Technical Note

Design of a Control Slide for Cyanoacrylate Polymerization: Application to the CA–Bluestar Sequence

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Abstract: Casework experience has shown that, in some cases, long exposures of surfaces subjected to cyanoacrylate (CA) fuming had detrimental effects on the subsequent application of Bluestar. This study aimed to develop a control mechanism to monitor the amount of CA deposited prior to the subsequent treatment. A control slide bearing spots of sodium hydroxide (NaOH) of known concentrations and volume was designed and validated against both scanning electron microscopy (SEM) observations and latent print examiners' assessments of the quality of the developed marks. The control slide allows one to define three levels of development that were used to monitor the Bluestar reaction on depleting footwear marks left in diluted blood. The appropriate conditions for a successful application of both CA and Bluestar were determined.

Introduction

Good practice and laboratory accreditation may require that forensic laboratories develop quality assurance methods to demonstrate, on a case-by-case basis, the accuracy of applied methods and the exactness of the results obtained. Operative tests (positive and negative controls) are then often conducted, jointly with the treatment of evidence, as a demonstration of the

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appropriate application, but also as a test to show the method or chemicals of a given technique are really working. In the context of fingermark detection techniques, most efforts have concentrated on amino acid visualizing reagents. Spots of amino acid of varying concentrations [1] or fingermarks (deposited by a technician or printed with artificial sweat [2]) are two of the most used control samples encountered in this area.

For this study, we were specifically interested in the optimization of the cyanoacrylate (CA) fuming technique (also named "superglue") in the context of its use in conjunction with Bluestar (ROC Imports, Monaco) by developing a specific control slide, ensuring an optimized polymerization level.

CA fuming is one of the fingermark detection methods of choice to be applied on nonporous surfaces [3]. However, the nature of the initiators and the role played by environmental conditions are not yet very well defined [4–6].

Because monomeric species are toxic by inhalation, the CA fuming process is most likely to be used in a fuming cabinet in a laboratory. Nevertheless, it can also be applied at crime scenes with proper ventilation. Because there are various types of forensic evidence at crime scenes (e.g., fingermarks, bloodmarks, fibers, shoemarks), it is important to detect and preserve these various types of evidence by finding a compromise between the different techniques or methods to be applied and to establish an optimized sequence of treatments. In the case of fingermark detection, the techniques dedicated to the detection of blood (whether latent or washed) are generally considered after the application of the techniques for detecting latent fingermarks.

CA fuming allows the detection of aged marks and can be further enhanced by staining methods. The chemical reaction can be divided into three phases: the initiation (or priming), the propagation, and the termination.

The polymerization is preferentially initiated by anionic compounds (e.g., Lewis bases). However, the sensitivity to carbocations, radicals, or transition metals is also well known [7]. The initiation of the polymerization reaction has been studied by considering alkanes [4], amines, and carboxylic acids [5], with a better qualitative productivity for carboxylic acids. It has also been determined that the quantitative yield is also influenced by the type of initiators [8]. With more than 300 compounds contained in the papillary secretions [9], fingermarks can constitute good initiating sites for the polymerization of CA through many different primers.

Environmental conditions also play a significant role: relative humidity [10], pH [11], and viscosity and thickness of the substrate [4] are some of the parameters that influence the polymerization process.

Finally, the ending of the polymerization process is not fully under the control of the practitioner, because it can occur by a lack of CA monomers or through the protonation of the carbanion site because of external reactants polluting the reaction [12].

To control the process of CA fuming, practitioners usually deposit a fresh fingermark on a glass plate that is placed in the CA fuming chamber next to the item bearing the potential marks of interest. Often, the control mark has a rich quantity of fingermark residue. Using this type of control mark verifies that the polymerization process occurred, but fails to inform about the overall sensitivity of the process [13]. It is expected that sensitivity is highly dependent on conditions that are not challenged during the one positive control process described above.

