Laboratory Procedure for Microchemical Crystal Tests of Inorganic Ions

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Abstract: This forensic science laboratory procedure explains the process of performing microchemical testing to determine the presence of a particular inorganic ion. Utilizing the bridge method, reagents are dragged into a location on a microscope slide containing a sample with an ionic compound, resulting in crystals, which can be used to identify an ion, based on their color, morphology, and optical properties.

Keywords: Forensic Microscopy, Microchemical Crystal Test, Forensic Chemistry, Forensic Science Laboratory

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Introduction

Microchemical crystal testing is an inexpensive, quick, and simple technique utilized to identify particular ions or compounds by the characteristic crystals that are formed after a reaction. Reactions between reagents and ionic compounds can be carried out in a various number of ways, however Chamot's bridge drop method, or reagent mixing, provides the most ease in completion along with fascinating and reliable results (1). In this method, separate drops of sample and reagent are placed next to each other on a microscope slide. Then the reagent drop is dragged into the sample drop, creating a channel between the two, and allowing the reaction to occur.

Forensic scientists working in trace analysis or drug chemistry often utilize microchemical crystal tests as a screening tool to help decide what the next steps in testing will be. This makes microchemical crystal testing a presumptive test, or a test that can only indicate a particular analyte *may* be present. Further confirmatory testing using instrumentation, such as a mass spectrometer, should be completed after microchemical testing to ensure the target analyte is truly present.

Descriptions of the resulting crystals formed in microchemical testing include color, morphology, and optical properties. While morphology varies and can be difficult to describe, crystals formed from microchemical crystal testing typically can be explained with the following: needle, rod, blade, plates, tablet, prism, rosette, burr, tuft, fan, star, cross, sheaf, cluster, bundle and dendrites. **FIGURE 1** provides a visual for each morphology mentioned (2,3). Since each test in this procedure is targeting a singular ion, the crystal morphology should be consistent throughout the sample. In drug chemistry applications, drugs themselves are compounds and comprised of multiple elements, and therefore ions. In this instance, it would be expected to see multiple different morphologies in a microchemical crystal test.

Crystal Morphology Descriptors

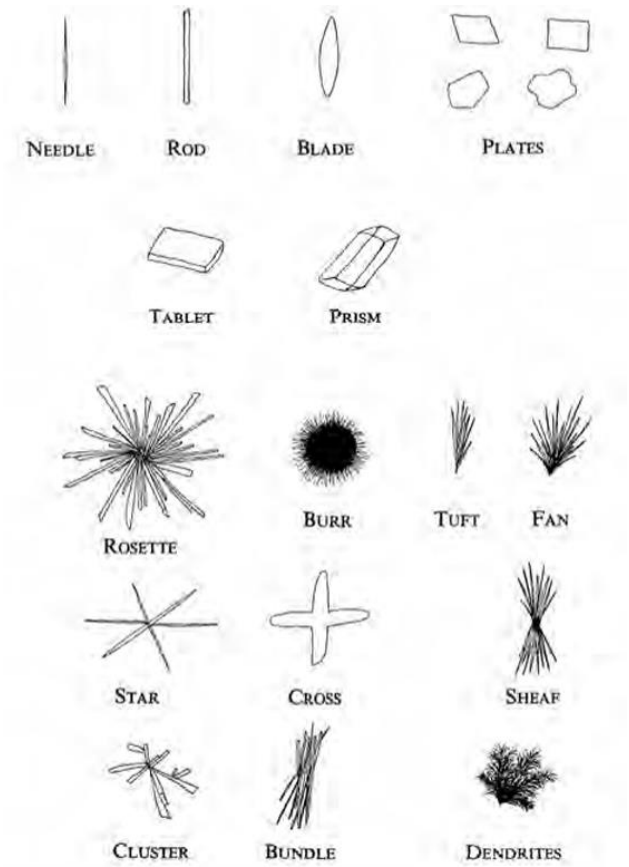

FIGURE 1 Visual of crystal morphology descriptors (2,3).

