**Article**

# Fingermark Simulants and Their Inherent Problems: A Comparison with Latent Fingermark Deposits

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**Abstract**: Commercially available fingermark simulants were compared to latent fingermark deposits to assess their efficacy as standards for a quality control assessment of fingermark development reagents. Deposits of the simulants and latent fingermarks were made on paper substrates and were developed using reagents that target amino acids (ninhydrin, 1,2-indanedione) and sebaceous secretions (Oil Red O, physical developer). The resulting marks were compared for visibility and color. Significant differences were observed between the simulants and latent fingermarks in response to the fingermark development reagents. Infrared spectroscopic analysis of the simulants compared to untreated latent fingermarks revealed differences in chemical composition. These results indicate that these simulants are not well suited as quality control standards in forensic laboratories and should be used with extreme caution in any form of research into latent fingermark detection.

## Introduction

Routine testing of fingermark development reagents is required to overcome performance issues arising from degradation of aged reagents, incorrect preparation, or contamination [1, 2]. A notable shortcoming in such procedures is the lack of analytical

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standards for quality control testing [1, 3, 4]. A common practice is to test reagents on latent fingermarks gathered from immediately available donors. However, this leaves the assessment of a reagent's efficacy prone to error because of the natural variability of skin secretions. Latent fingermarks are known to vary significantly in composition between individuals, and compositional differences have been observed between fingermarks from the same individual over time [3, 5]. This so-called donor effect also prevents truly meaningful comparisons between separately located facilities and the effects of laboratory protocol, reagent formulation, climate, substrate, and so forth [3, 5].

There have been several preliminary attempts towards producing a reproducible artificial fingermark that may be used as a uniform standard [1, 4, 6–10]. Nielson [5] proposed three criteria for such standard tests: (1) They must allow quantitative and qualitative testing. (2) They must reasonably reflect fingermark composition. (3) They must be easily reproducible. These investigations have produced a number of spot tests or test strips, where known concentrations of target compound deposited onto paper by either micropipette or inkjet printer are treated with the relevant development method. Such tests have been developed largely for amino acid-sensitive reagents, although similar tests for physical developer (PD) have been reported [1, 4, 6–10]. The simple composition of these tests cannot accurately represent or reflect the complex matrix of a latent fingermark and are therefore limited to assessing only a few reagents at best [3, 4, 11]. In the case of PD, spot tests have been developed using compounds that react rapidly with the working solution but are not present in latent fingermarks, due in part to a lack of understanding of the target compound(s) of this reagent [1, 11].

Numerous artificial sweat and sebum formulations have been reported in the medical literature as models for skin chemistry and permeation by exogenous substances, including drugs, cosmetics, and allergens [12, 13]. These formulations are based on reports of the composition of human skin secretions and often contain numerous compounds, although these formulations rarely concur in the number and concentration of components [3, 14]. To the best of the authors' knowledge, there has been no attempt to use such artificial skin secretions as latent fingermark standards.

Commercially available fingermark simulant reference pads appear to offer an alternative to spot tests to mimic latent fingermarks upon treatment. Examples of such simulants are the Latent

Print Reference Pads (Forensics Source, Jacksonville, FL), which are available in two versions: amino acid based and sebaceous oil secretions. These are intended to be used in the same manner as ink pads to deposit latent fingermarks "spiked" with a sufficient amount of simulant. Reference materials (which these pads are marketed as) have been defined as:

> A substance or mixture of substances, the composition of which is known within specified limits, and one or more of the properties of which is sufficiently well established to be used for the calibration of an apparatus, the assessment of a measuring method or for assigning values to materials. [15]

The intent of these pads is for the second of these three purposes—the assessment of a measurement method—and the supplier states explicitly that the use of one of these pads "provides assurance chemicals are working appropriately" [16].

Indications of anomalous results arising from the use of these pads [7] led to questions concerning the reliability and validity of these simulants as alternatives to latent fingermarks for quality assurance purposes. This article describes investigations into the development of marks made by these simulants on paper surfaces with a variety of latent finger mark development methods to assess their suitability as quality control references.

