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Quality Control for Amino Acid Visualization Reagents

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ABSTRACT: A method is described that quantitatively evaluates development conditions and reactivity of amino acid visualization reagents. Ninhydrin was the primary reagent used in this study. The method consists of spotting a serial dilution of amino acids in a numbered grid on paper. Examples of the variability of development as a result of atmospheric conditions are illustrated.

KEYWORDS: forensic science, amino acids, ninhydrin, fingerprints, latent prints

Amino acids have been found to be some of the most stable constituents of eccrine and sebaceous secretions. Over long periods of time they can be easily visualized when examining porous surfaces to determine if friction ridge impressions are present. Ninhydrin chemically reacts with amino acids and other constituents of the latent print deposit to form a blue- to purple-colored complex. It has been widely used for the detection of latent prints on paper and other porous surfaces since the 1950s.

Both temperature and humidity can have an effect on the rate and degree of development [*1,2*]. Indeed, a deposit which would produce an intense color reaction under suitable development conditions will show little or no development under adverse conditions. Because of the natural variation in the concentration of ninhydrin-sensitive constituents in a latent print deposit, the degree of development can not be used to judge the suitability of either the development conditions or the reactivity of the reagent. As a result, in the absence of a known control or standard, some question can (or should) arise as to the suitability of the development conditions when a negative result is obtained. Such questions can be answered if a quality control standard is used.

No widely accepted quality control method that provides for qualitative and quantitative testing of both reagents and development conditions has been reported in latent print literature. Olsen suggested a qualitative test [*3*], a variant of which has been adopted by at least one state forensic science laboratory system. Linde [*4*], in reporting the efficacy of various ninhydrin formulations, indicates the use of several concentrations of glycine to test strength of development; however, there is no indication that the procedure was suggested as a standard for ordinary use. The concentrations used by Linde do not approach the threshold of detectability for either ninhydrin or the zinc chloride/laser methods.

Three criteria were considered in designing a standard for amino acid reagents: the standard must provide for qualitative and quantitative evaluation; the standard must use materials which simulate materials ordinarily encountered in case work; and the standards must be

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easily reproducible. Both the chemicals which will react with the visualization reagent and the substrate on which these chemicals are placed were investigated.

Materials and Methods

Substrate

A number of papers were investigated for use as a substrate, including various chromatography papers, white bond papers, mimeograph and photocopy papers, and several types of card stock. Although several grades of chromatography paper gave good spot size, they ultimately were rejected on the basis that a printed grid used for reference purposes could not be reproduced economically on the paper. The remaining papers, with one exception, did not allow absorption within a reasonable period of time, showing drying times in excess of 1 h. In addition, absorption was uneven, causing a variability in spot size and a difference in concentration of the amino acids across the spot.

The remaining paper was designed for use in continuous roll photocopy machines. An obvious advantage of the xerocopy paper is the ease with which the form can be reproduced photomechanically. Eight grids can be produced on each 8 1/2- by 11-in. (21.6- by 28-cm) sheet of paper. A several years' supply of the grids were prepared and held for use. (Production of that paper was discontinued in 1983, and an acceptable xerographic paper substitute is being investigated.)

Amino Acid Standard

The relative abundance of individual amino acids present in eccrine perspiration has been studied [*5–7*] and summarized [*8*]. The mean relative abundance (serine ratio) of the three cited studies is summarized in Table 1.

The listed L-amino acids were obtained from Sigma Chemical Co. (Kit LAA-21 and L-Orinthine, No. O-2375) and mixed in the proportions given. A stock mixture was prepared by weighing out each amino acid as given in Table 1. This mixture was ground to a fine powder to assure an even distribution of each component. Since amino acids degrade in solution, a stock solution is not acceptable. In the preparation of test strips, 0.25 g of the stock mixture was dissolved in 25 mL of distilled water, and 18 doubling serial dilutions

TABLE 1—*Mean relative abundance (serine ratio) of amino acids in fingerprint deposits (from Knowles* [8]*).*