This study did not aim to propose a mixture of chosen organic and inorganic compounds to be used as a simulated sweat secretion found on friction ridge skin (so that the practitioner would no longer need to deposit his own fingermark). The aim was first to define what an optimal development is (in terms of ridge details and contrast). To appreciate the efficiency of a CA polymerization process, Burns et al. [14] showed that Fourier transformed infrared spectroscopy (FTIR) could be applied to quantify the polymerization level. Other techniques, such as Raman spectroscopy, could also be used [12]. In this work, following Lewis et al. [15] and Mankidy et al. [16], we chose an optical method – scanning electron microscopy (SEM) – to monitor and assess the polymer morphology at different exposure times to CA fuming. The establishment of a link between the expected polymer morphology on the dactyloscopic trace and the visual aspect of the developed control slide should lead to the determination of an optimal fuming time, indicating the best moment to stop the fuming process.

The polymerization reaction is mainly activated by anionic or nucleophilic compounds. Therefore, sodium hydroxide (NaOH) was chosen for our control product, because it has already been used as a catalyst for CA fuming [17].

Bluestar is a reagent for visualizing latent bloodstains. It reacts with the heme found in blood and gives a blue chemiluminescence signal. Despite the need to work in shadowy light, Bluestar is easy to use by spray, very sensitive, and compatible with DNA analysis.

The forensic laboratory of the French Gendarmerie (IRCGN) routinely uses the following sequence for bloody traces at crime scenes: CA fuming [18] followed by Bluestar [19–21]. Scientific publications have covered issues related to the compatibility between the application of Bluestar [21] or CA fuming [22] and subsequent DNA sampling. However, none of them studied the effect of CA fuming on subsequent Bluestar treatments. McCarthy and Grieve [23] reported that CA fuming would not inhibit the subsequent blood-specific reagents (i.e., Coomassie blue, Crowle's stain, or amido black) for fingermark detection. However, our casework experience has shown that CA fumigation on washed blood can inhibit the subsequent application of Bluestar.

The goal of this research was to develop an optimal control slide for CA development, to understand how the polymer interacts on washed blood, and to use the control slide to minimize the potential inhibiting effects of CA fuming on Bluestar.

Material and Methods

The control slides were made with Mensel-Glaser microscope slides (Braunschweig, Germany) and Foster & Freeman acetate sheets (Worcestershire, United Kingdom). The sodium hydroxide solution was prepared using Prolab NaOH (France) and deionized water. The Bluestar Forensic was from ROC Import (Monte-Carlo, Monaco). The CA was Cyanobloom from Foster & Freeman. Projectina (Switzerland) provided the fumigation chamber. SEM analyses were carried out taking advantage of a vacuum metal deposition using a Sputter Coater S150B from Edwards (United Kingdom) and a scanning electron microscope S360 from Cambridge (United Kingdom). The slides were also observed with a Foster & Freeman Crimelite horizontal light.

The control slides were designed as follows: a 25 x 75 mm adhesive acetate strip was punched with seven holes of 6 mm diameter (done with a hole puncher) and was further apposed to a microscope slide. The first hole was used as a blank. The following was applied to the remaining six holes: 5µl of NaOH 1 mol/l in two holes, 5µl of NaOH 0.1mol/l in two holes, and 5µl of NaOH 10 mol/l in two holes. It was important to use the same quantity of solution in each hole so that the thickness of the NaOH substrate would not influence the polymerization result. The deposition also needed to be homogeneous to facilitate the optical examination.

The range of concentrations (from 0.1 M to 10 M) allowed assessing the level of CA polymerization by observing the transformation of originally translucent spots to white ones with the oblique light on the entire area of interest. (After being dried for 8 hours, the control slide can be stored at room temperature, but no longer than 36 hours. Beyond 36 hours, the visual aspect and physical properties of the NaOH spots did not allow reproducibility during the tests.) Essentially, the various concentrations that were used allowed obtaining different quantities of NaOH crystallized on spots of fixed areas.

