

# Processing Evidence Using Ninhydrin

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# Processing Evidence Using Ninhydrin

## 1 INTRODUCTION

This procedure is used by trained personnel to ensure consistency and transparency of methods during the processing of footwear and tire evidence received in the Questioned Documents Unit (QDU).

Footwear or tire impressions may be present on the interior of clothing worn by a person who is subjected to significant force by being stomped, kicked, or run over. Due to the degree of force of these actions, skin cells can be transferred from the victim to the inner most surface of an item of clothing in the form of a footwear or tire impression. These impressions may be visible if the skin cells contrast with the color of the garment or they may be latent requiring chemical processing to develop contrast between the impressions and the fabric substrate.

## 2 SCOPE

These procedures are used by forensic examiners in the QDU to develop impressions on footwear and tire evidence using ninhydrin.

## 3 APPLICABILITY

Ninhydrin is used on porous or semi-porous substrates, including fabric, paper, cardboard, and raw wood.

## 4 DEVELOPMENT

Ninhydrin reacts with amino acids to produce a dark purple color (Ruheman's purple). Development is patent on light colored substrates.

Ninhydrin may develop impressions on dark colored substrates, but visualizing these impressions requires the use of an alternate light source and/or digital image processing.

## 5 SENSITIVITY/SPECIFICITY

Ninhydrin is a non-specific amino acid reagent that reacts with biological matrices, including skin cells, perspiration, and blood.

## 6 EQUIPMENT

- Acetone
- Adobe Photoshop (or comparable software)
- Aerosol sprayer
- Alternate light source
- Amino acid reference pad
- Digital camera
- Filter paper
- Flatbed scanner
- General laboratory supplies
- Humidity chamber
- Ninhydrin

- Specialized imaging device
- Steam iron

## 7 STANDARDS AND CONTROLS

Control samples are used to verify the effectiveness of the reagent. A positive control sample will consist of a substance the reagent is expected to react with on a surface appropriate for testing (e.g., filter paper).

- Prepare a positive control sample.
  - A control sample may be prepared at the time that the reagent is tested or multiple samples may be prepared for routine testing prior to testing the reagent.
- Record the results of the reagent check in the Chemical Enhancement and Control Logbook in the footwear and tire laboratory.

## 8 PROCEDURE

### 8.1 Preparation

Personnel shall prepare the working solution as follows. Personnel may prepare other quantities but the ratio of chemicals must be retained.

- Combine the following chemicals.
  - Ninhydrin – 6 g
  - Acetone – 1000 mL
- Stir the solution until the ninhydrin dissolves.

Record the information associated with the batch in the Chemical Enhancement and Control Logbook in the footwear and tire laboratory.

### 8.2 Storage

- Store the working solution in a dark glass bottle.
- Label the bottle with the following information.
  - Reagent name
  - Batch #
  - Preparer's name
  - Date prepared
  - Expiration date
- Apply the following pictograms to the bottle.



### 8.3 Shelf Life

The working solution has a shelf life of 1 year provided any one of the individual components don't expire with a year.

Assign an expiration date to the solution as 1 year from the date of preparation or the earliest expiration date for its components, whichever comes first.

Discontinue use of the working solution if it does not pass a reagent check or its expiration date has passed, and discard it appropriately.

#### 8.4 Application

- A. Process the evidence item in a fume hood using the following steps in order.
  1. Apply the solution to the item using an aerosol sprayer or by pouring the solution over the item in a tray (glass or plastic).
  2. Allow the treated area to dry completely.
  3. Expose the item to heat and humidity using a steam iron or a humidity chamber.
    - Iron
      - i. Set the iron to the heat setting appropriate for the item and the lowest steam setting.
        - Using a lower heat setting is acceptable but the development may take longer.
      - ii. Place a piece of filter paper (larger than the treated area) on the item.
      - iii. Iron the treated area while moving the iron across the item continuously until the desired development occurs.
        - Check the development frequently to avoid overdevelopment of the impression(s) or background development.
        - Generally, the development should occur within 30 seconds to 1 minute.
    - Humidity chamber
      - i. Place the item in the humidity chamber set to 70%–80% relative humidity and 70–80°C for approximately 5 minutes, or until desired development occurs.
- B. Examine the item under white light.
- C. Examine items with low contrast to the developed dark purple color under an alternate light source.
- D. Digitally process images of items with low contrast to the developed dark purple color using the appropriate Photoshop tools and techniques.
  - Refer to section 2.8 of the [QDU Quality Assurance Manual – Part II](#) for further guidance on digitally processing an image using Photoshop.
  - A digital image may be submitted to the OPU for digital processing, as directed by the examiner or analyst.
- E. Record information and observations in the examination record, which can include a copy of the associated record in the Chemical Enhancement and Control Logbook.
- F. Refer to [IMPRS-300 \(Footwear and Tire Evidence Examinations\)](#) for further instruction.

## 9 LIMITATIONS

When treating large items, it may be necessary to treat one area at a time instead of applying the solution to the entire item at the same time.

## 10 SAFETY

See [Laboratory Division Safety Manual](#) for appropriate information, including safe work practices and procedures, personal protective equipment policy, and hazardous waste disposal.

Safety information concerning each of the chemicals used in these procedures are available from the Safety Data Sheets (SDS) on file in the footwear and tire laboratory.

## 11 REVISION HISTORY

Revision	Issued	Changes
00	10/16/2023	Original document issued