## Materials and Method

### *Chemicals*

The chemicals used in this experiment were ninhydrin (Optimum Technology, Australia), 1,2-indanedione (CASALI/ Optimum Technology, Australia), anhydrous zinc chloride (Sigma-Aldrich, St. Louis, MO), dichloromethane (Mallinckrodt Chemicals, Miami, FL), ethyl acetate (Univar Analytical, Australia), glacial acetic acid (CSR Chemicals, Australia), absolute ethanol (CSR Chemicals, Australia), 1-methoxynonafluorobutane (HFE-7100; 3M Novec, Australia), petroleum spirits 60–80 °C (APS Chemicals, Australia), Oil Red O (Sigma-Aldrich, St. Louis, MO), propylene (Sigma-Aldrich, St. Louis, MO), maleic acid (APS Chemicals, Australia), silver nitrate (Chem-Supply, Australia), ferric nitrate nonahydrate (Chem-Supply, Australia), ferrous ammonium sulphate hexahydrate (Sigma-Aldrich, St. Louis, MO), citric acid (Ajax Finechem, Australia), synperonic N, and n-dodecylamine acetate (Optimum

Technology, Australia). They were all used as received and were of analytical reagent grade unless otherwise stated. Amino acid based and sebaceous oil secretion Latent Print Reference Pads were purchased from Forensics Source.

*Preparation of Reagents*

Ninhydrin, 1,2-indanedione, and PD were prepared as recommended by the Australian Federal Police (AFP) [17]. The PD working solution was prepared fresh for each use and was used on no more than two batches of samples. Oil Red O (ORO) was prepared as described by Frick et al. [18]

*Collection of Latent Fingermarks*

Three or more latent fingermarks were collected on white copy paper (Fuji Xerox Professional) from each of the seven donors. Donors had not consumed food, handled chemicals, or washed hands for at least 30 minutes before providing samples. For comparisons with the amino acid-based simulant, uncharged fingermarks, requiring no preparation, were collected. Charged fingermarks (prepared by having donors rub their fingers on their face or hair immediately prior to fingermark deposition) were then collected for comparisons with the sebaceous oil simulant.

Fingermarks were also deposited as described above on stainless steel matrix assisted laser desorption ionization (MALDI) plates, a gold-plated stainless steel MALDI plate, and gold-plated glass for analysis by infrared spectroscopy (all donated by D. Berryman, State Agricultural Biotechnology Center, Murdoch University, Perth, Western Australia).

*Deposition of Fingermark Simulants*

Simulants were deposited on white copy paper (Fuji Xerox) using custom-made polymer stamps (City Rubber Stamps, Perth, Western Australia) (Figure 1). To avoid cross-contamination by donor secretions, two separate stamps were used to deposit each simulant (amino acid and sebaceous oil reference pads). The stamps were cleaned thoroughly after each use with industrial grade detergent (RBS pF, RBS Chemical Products), ethanol, and deionized water.

*Figure 1*
*Polymer stamp used to deposit simulants.*

*Development of Latent Fingermarks and Simulants*

Ninhydrin and 1,2-indanedione treatment of uncharged latent fingermarks and the amino acid-based simulant were carried out as described by the AFP [17]. PD treatment of charged latent fingermarks and the sebaceous oil secretion simulant was also carried out as described by the AFP [17] with one minor modification: the maleic acid pretreatment step was increased from 5 minutes to 30 minutes, as recommended by Salama et al. [19] Treatment of charged latent fingermarks and the sebaceous oil secretion simulant with ORO was carried out as described by Frick et al. [18]

*Photography of Treated Samples*

Samples were photographed using a Nikon D300 camera mounted on a Firenze Mini Repro tripod and connected to a computer using Nikon Camera Control Pro Version 2.0.0. 1,2-Indanedione-treated samples were photographed in luminescence mode; ninhydrin-, ORO-, and PD-treated samples were photographed in absorbance mode. The camera settings that were used are given in Table 1. Illumination in luminescence mode was achieved using a Rofin Polilight PL500 (Rofin, Australia), with an excitation wavelength of 505 nm and an orange camera filter attachment (550 nm barrier filter). Illumination in absorbance mode was achieved using incandescent light bulbs with no camera filter attachments.

| | Absorbance Mode | Luminescence Mode |
|---|---|---|
| Focal Length/mm | 60 | 60 |
| Exposure Mode | Manual | Manual |
| White Balance | Auto | Auto |
| Shutter Speed/s | 1/20 | 2 |
| Aperture | f/11 | f/11 |
| Sensitivity | ISO 200 | ISO 200 |

*Table 1*

*Photographic conditions for absorbance and luminescence mode photographs.*

### *Visual Assessment of Developed Samples*

Treated samples were graded using a system based on that used by the Home Office Police Scientific Development Branch (HOPSDB) (Table 2) [20]. Later adjustments of the images were performed on Adobe Photoshop CS4 Version 9.0. Adjustments of photographed samples were performed only for clarity of the figures in this article. Evaluation of fingermark development was carried out on raw images.