Amino Acid	Molar Ratio	Molecular Weight	Weight %
Serine	100	105.09	28.8
Glycine	58.5	75.07	12.0
Orinthine	40.5	132.16	14.7
Alanine	28.0	89.10	6.8
Aspartic acid	19.8	133.11	7.2
Theonine	15.5	119.12	5.1
Histidine	15.0	155.16	6.4
Valine	9.5	117.15	3.1
Leucine	8.5	131.17	3.1
Iso-leucine	7.0	131.17	2.5
Glutamic acid	7.0	147.13	2.8
Lysine	7.0	146.19	2.8
Phenylalanine	5.5	165.19	2.5
Tyrosine	4.5	181.19	2.2

(1 : 1) were prepared from it. Two blanks (unspotted and distilled water only) and each of the eighteen dilutions were spotted in sequence at a 5-μL volume using either a SMI Adjustable Volume Digital Micro Pipettor or a Hamilton Repeating Dispenser, a 250-μL syringe, and disposable tips. The sample amount for each dilution is shown in Fig. 1.

Approximately 40 8½- by 11-in. (21.6- by 28-cm) sheets (320 strips) were spotted at a time. The paper was allowed to dry and the 8½- by 11-in. sheets were sealed in plastic in 15-sheet packages and stored at −90°C until needed. When needed, a single grid (strip) was cut from the 8½- by 11-in. sheet and treated. After repeated testing to establish the strip's reliability, it was found that a 15-sheet package stored at room temperature would last 3 to 4 months (depending on frequency of use). No deterioration in intensity or degree of development was noted from the first to the last sheet. No attempt has been made to determine maximum shelf life at room temperature.

Results and Discussion

The prepared standards were subjected to various ninhydrin formulations and placed in a chamber at 30 to 35°C 80% relative humidity (RH) for 24 h. Typical development is shown in Fig. 2. Additional examinations were conducted using zinc chloride [*9*] with laser examination using a Plasma Kinetics 10-W copper vapor laser; the extended limits of visualization can be observed in Fig. 3.

The amino acid serial dilution standards have been used to confirm the reactivity of each newly prepared batch of ninhydrin reagent and are used with case work to confirm that optimum development has taken place. The results may be evaluated quantitatively by visual inspection using a reference standard or by observing the dilution at which the first significant development takes place. Strips are placed directly in the development environment with the processed items to monitor development conditions.

Figure 4 depicts four strips which were treated simultaneously with the same batch of ninhydrin reagent; only environmental factors during development were varied. Strips *a* and *d* were placed in an environment of 25°C at 60% RH for 18 h before treatment. Strips *b* and *c* were placed in a desiccator over Drierite® ($CaSO_4$) for 18 h before treatment. All strips were treated at the same time by dipping them in a 1% ninhydrin : Freon 113 solution [*10*]. After

