

## Comparison of ThermaNin and Ninhydrin Diluted Solutions to Develop Fingermarks on Thermal Paper

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**ABSTRACT:** Issues regarding blackening of thermal paper during fingerprint evidence development and collection have been encountered by national and international experts. To effectively develop fingerprints of high clarity, it is necessary to adjust the formulation of ninhydrin, reduce the surface damage of thermal paper by using contactless methods, or remove and rinse off black interferences using strong solvents. This research specifically targeted forensic laboratories with simple and inexpensive equipment, and without special vacuum instruments. Considering this and the diverse variety of thermal papers, facsimile thermal paper which can easily discolor and is unsuitable for long-term storage was selected as the experiment material. This research was aimed to compare the overall quality of latent fingerprint development techniques using the original petroleum ether-based ninhydrin and commercially available ThermaNin. Using a six-time diluted original petroleum ether-based ninhydrin and ThermaNin (in isopropanol or petroleum ether), the diluted original petroleum ether-based ninhydrin had the best development of latent fingerprints on thermal paper. The diluted original petroleum ether-based ninhydrin was also more convenient to store and prepare compared to ThermaNin. Hence, for the development of latent fingerprint evidence on thermal paper, forensic crime laboratories without special equipment can use the original ninhydrin formulation coupled with the principles of dilution and control of the organic solvent used (by applying the original formulation and adjusting the dilution ratio, as well as dispensing the treatment solution on the back of the paper with a pipette dropper) to achieve high effectiveness, increased storability, and increased safety.

**Keywords:** forensic science; fingerprint; thermal paper; ninhydrin; ThermaNin

### Introduction

Nowadays, commercial transactions can occur anytime and anywhere. The use of thermal paper has thus become more frequent given its convenience and simplicity. Thermal paper is commonly coated with a special heat-sensitive ink which turns black when exposed to high heat. It was initially used in fax/facsimile machines which applies heat to thermal paper in order to create the text. Gradually, the uses of thermal paper now include, but is not limited to receipts, ATM bills, credit card bills, and convenience store invoices. In addition, examples of thermal papers also include receipts for fraud cases, robbery cases, murder cases and purchase of murder weapons. Complex, multi-purpose/function thermal paper is also used for lottery tickets, high-speed railway tickets, and flight tickets. As such, thermal papers are frequently encountered as

evidence by investigators due to its wide and common use.

The increased functionality and storability of thermal paper are associated with higher complexity and increased variation of its internal structure. Thermal paper has been frequently encountered by forensic experts and has been collected as evidence. However, issues regarding latent fingerprint visualization and enhancement have also surfaced, owing to the diverse variety of thermal paper. For instance, amino acid reaction reagents (e.g., ninhydrin, 1,8-diazafluoren-9-one [DFO], 5-methylthioninhydrin [5-MTN], and 1,2-indanedione) on a paper substrate might cause damage to the surface layer and thus blacken thermal paper, leading to failure in latent fingerprint visualization and development [1-3]. However, this does not occur in all cases, and it often confuses first-line investigators during latent fingerprint development and

evidence collection. The main issue regarding latent fingerprint development on thermal paper, which is whether it would also turn black, is also closely linked to the application method used for the chemicals.

The polar solvent present in fingerprint development reagents may react with the components of thermal paper and blacken thermal paper. This issue has been a global concern among experts in this field of forensics. According to a literature review, different interventions/techniques used were differentiated and categorized as follows:

- i. Utilization of original ninhydrin method [4], coupled with fluid 3M™ Novect™ Hydrofluoroether products (HFE-7100, HFE-71IPA, etc.) in the formulation, with an appropriate ratio to effectively reduce the damage to the surface layer of thermal paper. The disadvantages of this method are that the cost of HFE-7100 is higher than that of commonly used petroleum ether, and real-life practice still shows uncontrollable blackening of thermal paper having simple structures (e.g., ATM receipts and parking receipts).
- ii. Utilization of the “sandwich,” i.e., blotter or dry contact method [5-6]. The development reagents (dimethylaminocinnamaldehyde [DMAC] and 1,2-indanedione) are added to the blotter paper, and the solvents allowed to dry. Then, evidence samples to be processed are placed in between blotter papers to form a “sandwich” in a zipper bag. The development process takes a few days to complete. The disadvantages include the difficulty controlling the appropriate quantity of reagents added. An excessive amount will cause blackening, while an insufficient amount will result in uneven treatment and ineffective latent fingerprint enhancement.
- iii. Utilization of the chemical (e.g., DMAC, ninhydrin, and iodine) fuming method [7-10] with the help of special equipment (chamber, vacuum, or heating) facilitates the heating and vaporization of DMAC (reacts with urea present in a fingerprint), ninhydrin, iodine, etc. The use of low-vacuum technology for ninhydrin vaporization does not require the presence of an organic solvent. Ninhydrin reacts with amino acids present in a latent fingerprint, thus allowing visualization of friction ridge details. However, the equipment required is less readily accessible and it is less convenient to use during crime scene investigation.
- iv. Utilization of organic solvent fuming or solvent-free low-temperature heating method allows for fingerprint development. Typically, thermal papers will have a layer of protective coating which prevents unintended discoloration due to heat from external sources, such as the ambient temperature. Hence, the absence of a protective layer would make thermal paper prone to discoloration. Chemicals such as acetic acid (which are included in the formulation of ninhydrin solutions) have a high volatility. Thus, they may damage the protective layer on the thermal paper, making it more susceptible to discoloration. Friction ridges could be visualized as the escaping vapors of such liquids react with components at the locations where fingerprints reside [11-12]. Moreover, following the application of a solvent-free low-temperature heating technique (e.g., a hair dryer), fingerprints could be developed on thermal paper [13-15]. However, both approaches are considered potentially destructive to evidence, and the extent of reaction is difficult to control. Over-treatment can easily occur and cause blackening of the entire documentary evidence. This can cause damage to the protective layer of thermal paper, reduce its protective function, and increase likelihood of the reaction occurring at the inner reactive layer.
- v. Bleaching is divided into different approaches of treatment as follows: rinsing off the darkened components prior to visualization of fingerprints; development of fingerprints with commonly used reagents before rinsing off the darkened components; or introduction of reagents that help prevent blackening [16-20]. Concerns regarding this method include fingerprint ridge fading and weakening.
- vi. Ninhydrin derivative (ThermaNin) [21] is commercially manufactured by BVDA International. ThermaNin is a hemiketal generated by a heated reaction between ninhydrin and alcohol. It is now used for fingerprint development on thermal paper.
- vii. The use of near infrared (NIR) visualization method is considered non-invasive and non-destructive to the evidence on the thermal paper being

- examined. Nevertheless, the requirement of special instruments and controlled operational conditions makes the technique unsuitable for real-life laboratory practice [22-23].
- viii. Utilization of a dilution method is currently applied [24]. Although this method is as well categorized as formulation dilution adjustment, what makes it special is that in this method, the concentration of polar solvents used is known to contribute to the blackening of thermal paper. Thus, this method is suitable for development processes that involve reactions with amino acids (e.g., 1,2-indanedione, DFO, and 5-MTN). The original formulation of ninhydrin enables latent fingerprint development on thermal paper without it turning black, by diluting the corresponding key solvent, which is associated with blackening, below a certain concentration. This method is considered highly flexible and convenient for laboratory practice.

Although ninhydrin has many disadvantages, if the blackening of thermal paper which leads to obscured fingerprint ridges can be overcome, it remains a highly sensitive amino acid reaction reagent for the development of latent fingerprint on thermal paper. Hence, this study aims to analyze and compare different methods for latent fingerprint development on thermal paper without substrate blackening. This study especially targets forensic laboratories with simple and inexpensive equipment and without a special vacuum instrument. Considering this and the diverse variety of thermal paper, facsimile thermal paper which easily discolors and is unsuitable for long-term storage was selected as one of the experiment materials. This research compares the overall quality of latent fingerprint development using original petroleum ether-based ninhydrin, diluted formulation, and commercially available Thermanin. This study provides suggestions regarding thermal paper evidence collection for agencies with small operational forensic crime laboratories.

## Materials and Methods

### *Chemicals and materials*

Ninhydrin and Thermanin were manufactured by BVDA International (Haarlem, the Netherlands). All solvents used were first-grade (extra-pure) reagents. Acetone,

isopropanol, and petroleum ether were manufactured by Shimadzu Chemical (Osaka, Japan). Ethyl acetate and acetic acid were manufactured by Katayama Chemical (Osaka, Japan). Ethanol was manufactured by Taiwan Tobacco & Liquor Corporation (Taipei City, Taiwan).