Control prints were prepared on glass slides. To obtain them, four donors deposited one fingermark at the intersection point made by four glass slides placed side by side (Figure 1). That allowed obtaining four quarters from one mark to be processed up to four different levels of CA fumigation. All marks were freshly prepared and treated 12 hours after deposition. This sample of controlled prints was examined with SEM.

In order to implement the process, 22 marks originating from 9 donors were produced as previously indicated. These marks were used to correlate the levels of development with an assessment of their quality according to latent print experts (LPE). Each practioner, through a questionnaire, had to evaluate the quarter of the mark he preferred to use in the hypothesis of a future comparison. They were requested not to use a numerical standard or to focus on supposed rareties of minutiae, but only on the development quality (e.g., the background noise reduction, the ridge detail). The aim was to appraise the level of CA polymerization each LPE preferred to use, to identify the one of a general agreement.

The next step was to try to establish a relationship between the opinion of the LPE and the degree of CA polymerization. We used the same approach that Burns applied in 1998 using FTIR [14]. In his study, he explored the capacity to use the FTIR technology to assess the efficiency of the exposition of the latent mark to ammonia before the CA polymerization process. To reduce the subjectivity of the technician usually assessing the quality of CA polymerization development, Burns proposed to use FTIR measurements to establish a correlation between the subjective evaluations of technicians he tested and the objective results of FTIR. A similar approach was carried out here to link the “eye of the practionner” and a reproducible device result, FTIR being replaced by SEM.

Figure 1

Illustration of the deposition of one mark on the intersecting points of four glass slides.

For the second objective of the study – dealing with the interaction between CA fuming and Bluestar – diluted blood at 1/100 (in water) was chosen to mimic as much as possible the dilution of blood found in cases involving attempts to wash the stains. Fresh blood (without anticoagulents) from a nearby military center was used by the researcher within five minutes of collection. The concentration of 1/100 is seen as the upper level concentration at which blood cannot be distinguished on colored garments [24].

Three volumes of diluted blood of 1, 3, and 6 µl were deposited on glass slides equipped with fixed dimension holes similar to the control slide. The goal was to study the influence of the thickness of blood on the CA fuming (operated at different polymerization levels).

These CA-treated blood spots were subsequently sprayed with Bluestar. An SEM analysis was conducted on developed samples in order to correlate the polymerization process with the efficiency of the Bluestar reaction.

In addition to the use of diluted bloodstains left on glass slides, a set of footwear marks contaminated with 1/100 diluted blood was left on acetate sheets. Each set was made of a series of four footwear marks left successively, in a depletive way, on four acetate sheets (without loading with blood between each deposition). For each level of CA development, a set was prepared. To test the efficiency of Bluestar with or without CA fuming, each mark was cut in half, the left part being submitted to CA and the right part left untreated. Then both parts were sprayed with Bluestar.

Results and Discussion

Development of a Control Slide

Levels of Development

The appearance of visible CA polymerization on the NaOH spots did not show direct correlation with the NaOH concentration of the spots (Table 1). It could then be inferred that another factor has to be taken into account. The physical properties of NaOH spots could indeed have an influence on CA polymerization, as reported by Czekanski et al., working originally on alkanes [4], who suggested that viscosity could modify CA polymerization. In our case, the viscosity could vary through a drying stage, changing the spots' appearance and modifying their properties.

Three levels of development based on the observation of the control slides placed in the fuming chamber were defined (Figure 2). The levels were determined by the number and intensity of CA-developed spots, as shown in Table 1.

Levels of Development	Observations Made on the Slides
Level 1	1M and 0.1M spots: weak development 10M: no development
Level 2	1M and 0.1M spots: strong development 10M: weak development
Level 3	1M, 0.1M and 10M spots: strong development

Table 1

Levels of development.