Crystals as a result of microchemical testing can be assessed using the theories and practices of optical mineralogy. In order to assess optical properties of crystals, a polarizing light microscope must be used. This light microscope has the unique ability to control the direction of light that is being used to illuminate and visualize the sample. First, light passes through a component called the polarizer, which orients all light rays in the East/West direction, before it hits the sample. After light hits and passes through the sample on the stage, it reaches the ocular lens which creates the image. Another component of the polarizing light microscope, which can be introduced into the light path, is the analyzer. The analyzer also orients light in a particular direction, the North/South direction once it has passed through the sample, but before it reaches the ocular.

With just the polarizer in the light path, the light results in plane polarized light (PPL). Under plane polarized light, pleochroism can be determined, or the change in inherent color when a sample is rotated under plane polarized light.

When the analyzer is inserted, the systematic light is referred to cross-polarized light (XPL). When describing the color of crystals, it is important to note what light setting is being used and if colors recorded are inherent (seen in plane polarized light) or interference colors (seen in cross-polarized light).

When under cross-polarized light, the recombination of light after it interacts with the sample can be seen, resulting in interference colors. A third additional component of polarizing light microscopes is the first-order waveplate, at 550nm. This acts as a filter with a known and constant wavelength of light that is added into the light path. When this additional wavelength of light is added while in cross-polarized light, interference colors will change, and this change can be used to determine the birefringence of the crystals, or the relative difference in the refractive index values of the crystal. Utilizing the width of the crystal and the interference colors, a Michel-Lévy chart (**FIGURE 2**) can be used to determine the birefringence. Highest order interference colors should be utilized for determining birefringence. Sign of elongation (SOE) can also be determined by observing the change in interference colors once the first-order wave plate is inserted under cross-polarized light. Note that orienting the microscope slide in the Northeast/Southwest position does not guarantee the optical axis of the sample is also oriented in the same direction. Samples should be oriented in between extinction points, which ensures that observation of interference colors are at the maximum. To determine SOE, either color in the form of wavelength will be added or subtracted from the sample's interference colors. Typically, this is used when a location on the crystal is located under cross-polarized light that looks gray, then the first-order wave plate is inserted. If the grey spot interference color changes to yellow, subtracts wavelength, after inserting the first-order waveplate, then the SOE is negative. If the grey spot interference color changes to blue, adding wavelength, after inserting the first-order waveplate, then the SOE is positive.

Michel-Lévy Chart (4)

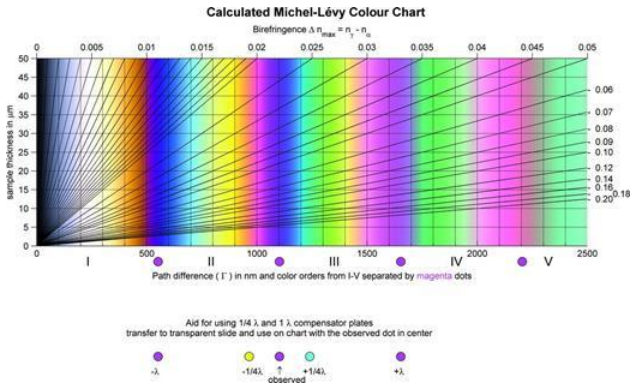

FIGURE 2 Michel-Lévy chart (4).

An important aspect of all laboratory analytical testing is quality assurance and quality control. A quality assurance program consists of policies and procedures put in place to ensure accurate and reliable results are being obtained. In those procedures, quality control is explained. Quality control includes the action of running positive and negative controls during testing. A positive control is used to ensure all reagents and equipment are working properly and provide a true positive test outcome. This type of quality control is to prevent false negative tests. A positive control utilizes a reference sample with regents and equipment being used. Negative controls are used to ensure that there is no contamination across reagents and equipment used in testing. This type of quality control shows what a true negative test will produce and prevents false positives. A negative control will only utilize the reagents and equipment used in the test; no sample is used in this type of quality control. Forensic testing consists of a questioned or unknown sample that is always compared to a reference or known sample. When working with unknowns in this procedure, it is crucial to test positive and negative controls to compare the unknown sample to.