For photography, a Nikon D90 DSLR camera (Tokyo, Japan) was used with a 60-mm AF Micro NIKKOR lens (Nikon). The parameter settings were as follows: M-mode, shutter speed, 1/250; aperture, F/8.0; ISO 200. Facsimile thermal paper from the Japanese brand Howen was used. This brand of thermal paper is frequently used in fax machines in Taiwan and turns black easily. Hence, if the issues for fingerprint development in thermal paper can be overcome, the solution will be suitable for use in operational units in Taiwan. For pseudo-operational trials, we also collected receipts for payment, cash withdrawal, and parking.

### *Fingerprint deposition*

Three male participants were recruited as fingerprint donors. The donors' participation was voluntary. Thermal paper substrates were cut into rectangular stripes. Before fingerprint deposition, participants washed their hands with detergent. They were then instructed to climb the stairs until they sweat profusely. After which, fingerprints were deposited consecutively on prepared thermal paper. Each fingerprint deposition lasted for approximately 1 second. As it is not possible to standardize the composition of every deposited fingerprint, a serial depletion for fingerprint deposition is used to obtain the most suitable fingerprint. In addition, chemicals with amino acid reaction have little effect on the quality of fresh or old fingerprint.

To meet the standards in real life practice, the deposited fingerprints are not immediately processed and analysed until after one week of storage. Only the first two consecutive fingerprints in the depletion series were collected and analyzed, since the white protective layer on the surface of thermal paper would adhere to the fingertip after each contact, thus limiting the number of consecutive depositions. Each fingerprint was left to age for 1 week to simulate operational conditions and was then cut into two halves, with each half receiving a different treatment. Following the treatment, the fingerprints were left to develop for 24–48 h. The fingerprints were then

compared side-by-side with its corresponding half and photographed for further analysis and evaluation of overall development quality.

### **Preparation of solutions**

As shown in Table 1, the three commonly used (in real-life practice) reagent formulations were

utilized for fingermark development. Each formulation was applied in the following two different ways: pipette dropper dispensing or direct soaking. Following latent fingermark deposition, fingermarks were cut into half, treated, and compared. The results were examined and analyzed in four aspects as presented below.

Table 1: Formulations of three different solutions employed.

	Chemicals	Ethanol	Acetic Acid	Ethyl Acetate	Isopropanol	Petroleum Ether
ThermaNin solution [21]	4 g ThermaNin			15 mL	5 mL	To 1 L
Original Petroleum Ether-Based Ninhydrin Solution [3]	5 g ninhydrin	45 mL	5 mL	2 mL		To 1 L
Petroleum Ether Diluted Solution [24]	Adopted the original formulation but create a 1, 2, 3, 4, 5, 6 times diluted series of the same volume using petroleum ether					

### **Experiment design**

#### *Experiment 1: Comparison of dropping and soaking methods*

Experiment 1 involved the comparison of different application methods (dripping or direct soaking). This research compared the result quality, especially with regard to the degree of damage caused by pipette dropper dispensing or direct soaking by comparing the quality of developed fingermarks using these two methods of application. It was found that for thermal paper, using a dropper is less destructive and less blackening than soaking. However, the more destructive soaking method will result in a better dilution ratio of the formulation, which is more suitable for development of latent fingerprint on thermal paper in practice. Therefore, in experiment 2, the method of soaking was used instead. In this study, the spraying method is not utilized as there are safety concerns regarding the low ignition point.

#### *Experiment 2: Comparison of different development reagents*

Experiment 2 involved the comparison of different development reagents (original petroleum ether-based ninhydrin vs. petroleum ether dilution vs. ThermaNin). Latent fingermarks were assigned to three different groups, and each group received different development reagent treatments by soaking. Fingermarks were cut into half for analysis and objective comparison.

#### *Experiment 3: Comparison of Different ThermaNin formulations*

Experiment 3 involved the comparison of ThermaNin formulations. In 2016, the official website of BVDA provided the recommended formulation of ThermaNin (4 g/L ThermaNin, 0.5% v/v isopropanol, 1.5% v/v ethyl acetate)<sup>9</sup>. However, earlier in 2004, it recommended the use of non-polar solvent solely in the formulation (4 g ThermaNin added to 1 L petroleum ether). Therefore, the two formulations were compared for its ability to develop quality fingermarks.

#### *Experiment 4: Comparison of different substrate samples*

Experiment 4 involved the comparison of experiment substrate samples. For pseudo-operational trials, the fingermark development results were compared among various thermal paper samples collected from daily activities. These thermal paper samples were sourced from receipts (for payment, cash withdrawal, and parking) that are commonly found in Taiwan.

