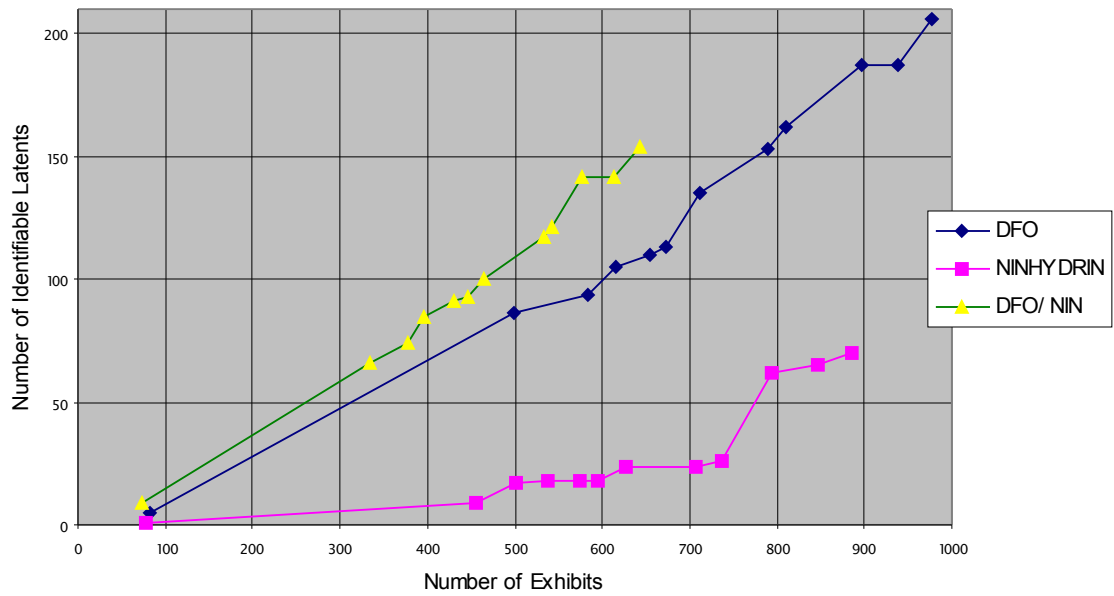


Figure 7b: National performance for DFO, ninhydrin and DFO followed by ninhydrin (identifiable latents).

Discussion.

It should be noted that RCMP Forensic Identification Sections are not routinely equipped with ovens that can accurately maintain the temperature and relative humidity. Paper exhibits that require heat are often left in dark storage for a few weeks to develop at a slower reaction rate, or heated cautiously in a heat press set at 100 °C for 10 minutes. This lack of standardized development conditions could well influence the field trial results. However, since these are the working conditions frequently experienced in Canada, a comparison of the different reagents for the recovery of fingerprints from porous exhibits under such circumstances was felt to be worthwhile.

All data was analyzed for inconsistencies between the number of exhibits processed and the number of fingermarks recovered. The data from Moncton required further examination as shown in Table 1. However a comparison between the uncorrected data (Figure 4a) and the corrected data (Figure 4b) showed no significant difference between them. All subsequent graphs use the raw data.

The results observed in the national field trial (Figure 7) suggest that more fingermarks will be recovered when porous exhibits are treated first with DFO and then with ninhydrin. All data suggest that DFO is more sensitive than ninhydrin with the Orillia results indicating equal performance of DFO compared to the sequence DFO followed by ninhydrin. Although DFO is consistently shown to be more sensitive than ninhydrin, additional fingermarks

are frequently recovered when exhibits processed with DFO are further treated with ninhydrin (Figure 5c).

Conclusion

As a result of this field trial the RCMP will be recommending the sequential use of DFO followed by ninhydrin. The DFO and ninhydrin formulations described in this paper are recommended as the RCMP national standard. When using DFO it is essential to heat the exhibits in a dry environment and to examine the exhibits under the blue and green light of a Forensic Light Source using orange (KV 550nm) and red (Wratten #29) viewing goggles respectively.

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List of Tables:

Table 1: Adjustments to Moncton Data.

Table 2: Performance after 600 exhibits.

Table 1: Adjustments to Moncton Data.

Month/ Protocol	No. of identifiable fingerprints	No. of non- identifiable fingerprints	No. of exhibits	No. of identifiable fingerprints per exhibit	No. of non- identifiable fingerprints per exhibit
May/ #1	8	39	3	3 (8/ 3)	13 (39/3)
Aug/ #1	15	69	22	0.7 (15/ 22)	3 (69/22)
Aug/ #2	5	87	23	0.2 (5/ 23)	4 (87/ 23)
Nov/ #2	27	72	28	1 (27/ 28)	3 (72/ 28)

Table 2: Performance after 600 exhibits.

Protocol	DFO/ NINHYDRIN (Protocol #3)	DFO (Protocol #1)	NINHYDRIN (Protocol #2)
No. of exhibits	600	600	600
No. of fingermarks	460	370	180
Performance Rank	1 st	2 nd	3 rd