These levels allowed the defining of the extent of CA development that can be applied to marks. In order to correlate the above-defined levels with the quality of the friction ridge developed, the marks left on glass slides were used. For each of these marks, one quarter remained untreated, and the three remaining were developed using the control standard and stopped at level 1, 2, and 3. These marks (or portion thereof) were observed and assessed. Figure 3 illustrates the end results for one mark.

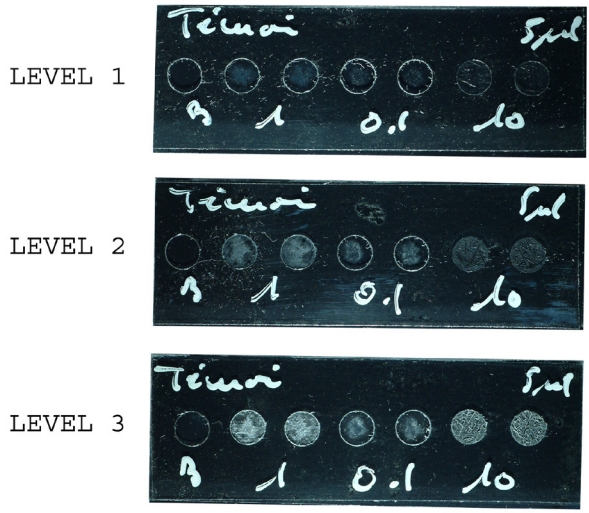


Figure 2

Oblique lighting observation of the three development stages on the control slide.

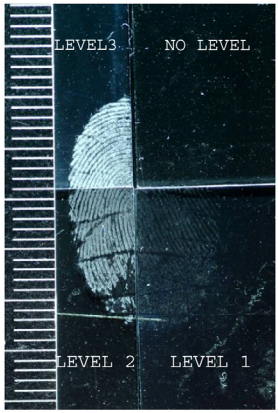


Figure 3

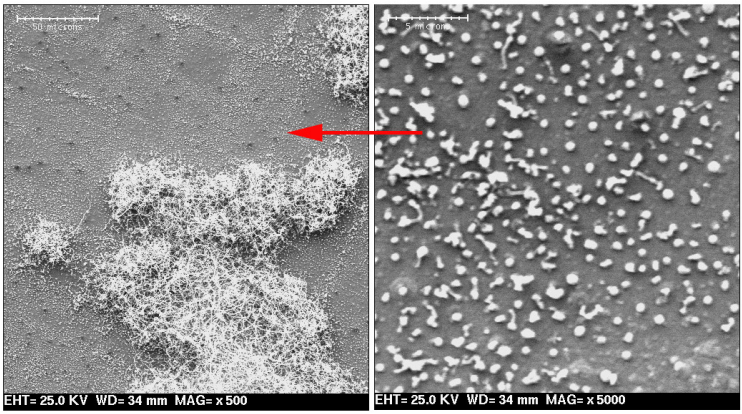
Development at three levels of one mark. One quarter remains undeveloped, and the three remaining quarters were exposed to level 1, 2, and 3. Levels fixed according to the behavior of the NaOH control slide.

Observations with SEM

In order to relate the levels of development with the observations of the polymer chains, SEM was used as in Lewis et al. [15] and Mankidy et al. [16]

The observations on the control marks confirmed that the CA polymers on fingermarks grow in the form of noodles. In addition, a development at level 3 led to overdeveloped CA marks because of the initiation of polymerization between ridges (Figure 4).

The optimum level of development corresponds to level 2. On the SEM observations, at that level, no initiation of the polymerization occurred between ridges.



(a) *(b)*

Figure 4

Area of ridges developed with CA fuming at level 3 and observed at 500X. Noodlelike development is abundant on the ridge. (a) Between ridges, the polymerization already started in the form of studs (that might grow towards noodle if fuming continues). It is shown here at 5000X (b).

Assessment of the Quality of Development by Latent Print Examiners

To correlate the observations made by the SEM with the perceived level of quality by latent fingerprint examiners, a set of marks was produced and submitted to CA fuming according to the levels defined above.