## **Results and Discussion**

### **Comparison of dropping and soaking methods**

For fingermarks that were treated with the original ninhydrin formulation, results showed that the soaking method caused complete blackening of thermal paper, while the dropper dispensing method caused partial blackening and the appearance of tiny black dots. Among fingermarks that were treated with petroleum

ether-diluted formulations (1-, 2, 3-, 4-, 5-, and 6-time dilutions), those treated with 5 times petroleum ether-diluted reagents via the dropper dispensing method appeared clear and non-blackened. Moreover, the soaking method caused complete blackening of thermal paper when using 5 times petroleum ether-diluted

reagents, and clear and non-blackened fingermarks were only obtained with 6 times petroleum ether-diluted reagents (Figure 1). The spraying method is not used and considered in this study as there are safety concerns due to low ignition point.

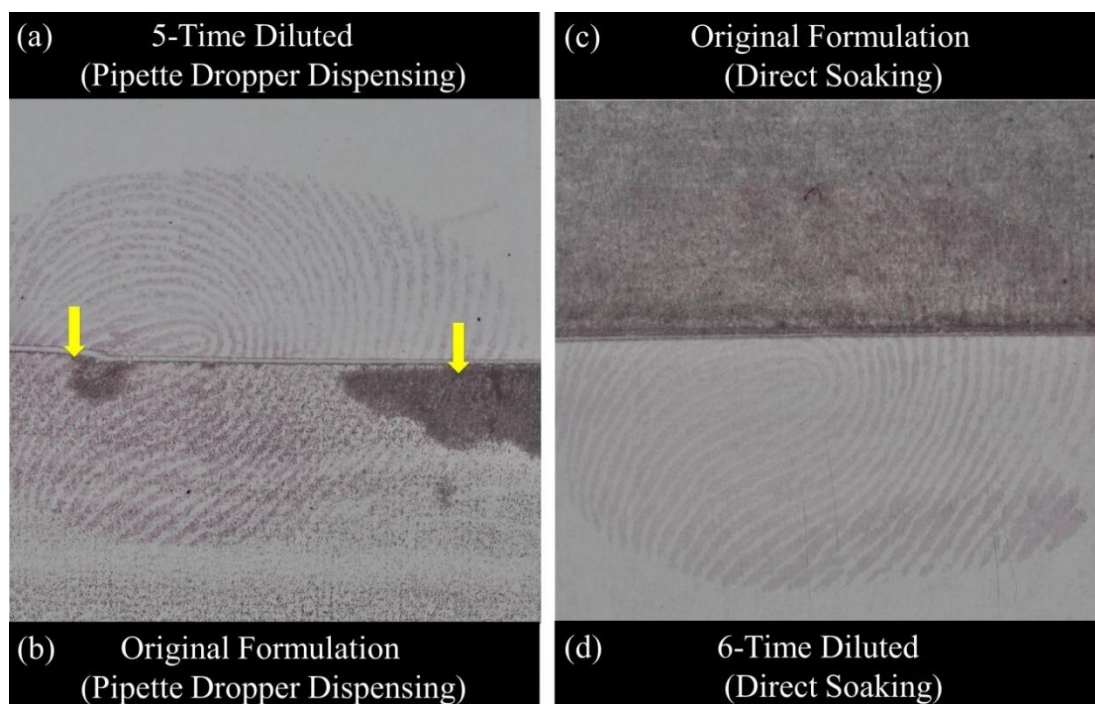


Figure 1: Different development and application methods had different effects on thermal paper blackening. Dropper dispensing method might create uneven distribution and treatment, (a) clear development result observable in petroleum ether 5-time diluted reagents treated fingermark; (b) but leading to partial blackening. Completely blackened thermal paper was seen in original ninhydrin formulation-soaked fingermark (c); clear development result observable in petroleum ether 6-time diluted reagents-soaked fingermark (d).

The results suggest that different treatment methods had different impacts on the development of latent fingermarks on thermal paper. Therefore, for cases where the type of thermal paper cannot be determined or the same type of receipt cannot be obtained as a control, the selection of methods that allow clear fingermark development and produce less substrate blackening is always recommended. As the direct soaking method caused blackening of the thermal paper, the dripping method is more recommended. To minimize the appearance of tiny black dots with the dripping method, a dropper can be used to dispense reagents at least twice on the back of thermal paper (opposite side of the deposited fingermark) to minimize potential damage, ensure even treatment, and obtain optimal results.