| Grade | | Description |
|---|---|---|
| 0 | No development | No visible ridge detail |
| 1 | Weak development | Patchy ridge detail with low contrast |
| 2 | Medium development | Near-continuous ridge detail with good contrast |
| 3 | Strong development | Continuous ridge detail with strong contrast |

*Table 2*

*Grading system employed for developed fingermark samples.*

### *Infrared Microscopy*

Spectra were collected using a Bruker Hyperion 2000 microscope (Bruker Biosciences Corporation, Bruker, Australia) fitted with a liquid nitrogen cooled mercury cadmium telluride (MCT) detector and coupled to a synchrotron-sourced Vertex V80v spectrometer. An objective with a 36X magnification was employed with an aperture size of 10×10 µm, and 256 scans at a resolution of 4 $cm^{-1}$ over the range of 3800–700 $cm^{-1}$ were averaged. All spectra were acquired and processed using OPUS v6.5 software (Bruker Biosciences Corporation).

## Results and Discussion

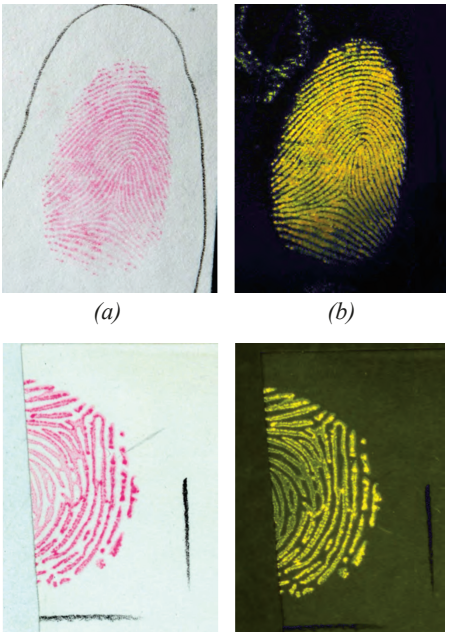
### *Comparisons between the Amino Acid-Based Simulant and Latent Fingermark Development with 1,2-Indanedione and Ninhydrin*

Marks produced by the amino acid-based simulant gave colored marks when developed with both ninhydrin and 1,2-indanedione. There appeared to be a higher concentration of amino acids in the amino acid-based simulant than in latent fingermark samples; the simulant often displayed much greater color intensity and in the case of 1,2-indanedione, luminescence upon treatment (Figure 2). Latent fingermark development (grades between 1–2) was typically poorer than that of the developed simulant (grade of 3) impressions. This apparent discrepancy in amino acid concentration is of concern, because good development obtained using the simulant will only indicate that an amino acid-sensitive reagent works and not necessarily whether it is sufficiently sensitive to develop latent fingermarks.

Additionally, the ninhydrin-treated samples produced significant differences in coloration. The purple color (Ruhemann's purple) produced from the reaction between ninhydrin and amino acids was consistently absent in the treated simulant samples, which instead appeared orange-red in color (Figure 3). It is well known that the addition of metal ions to the Ruhemann's purple product can result in a coordination complex that displays different absorption and emission spectra that result in a color change, depending on the metal post-treatment [21]. A possible explanation to these observations is that the amino acid-based simulant contains metal salt ions and these give rise to the shift in the absorption wavelength.

### *Comparisons between Sebaceous Oil Simulant and Latent Fingermark Development with ORO and PD*

ORO development of the sebaceous oil simulant was slightly stronger (grade of 3), compared to fingermark samples (grade of 2–3) (Figure 4). As with the amino acid-based simulant, this stronger development with the sebaceous oil simulant may give a false sense of security, when the tested reagent may be unable to detect more lipid-poor fingermarks. In contrast, marks left by the sebaceous oil secretion simulant were barely detectable with PD (Figure 5)



*(a)* *(b)*

*(c)* *(d)*

*Figure 2*

*Visual comparison between latent fingermarks viewed in (a) absorbance mode and (b) luminescence mode. Amino acid-based simulant (c) absorbance mode and (d) luminescence mode following treatment with 1,2-indanedione.*