Nine donors were asked to deposit 22 fingermarks on glass slides according to the protocol used previously. For each mark, one quarter was left untreated, and the three others were exposed at the three different development levels that were previously identified. The decision to stop the CA fumigation at a given level was driven by observing the control slide.

The developed quarters were submitted to nine latent print examiners (experience ranged from 4 to 15 years) of the French Gendarmerie laboratory [Institut de recherche criminelle de la gendarmerie nationale (IRCGN)]. For each of the 22 marks, they were asked to decide, based on their training and experience, which of the three quarters (level 1, 2, or 3) was of the highest quality for identification purposes. Combined results on the marks and for all examiners considered jointly show that level 2 was chosen as the highest quality in 55% of the cases, level 3 followed with 34%, and level 1 received 11% (Figure 5). The breakdown according to each examiner is given in Figure 6. Some variation can be noticed between examiners number 4 and 6. The choice of level 2 was consistent with the SEM observations.

According to discussions with LPEs that followed the assessment, it appeared that the main criteria of choice were the lack of background noise, the contrast, and the clarity of the shape of ridges and pores.

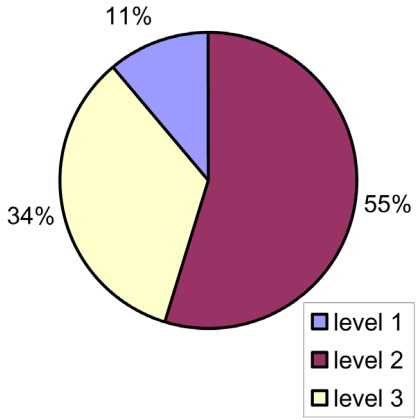


Figure 5

Distribution of the levels according to the best quality criteria for all 22 marks and all 9 LPEs.

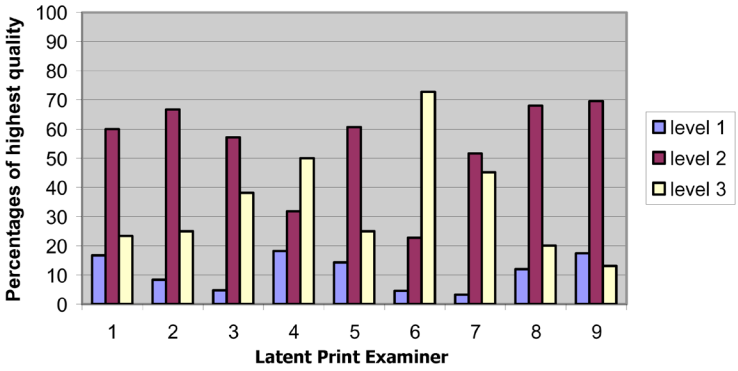


Figure 6

Distribution of the levels according to the best quality criteria for all 22 marks, for each LPE.

Impact of CA Development and its Levels on the Bluestar Reaction with Diluted Bloodstains

Application of CA Fumigation to Diluted Bloodstains

The slides bearing spots of diluted blood (1, 3, and 6µl of 1/100 diluted blood in water) were subjected to the three levels of CA development. SEM observations of the developed stains showed that some blood components can act as initiation sites for CA polymerization and that blood thickness affects the efficiency of the CA polymerization (Figure 7). That has been observed at all levels of CA development.

Blood is composed of three different types of cells (i.e., 99% erythrocytes, 0.2% leucocytes, and 0.8% thrombocytes). The dimensions, shapes, and general aspects (under SEM) of the blood cells covered with CA polymers are consistent with erythrocytes (Figure 8).

As the polymerization progressed, a dense polymer mesh developed on erythrocytes, from studs to the noodlelike polymer chains. Nevertheless, blood thickness had an influence on the CA polymerization. Observations made on different blood thicknesses (here defined by the volume of the same concentration) showed that CA has a slower efficiency of polymerization for higher thicknesses.