#### *Comparison of different development reagents*

For fingermarks that were treated with original ninhydrin formulation soaking, the substrate thermal paper turned completely black immediately after treatment. Fingermark ridges were still observable to a certain extent in some cases but were largely distracted/disturbed by blackened background substrate. On the other hand, Thermanin (0.5% v/v isopropanol, 1.5% v/v ethyl acetate) soaking resulted in clear fingermarks, and ridges could be observed in the Thermanin soaked group without blackening of thermal paper (Figure 2). Among fingermarks treated with petroleum ether-diluted formulations (1-, 2-, 3-, 4-, 5-, and 6-time dilutions of the original ninhydrin formulation of the same volume), those soaked in 1-, 2-, 3-, 4-, and 5-time diluted reagents

showed blackening of thermal paper. Clear fingerprint ridges with non-blackened background could only be observed when

reagents were 6-time diluted, although the developed purple ridge color was relatively light (Figure 3).

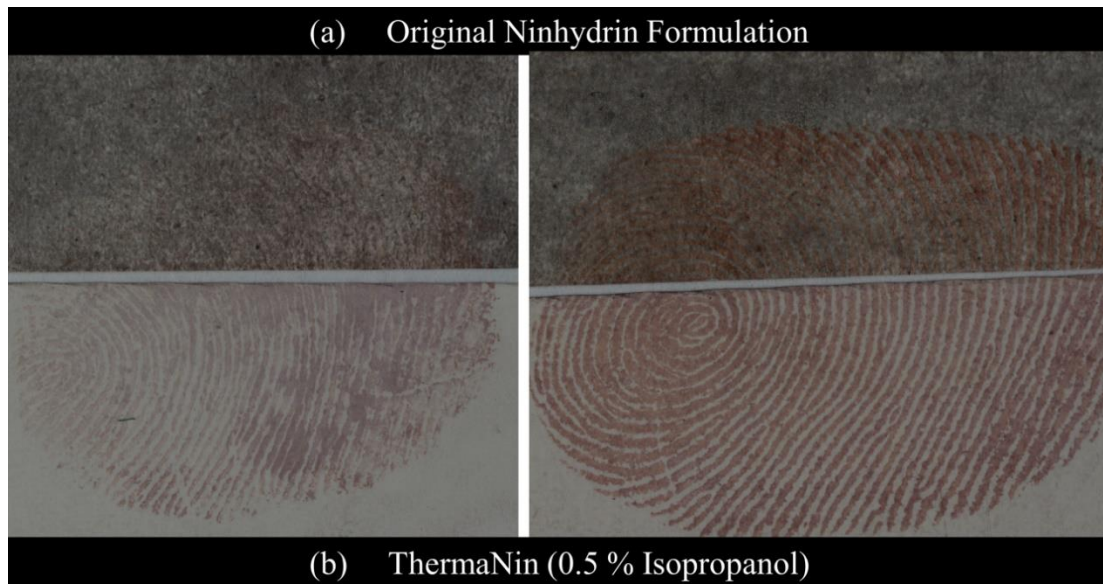


Figure 2: Comparing different fingerprint development reagents with different donors: (a) original ninhydrin formulation vs. (b) ThermaNin.