*(a)* *(b)*

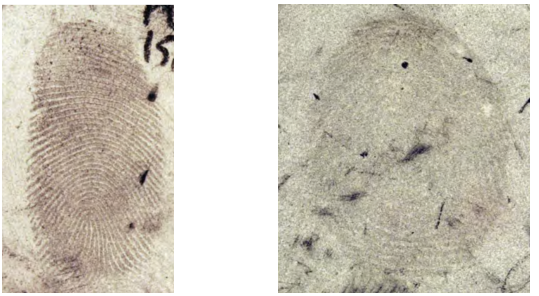
*Figure 3*

*Visual comparison between (a) latent fingermarks and (b) amino acid-based simulant following treatment with ninhydrin.*


*(a)* *(b)*

*Figure 4*

*Visual comparison between (a) latent fingermark and (b) sebaceous oil secretion simulant following treatment with ORO.*



*(a)* *(b)*

*Figure 5*

*Visual comparison between (a) latent fingermark and (b) sebaceous oil secretion simulant following treatment with PD.*

The differences in the response of the sebaceous oil simulant to ORO and PD may be explained by the development mechanisms of the two reagents. ORO develops fingermarks by simple diffusion of the dye from the solvent into neutral lipids (i.e., triglycerides). The formulation used in this study is based on that used to demonstrate lipid content in adipose tissue [18]. The development mechanism of PD, on the other hand, is rather more complex. Although there is still a great deal of uncertainty regarding the target compounds of PD, it is thought to target protonated compounds (i.e., fatty acids or proteins under reaction conditions), rather than neutral lipids [22]. The observed variations indicate significant compositional differences between the real sebaceous-rich fingermarks and the simulant.

*Infrared Microscopy Analysis*

Infrared spectra arise from the interaction of infrared radiation with materials at the molecular level. Molecules vibrate (stretch and bend) in a manner that is characteristic of the groups of atoms (functional groups) that they are composed of and their stereochemistry. Some, but not necessarily all, of such vibrations can absorb infrared radiation and consequently render an infrared absorption spectrum that reflects the molecule's structure and is characteristic of its functional groups [23–25]. In this study, we mainly focused on the nitrogen-hydrogen (N-H), carbon-hydrogen (C-H), and carbon-oxygen (C=O, where = signifies a double bond) interactions.

Latent fingermarks can be thought of as thin films of material on the substrate, and conventional, glow bar sourced IR spectrometers may not display adequate sensitivity and spatial resolution required for the analysis of the impressions. A number of investigations have shown that synchrotron sourced IR microscopy (IRM) is a viable technique, with significant advantages over conventional source IR, for the chemical analysis of latent fingermarks [26–31]. A synchrotron is a large facility where electrons can be accelerated to almost the speed of light [32]. As they are deflected around a ring by a very strong magnetic field, extremely bright light is generated across a wide range of wavelengths, which is subsequently channelled down various beam lines for experimental purposes [32, 33]. The much greater brightness of a synchrotron source enhances significantly the signal-to-noise ratios for IR spectroscopy and thereby reduces spectra acquisition times when compared with a conventional IR light source, as well as having improved spatial resolution [26–28].

To further investigate the chemical differences between the simulants and fingermark deposits, synchrotron-sourced IR spectrometry was employed using the IR Microscopy Beamline at the Australian Synchrotron. Typical IR spectra of latent fingermarks and the fingermark simulants are shown in Figure 6, with band assignments given in Table 3. As seen in Figure 6, there are clearly significant differences between the fingermarks and the simulants.

| Fingermark Bands ($cm^{-1}$) | Simulant Bands ($cm^{-1}$) | Vibration | Sebaceous or Protein Assignment |
|---|---|---|---|
| - | 3312 | N-H stretch (2°amine) | Amino Acid |
| 3010 | 3010 | O-H stretch | Sebaceous |
| 2921 | 2938 | C-H stretch (1°carbon) | Amino Acid |
| 2924 | 2925 | C-H stretch (1°carbon) | Sebaceous |
| 2855 | 2883 | C-H stretch (2°carbon) | Amino Acid |
| 2855 | 2855 | C-H stretch (2°carbon) | Sebaceous |
| 1745 | 1744 | C=O stretching (ester) | Sebaceous |
| - | 1595 | N-H in-plane bend (2 amine) | Amino Acid |
| 1465 | 1458 | C-H bend | All |
| - | 1030 | C-N stretch (2° amide) | Amino Acid |

*Table 3*

*Band assignments and functional groups of latent fingermarks and fingermark simulants [24].*