Learning Objectives

The purpose of this laboratory exercise is to familiarize students with microchemical reactions and using a polarizing light microscope to visualize various crystal morphologies. This laboratory exercise should proceed a basic introduction to using and functionality of a polarizing light microscope.

At the completion of this laboratory exercise, students will:

- have basic knowledge of the microchemical theory
- determine differences between presumptive and confirmatory tests
- apply techniques to perform microchemical testing
- identify the presence of specific ions, and the use of polarizing light microscopy to analyze test results.

Student learning can be measured using a variety of techniques. Below are post-laboratory questions that can be used to assess student learning.

1. What are microchemical tests? Give the purpose and value of the tests.
2. List three reactions that are used for microchemical testing. Give an example of each.
3. If crystals are found under plane-polarized light, why is a search for crystals recommended under crossed polars?
4. Explain why a precipitate formed in some of the microcrystalline tests.
5. Why are microchemical tests not considered a confirmatory test for illicit drugs?
6. What are positive and negative controls? Why are they required?

Methods

Hazards and Safety

Hazardous chemicals are used in this lab. Reagents should be stored properly before, during, and after use, and proper personal protective equipment, including gloves and safety glasses, should be worn. Refer to the laboratory's SDS document for particular reagents' hazards. Be aware of extreme light intensity levels of the microscope to avoid eye damage.

Equipment

Equipment involved in this experiment includes; Lecia DM750P polarizing light microscope with objectives of varying magnifications (4x, 10x, and 40x) and a first-order waveplate (550nm), microscope slides, coverslips, inorganic ions, reaction reagents, wooden toothpicks, micro-spatula, distilled water, glass Pasteur pipettes, and pipette bulbs. A Lecia ICC50W camera was used to capture images, however, any camera would work to capture images.

Reagent Preparation

Reagents were prepared as follows;

- Silver Nitrate (10mg/mL): 0.5g solid dissolved in 50 mL of distilled water
- Potassium Mercuric Thiocyanate (10mg/mL): 0.5g solid dissolved in 50 mL of distilled water
- Dimethylglyoxime (25mg/mL): 0.5g solid dissolved in 20 mL distilled water
- Potassium Dichromate (24mg/mL): 1.2g solid dissolved in 50 mL distilled water

Procedure

Part I: Testing Known Samples

General Bridging Procedure

1. Label the microscope slide with ion, initials, and date
2. Place drop or suggested amount of sample ion on one side of microscope slide
    a. If sample is solid, use micro-spatula to place on slide, then dissolve in suggested solvent by using a Pasteur pipette to place a drop of solvent on top of the sample and mix until completely dissolved with toothpick
    b. Clean off micro-spatula with laboratory wipe and methanol between samples to avoid contamination
3. Place separate drop of reagent next to sample
4. Bridge the two drops with a clean toothpick, dragging from reagent into the sample ion, creating a channel between the reagent and the sample (**FIGURE 3**)
5. Top with coverslip, placing on reagent side first then dropping down
6. Visualize crystals under polarizing light microscope using the 4x, 10x, and 40x objectives
    a. Pick a single crystal and view under plane polarized light
        i. Determine if the crystal exhibits pleochroism
    b. Engage the analyzer to view the sample under cross-polars
        i. Determine if the crystal is isotropic or anisotropic
        ii. Observe and record any interference figures and/or extinction points
    c. Insert the first-order waveplate
        i. Determine the sign of elongation of the crystal

ii. Determine the birefringence of the crystal using the highest order interference color

Bridging Reagent to Sample

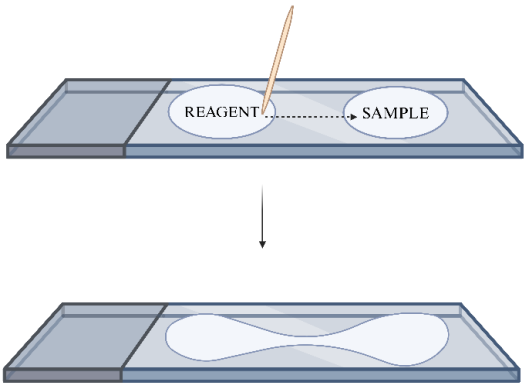

FIGURE 3 Cartoon depicting the placement of sample and regent drops on a microscope slide for microchemical crystal testing using Chamot's bridge method (1). Created in https://BioRender.com.