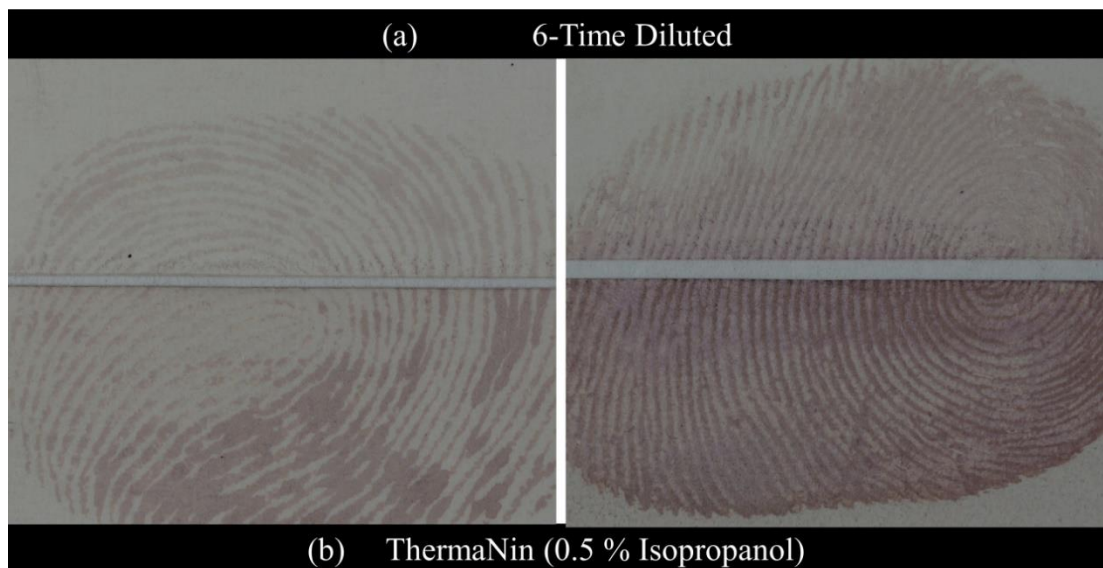


Figure 3: Comparing different fingerprint development reagents with different donors: (a) 6 times diluted ninhydrin formulation vs. (b) ThermaNin.

The same principle was adopted for the addition of polar solvent during reagent preparation. Different polar solvents had different impacts on the final development results. Ethyl acetate was considered less destructive, but had lower solubility; thus, it required addition of alcohol during preparation. Reagents should be prepared and used

following operational standards [10-11] (control or dilute ethyl acetate under 3.0% v/v and alcohol/acetic acid under 1.0% v/v). In the current study, the original ninhydrin formulation (5 g/L w/v ninhydrin, 4.5% v/v alcohol, 0.5% v/v acetic acid, 0.2% v/v ethyl acetate) was diluted. For all formulations, petroleum ether was used for dilution.

However, thermal paper still turned black after treatment with 2-time diluted reagents. Clear and non-blackened fingermarks could only be obtained when reagents were 6 times diluted. This study utilized a 6-fold dilution as the concentrations of the formulations are related to those mentioned in previous literature [10, 11]. It was observed that the original ninhydrin formulation is 4.5% v/v alcohol diluted to 1.0%. It requires further dilution of five times with the addition of acetic acid and ethyl acetate. The result of this dilution is similar to previous literature.

#### *Comparison of different ThermaNin formulations*

A comparison was made between the 2016 BVDA-recommended ThermaNin formulation (4 g/L ThermaNin, 0.5% v/v isopropanol, 1.5% v/v ethyl acetate) and its 2004 recommended formulation involving solely non-polar solvent (4 g ThermaNin added to 1 L petroleum ether). As shown in Figure 4, both developed clear, purple-colored fingermarks of similar quality, without the thermal paper turning black.

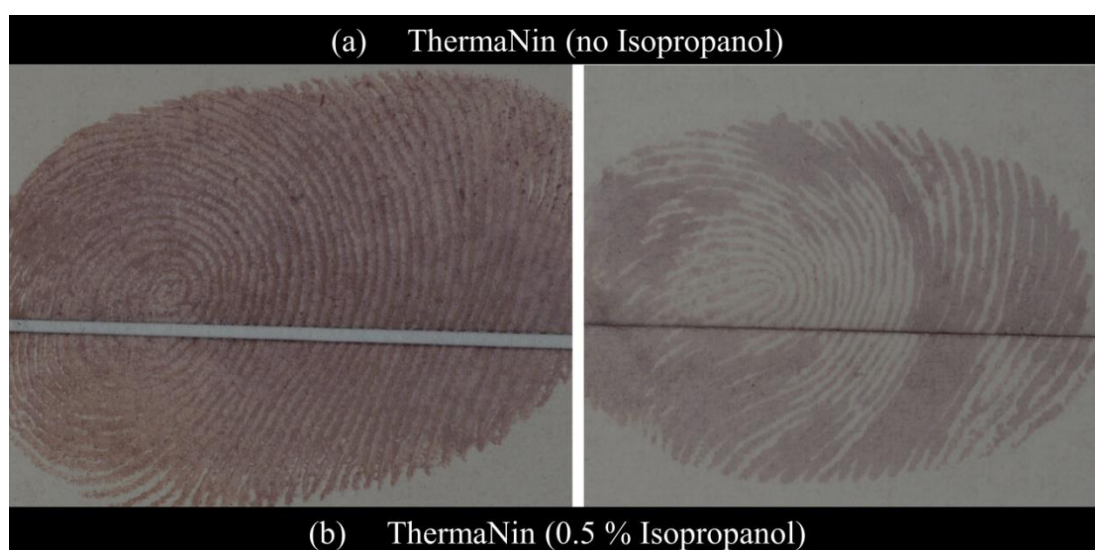


Figure 4: Comparing two different ThermaNin formulation developed fingermarks of similar quality after treatment: (a) ThermaNin without any solvent vs. (b) ThermaNin with 0.5% isopropanol.