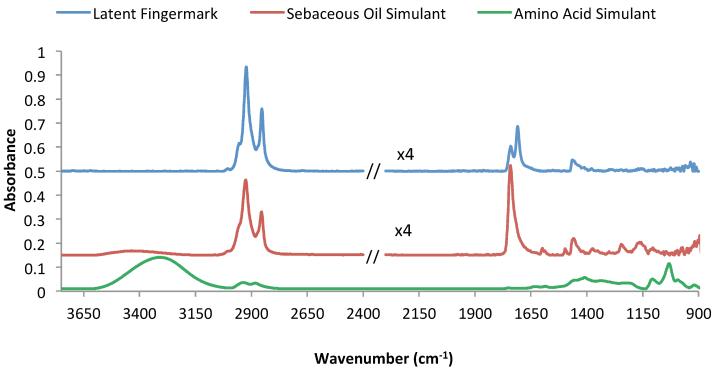



*Figure 6*

*Typical IR spectra acquired from a charged latent fingermark (0.5 AU offset), sebaceous oil fingermark simulant (0.15 AU offset),and amino acid-based simulant (0.01 AU offset). The intensity of the latent fingermark and sebaceous oil simulant spectra was amplified fourfold from 2300–900 cm-1. The amino acid-based simulant spectrum was scaled to half its original intensity for comparison purposes.*

The N-H stretching and bending peaks (~3300, ~1595, and 1030 $cm^{-1}$) are due to $NH_2$, NH, or CN groups present in amino acids. Although spectra of the very concentrated amino acid-based simulant clearly display these signals, they are not as apparent in the much fainter latent fingermark deposits. Further to this significant difference in the spectra arising from the amine groups, the C-H bonds also give rise to much sharper peaks (~2940–2850 $cm^{-1}$) in latent fingermark spectra. This clearly indicates that there is a significant difference in the composition of the samples. The presence of different amino acids or different ratios and concentrations of amino acids in the simulant may explain these findings, especially when taking into account that the matrix is much more complex in latent fingermarks.

The spectra obtained from charged and uncharged latent fingermarks differ only in their intensity, with charged deposits displaying significantly greater intensity. The sebaceous oil simulant renders a similar spectrum to that of charged latent fingermarks. The similarity can be explained by the fact that the C-H stretches are the major features in sebaceous oil spectra (due to their long-chain hydrocarbon component) and in latent fingermark deposits. There is a weak O-H stretch at 3010 $cm^{-1}$, possibly due to the presence of fatty acids, which can be seen in the sebaceous oil simulant, but is less pronounced in latent fingermark deposits. Whereas spectra of the sebaceous oil simulant display one intense signal for the C=O stretch (possibly due to the ester functional groups in the triglycerides), the fingermark spectra display split peaks. Further, the C-H bend in the sebaceous oil simulant is shifted relative to the fingermark samples (1458 versus 1465 $cm^{-1}$), and there is a difference in the relative intensity of nearly all bands in the spectra. This suggests the long-chain hydrocarbon lengths, unsaturation, and branching are different, indicating a difference in the oil compositions.

The C-H stretches and bends (~2930, ~2860, and ~1460 $cm^{-1}$) are the only signals that consistently exist in the amino acid and sebaceous simulants as well as in latent fingermarks because all contain $CH_x$ groups of one form or another.

Personal communication with the manufacturer confirmed that the amino acid and sebaceous oil simulants were composed of a mixture of L-amino acids and linseed oil, respectively. These are simplistic formulations and are not representative of actual latent fingermarks. Linseed oil is composed almost exclusively of triglycerides, with the main constituents being fatty

acids including linolenic acid, linoleic acid, and oleic acid [34]. Human sebum, on the other hand, contains a wide variety of lipid compounds as major components, including triglycerides, wax esters, free fatty acids, squalene, and cholesterol [35].

## Conclusion

Visual comparisons and infrared studies of the two fingermark simulants and latent fingermark samples show significant differences in response to development treatments and chemical composition. The intense development of these simulants with 1,2-indanedione and ORO indicates a far greater concentration of the target compounds in the simulant in latent fingermark deposits. The orange coloration produced with ninhydrin and the poor development with PD confirm that these simulants are too dissimilar to latent fingermarks to enable valid quality control assessments for these development techniques.

To date, latent fingermarks remain the best sample type for testing of fingermark development reagents. Spot tests are an option to validate reagent efficacy, although these should not be used to replace fingermarks completely. Though they are not homogenously reproducible as spot tests are, latent fingermarks can still be used to perform comparisons and to test reagent sensitivity in the form of "split prints" and depletion series, respectively. The commercially available fingermark simulant reference pads tested in this study cannot be recommended as reliable indicators of reagent performance.

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