One postulated reason is that, for thicker layers, erythrocytes migrate by gravitation to the bottom of the substrate, offering less polymerization sites for the CA (Figure 9).

These results suggest two postulates. The first one takes into account the reaction between CA and compounds present at the surface of the cellular membrane taking exchanges between the cytoplasm and the plasmatic contents. By their chemical nature, these compounds could be initiators of the reaction of CA polymerization. The second one suggests that CA molecules could cross the membrane layer of the red blood cell to react with a specific compound present inside to initiate the polymerization. Because hemoglobin represents 92% of dry weight of an erythrocyte (about 30 pg), the estimated number of hemoglobin molecules per cell is about 300 million. Hence, it is postulated that hemoglobin (containing four heme groups) can be the initiator component of CA polymerization. The heme group bears two carboxylic groups (COOH) that have been identified by Wargacki et al. [5] as a strong initiator of CA polymerization. This second postulate could seem a harder way for this reaction to occur because of physiological aspects of red blood cells, but

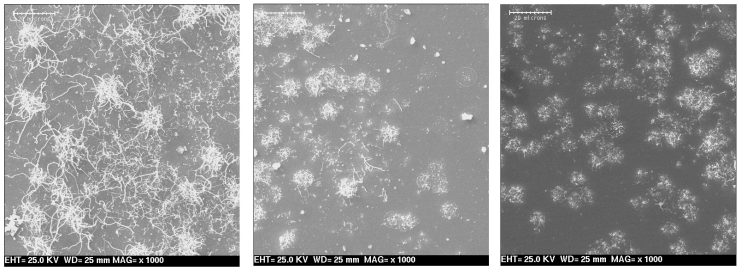


Figure 7

Polymerization of 1, 3, and 6µl of 1/100 diluted blood on a 6 mm diameter area, from left to right, respectively. CA-development level is 3.

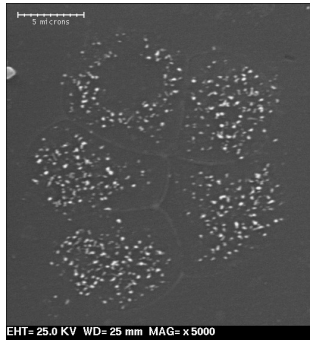


Figure 8

SEM view of erythrocytes showing polymerization of cyanoacrylate.

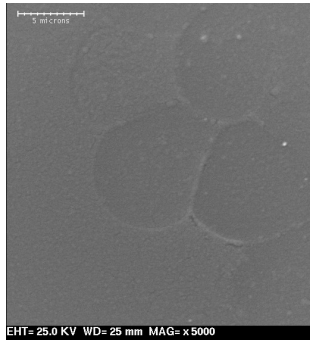


Figure 9

Illustration of eryhthrocytes showing no signs of polymerization for a 6 mL diluted bloodstain.

in 2006, Rothen-Rutishauser et al. demonstrated the possibilty of fine particles and nanoparticles to cross the membrane layer of red blood cells [25].

Effect on CA Polymerization on Subsequent Bluestar Hemoglobin Reaction

These heme groups are also the catalyst for the Bluestar reaction with hydrogen peroxide leading to a chemiluminescent product. Hence, the compatibility problem observed in casework may be due to the fact that CA monomers and Bluestar share the same chemical target.

Using the control slides previously developed, we aimed at finding the appropriate conditions for the CA fumigation that would not prevent a successful treatment with Bluestar. This was done on the set of footwear marks left in diluted blood.

Three sets of four footwear mark impressions were exposed (left half of each mark) to level 1, 2, and 3 of CA fuming, respectively. The development was monitored using the control slides. All marks were subsequently treated with Bluestar.

For level 1 of CA development, no difference of luminescence (in terms of starting time of the luminescence and its intensity) was observed between CA-pretreated marks and the untreated ones, regardless of the depletion states. At this level of CA development, the quantity of CA polymer was not high enough to impact on the Bluestar reaction.