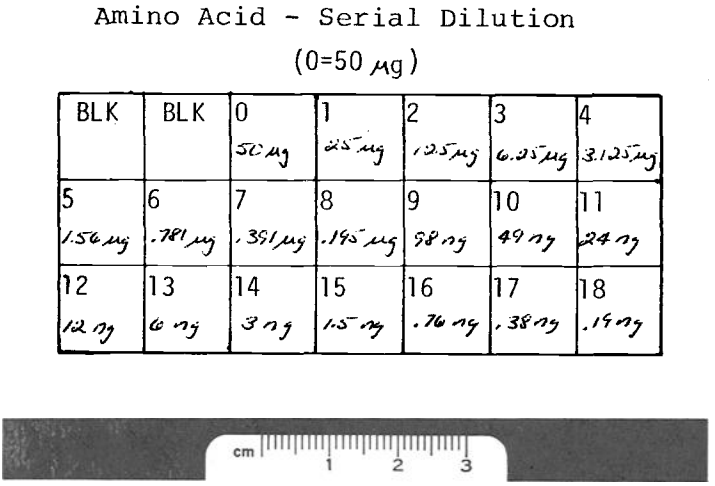

FIG. 1—*Amino acid sample size for each dilution (5-μL volume).*

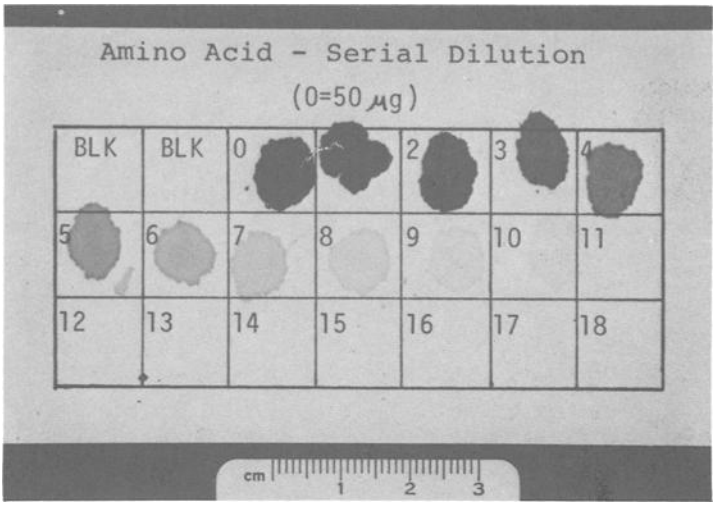

FIG. 2—*Typical optimum development.*

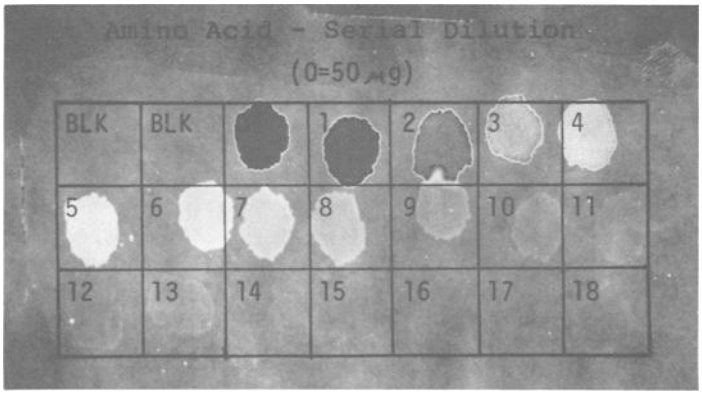

FIG. 3—*$ZnCl_2$—laser development. A ×8 to 16 increase in sensitivity is typically observed.*

being processed with ninhydrin, Strips *a* and *c* were placed in a chamber at 30°C, 75% RH for 24 h. Strips *b* and *d* were placed in a desiccator over Drierite. After 24 h, the strips were evaluated: *a* and *c* showed strong (normal) development. Strip *b* shows barely perceptible development of dilution 0 and *d* shows perceptible development to Dilution 2.

These results seem to indicate that pretreatment temperature/humidity conditions do not have a great effect on the strength of development (at least over short periods of time). Post-treatment temperature/humidity conditions, however, appear to be critical. A decrease of ×1000 in sensitivity between *a* (or *c*) and *b* and a ×250 decrease in sensitivity between *a* and *d* is seen here. The difference in development between *b* and *d* (*d* showing greater sensitivity) is attributed to the fact that some residual humidity was present in the paper immediately after processing which allowed development to commence before that moisture was removed by the desiccant.

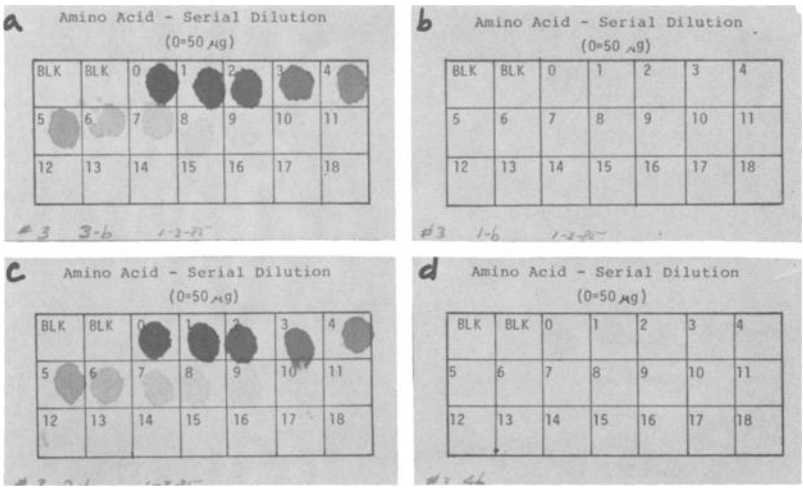

FIG. 4—*Effects of pre- and post-treatment environmental conditions:* (a) *prehumidified, treated, posthumidified;* (b) *predesiccated, treated, postdesiccated;* (c) *predesiccated, treated, posthumidified;* (d) *prehumidified, treated, postdesiccated.*

Although relative humidities approaching zero are not encountered in the environment, very low humidities are routinely encountered in arid or semi-arid climates, and humidities low enough to interfere with optimum development occur in almost any climate at some time. The degree of interference is unknown without a known and replicable standard against which to measure.