For the 2004 non-polar solvent formulation, 3 hours following reagent preparation, light pink/red crystals were seen precipitating, with white powdery particles diffusing inside the petroleum ether solution. The red precipitation became more obvious as storage time increased. For the 2016 polar solvent formulation, yellow solvent could be seen at the bottom, with crystal precipitation.

There were still risks with the use of polar solvents in fingerprint development on thermal paper. Although ThermaNin was developed and manufactured to have a short storage life of 1–3 weeks, the findings in the current study showed that fingerprint development results were relatively similar when comparing freshly prepared reagents with reagents stored for 45 days, although the red precipitation became much more obvious as storage time increased. It was only after 52 days of storage that the reagents failed to develop or enhance the latent fingermarks on thermal paper (Figure 5).

#### *Comparison of different substrate samples*

The experimental results were evaluated, and the findings could be divided into two categories. Thermal paper with simple structure is often thin, and the text has fuzzy or diffuse edges. This is frequently seen in receipts for parking, queueing, ATM, etc. These substrates turned completely black on treatment with the original ninhydrin formulation. However, clear and non-blackened fingermarks could be obtained with 6 times diluted reagents. Thermal paper with complex structures might have additional protective layers for long-term preservation. This is frequently seen in invoices or transactions at gas stations. In some cases, the original ninhydrin formulation was able to develop clear fingermarks on these substrates without turning the thermal paper black. The color of the developed fingermarks was lighter on treatment with 6 times diluted reagents than with the original formulation (Figure 6).

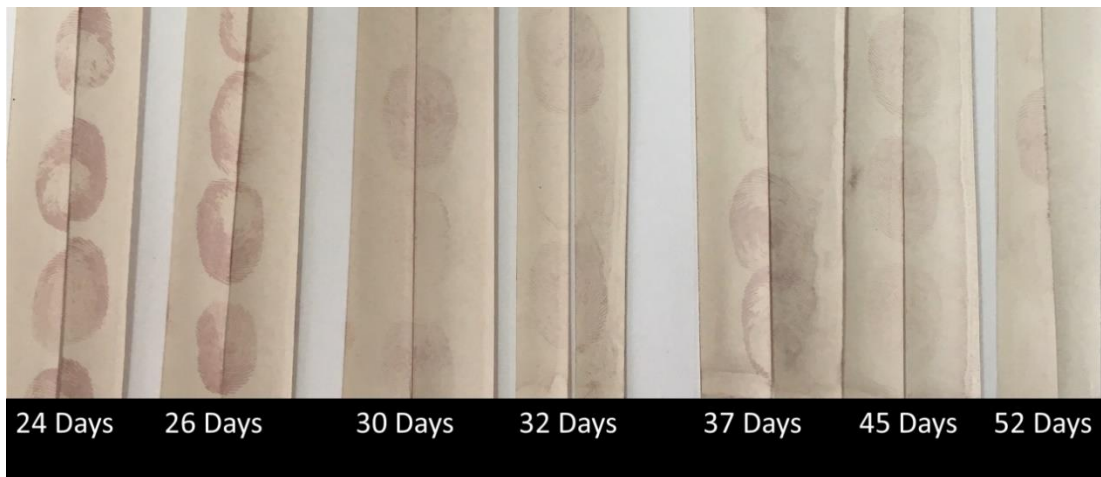


Figure 5: Latent fingerprint development results for non-polar solvent formulation treatment. For each strip, the left half received treatment of freshly prepared reagents altogether, and the right half received the assigned treatment with the same prepared reagents stored for (from left to right) 24, 26, 30, 32, 37, 45, and 52 days.