When marks were exposed to a level 2 CA development, the halves treated to CA gave a reaction with Bluestar that was as efficient but reacted later in time compared to the halves sprayed directly with Bluestar. In addition, the luminescence of the CA-pretreated halves lasted longer compared to the other (not CA-exposed) halves (Figure 10).

No luminescence was observed for the halves that were fumigated at level 3 (Figure 11). Hence, this level of development should be avoided if a subsequent application of Bluestar is required.

Level 2 of CA development was an optimal level, allowing the Bluestar treatment to be compatible with CA-fuming. Level 2 also offered the added benefit of a longer luminescence. We believe that this longer luminescence is due to the filter effect due to the polymer mesh created at a level 2 CA development, hence the reagent takes more time to reach the heme group.

We also noticed that a second application of Bluestar allowed one to re-observe the luminescence. A slight increase in the quality of details visible on the footwear marks was sometimes observed. Also, the detrimental effect of the spraying of Bluestar on the details of the mark was mitigated by a previous CA fumigation. The polymer seemed to act as a fixing agent. This represents a real interest from an operational point of view, when an area needs to be sprayed twice or when a vertical surface is to be treated.

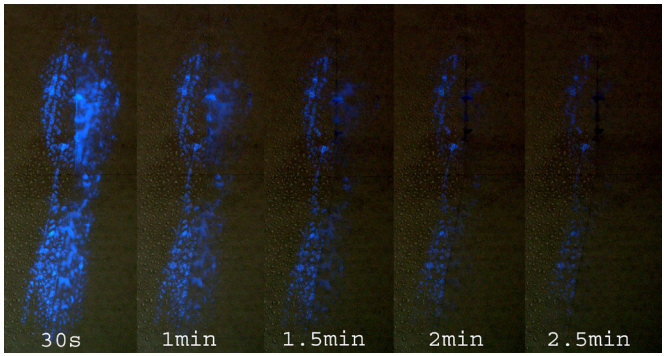


Figure 10

Observation of the Bluestar luminescence of footwear mark (4th depletion) made 30 seconds to 2.5 minutes after application. The left half of the mark was fumigated with CA up to level 2 (according to the control slide) before being treated with Bluestar. The right half was treated only with Bluestar.

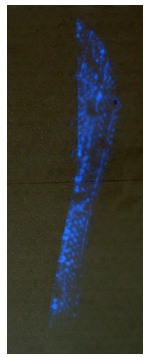


Figure 11

Observation of the Bluestar luminescence for one footwear mark (4th depletion). The left half of the mark was fumigated with CA up to level 3 (according to the control slide) before being treated with Bluestar. The right half was treated only with Bluestar.

Conclusion

This research aimed at putting together a control slide with adequate CA initiators that could help in setting the appropriate fuming time of CA monomer in order to ensure a successful subsequent treatment with Bluestar. Spots of NaOH of varying concentrations were successful as development controllers. Three levels (level 1, 2, and 3) of CA development were defined and checked against the development of fresh fingermarks left on glass slides. The quality of development was assessed using SEM and by consensual judgments made by latent fingerprint examiners. On that basis, it has been shown that a level 2 is adequate to detect fresh marks on glass slides. Note that the successful development with CA fuming of fingermarks may depend critically on the type of smooth surfaces and the age of the fingerprint residue. Hence, this level 2 indicated as appropriate on fresh marks may not apply to all casework conditions.

These defined three levels served as a means to understand the potential inhibition of CA fuming on the subsequent Bluestar reaction and to determine the limits of exposure, if any. It was shown that a level 2 exposure was the maximum CA development that still allowed a successful Bluestar reaction. In addition, the exposure of the surface to CA at that level increased the length of the Bluestar-induced luminescence.

The suggested mechanism is that erythrocytes act as initiator sites for the polymerization of CA. The adequate level of CA treatment still offers the possibility of the Bluestar reaction with the added benefit of a protective action of the marks of interest that extends up to the second application of the Bluestar treatment.

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