Figure 5 depicts four strips which were treated with the same batch of ninhydrin reagent (1%) on successive days in February and allowed to develop in ambient laboratory conditions. The building housing the laboratory was at that time less than one year old and contained up-to-date environmental controls which supposedly maintain constant humidity. No record of the ambient humidity was kept. Strips were treated in the order *a*, *b*, *c*, *d*. Of interest is the observation that development appears to be arrested even when a subsequent strip developed more strongly (*b*, compare *c*); however, the phenomena has been observed on more than one occasion. In any case, such variability in the intensity of development is common from October to April in temperate climates, when relative humidities may be low unless a controlled temperature and humidity environment is available.

A widely used method to cause rapid ninhydrin development is the use of a steam iron or other heat source to speed the reaction. However, a low level background development has been generally noted. When the zinc chloride extension has been attempted, the level of background development is sufficient to cause the background to luminesce intensely, causing prints to be obscured. For that reason, a low temperature development appears preferable if laser exam is anticipated. Since the low temperature method is humidity dependent, a standard can ensure that maximum development has occurred before the zinc chloride ($ZnCl_2$) extension.

Conclusions

The use of a reliable standard helps to ensure that a false negative result is not reported due to insufficient development. Daily variations in ambient conditions can be sufficient to

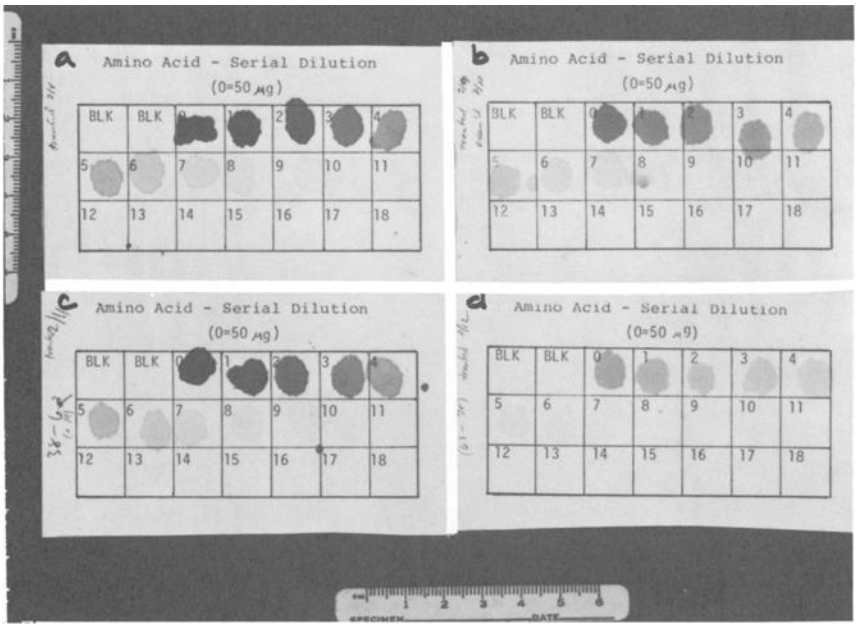

FIG. 5—*Effects of variation in ambient humidity conditions.*

alter profoundly the degree of development, causing as much as a ×1000 decrease in sensitivity. Unless the standard evaluates the reaction down to the limits of detectability (simulating ordinary physiological levels), false negatives may be reported inadvertently. The standard also provides an objective basis to determine optimal environmental conditions for development.

The standard described in this paper has proved reliable in several years' use. It is easy and economical to produce, chemically and physically similar to materials encountered in case work, and provides a quantitative monitoring of reagent strength and development conditions.

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