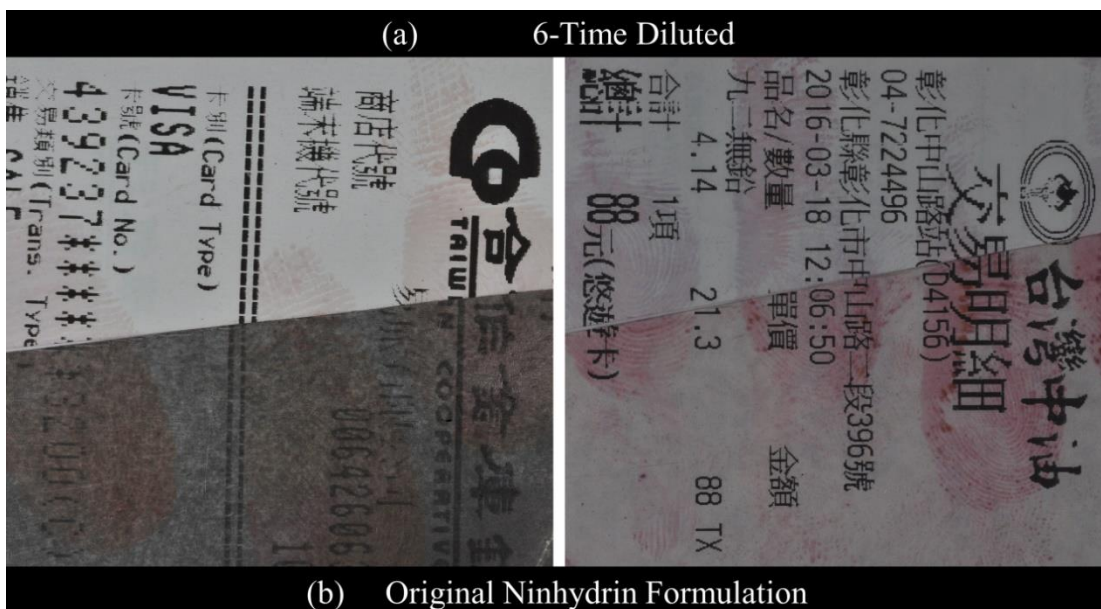


Figure 6: Latent fingerprint visualization results of different development techniques on different substrate samples: (a, b) 6 times diluted reagents developed clear and non-blackened fingerprints on ATM receipts, and both the original and 6 times diluted formulation developed fingerprints on gas station transaction receipts, with the latter yielding lighter colored fingerprint ridges.

As mentioned previously, there are various types of thermal papers. In cases where the type of thermal paper cannot be determined or the same type of receipt cannot be obtained as a control, latent fingerprint development techniques must be carefully selected. In real-life practice, it is important to follow the principles of dilution and adjust the quantity of the polar solvent used appropriately. Specifically, this is achieved by using ethyl

acetate (which is less damaging to thermal paper) with a small amount of alcohol in order to dissolve the ninhydrin, and then using PE or HFE 7100 as the main solvent. Moreover, based on the differential results, it would be suggested to pre-test the reagents in a small area at the bottom of the thermal paper evidence. The reagents/diluted formulation should be used only if no blackening is observed during the pre-test. Furthermore, the



dropper dispensing method should be considered. These steps will ensure optimal fingerprint development and collection on thermal paper. The different chemical reagents

and corresponding development results are provided in Table 2.

Table 2: Comparison between different reagents and development results.

Different formulations/ Development results	Result and Quality of Developed Fingermarks		Reagents Used	
	Ridge Color	Substrate/ background	Condition and Storability	Advantage/ Disadvantage
Petroleum Ether Six-Time Diluted Formulation	Light Purple	Clear, not blackened	Immediate preparation (on-demand), no need for storage	High convenience, suitable for operational crime lab
ThermaNin (0.5% isopropanol)	Light Purple	Blackened in some cases	Non-polar and polar layer separation; pale white solid powder; no storage time info	Need to purchase for ThermaNin chemicals; need to pay attention to the storage time
ThermaNin (Just dissolved in Petroleum Ether)	Light Purple	Clear, not blackened	Pale white solid (light-red colored precipitation seen the next day); about 1.5 months of storage	Need to purchase for ThermaNin chemicals; need to pay attention to the storage time

## Conclusion

The use of a diluted ninhydrin formulation produced fingerprints with better contrast and quality than ThermaNin formulations. Moreover, the preparation and usage of the original ninhydrin were more efficient than compared to the ThermaNin formulations. Hence, for the development of latent fingerprint evidence on thermal paper, forensic crime labs without special equipment are recommended to use the original ninhydrin formulation coupled with the principles of dilution and control of the polar solvent used to achieve high effectiveness, increased storability, and increased safety. As thermal paper is different from other substrates such as paper, the dilution factor used, the method of chemical application (dripping or soaking) and the lack of heating for the samples, are all limited due to the nature of thermal paper.

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