Best Practice for each Ion

Cations

- Potassium: dissolve enough solid Potassium Chloride (KCl) to fit on the tip of the beveled end of the micro-spatula (**FIGURE 4**) in 1-2 drops of distilled water, mix with a toothpick, bridge with 1-2 drops of Chloroplatinic Acid (H_2PtCl_6), crystals form immediately

Sample Amount

FIGURE 4 Photo showing the approximate amount of sample powder needed for microchemical crystal testing reactions. Micro-spatula is shown against a standard 25 mm x 75 mm x 1.0 mm microscope slide for reference.

- Silver: place 1-2 drops of Silver Nitrate ($AgNO_3$) onto microscope slide, carefully add just enough Zinc Powder (Zn) to fit on the tip of the beveled end of the micro-spatula into the drop of Silver Nitrate, DO NOT MIX, let sit for 30 seconds then gently top with cover slip, crystals will form as a precipitate immediately
- Lead: dissolve enough solid Lead (II) Nitrate ($PbNO_3$) to fit on the tip of the beveled end of the micro-spatula in 1-2 drops of distilled water and mix with a toothpick, bridge with 1-2 drops of Potassium Dichromate ($K_2Cr_2O_7$), wait 5-10 minutes to see crystals
- Nickel: dissolve enough solid Nickel (II) Nitrate ($Ni(NO_3)_2$) to fit on the tip of the beveled end of the micro-spatula in 1-2 drops of distilled water and mix with a toothpick, bridge with Dimethylglyoxime (DMG), wait 5-10 minutes to see crystals
- Zinc: dissolve enough solid Zinc Acetate ($C_4H_6O_4Zn$) to fit on the tip of the beveled end of the micro-spatula in 1-2 drops of distilled water and mix with a toothpick, bridge with 1-2 drops of Potassium Mercuric Thiocyanate ($K_2Hg(SCN)_4$), crystals will form immediately
- Copper: dissolve enough solid Copper Acetate ($Cu(CH_3COO)_2$) to fit on the tip of the beveled end of the micro-spatula in 1-2 drops of distilled water and mix with a toothpick, bridge with Potassium Mercuric Thiocyanate ($K_2Hg(SCN)_4$), wait 15-20 minutes to see crystals
- Cobalt: dissolve enough solid Cobalt (Co) to fit on the tip of the beveled end of the micro-spatula in 1-2 drops of distilled water and mix with a toothpick, bridge with 1-2 drops of Potassium Mercuric Thiocyanate ($K_2Hg(SCN)_4$), crystals will form immediately

Anions

- Sulfate: place 1-2 drops of Silver Nitrate ($AgNO_3$) onto the microscope slide, put 1 grain of solid Aluminum Sulfate ($Al_2(SO_4)_3$) into the Silver Nitrate, DO NOT MIX, wait 5 minutes until Aluminum Sulfate is dissolved and then top with coverslip to see crystals
- Chloride: dissolve 5 flakes of solid Cupric Chloride ($CuCl_2$) in 1-2 drops of 1M Hydrochloric Acid (HCl) and mix with a toothpick, bridge with 1-2 drops of Silver Nitrate ($AgNO_3$), crystals will form immediately
- Nitrite: dissolve enough solid Sodium Nitrite ($NaNO_2$) to fit on the tip of the beveled end of the micro-spatula in 1-2 drops of distilled water and mix with a

toothpick, bridge with Silver Nitrate ($AgNO_3$), crystals will form immediately

- Chromate: place enough solid Potassium Chromate (K_2CrO_4) powder to fit on the tip of the beveled end of the micro-spatula on to a microscope slide, bridge with 1-2 drops of Silver Nitrate ($AgNO_3$), wait 30 seconds then top with coverslip, crystals will form immediately

Part II: Testing Unknown Samples

1. Obtain unknown cation and anion samples
2. Perform both positive and negative controls, following the procedure outlined in Part I.
3. Analyze unknown ions to determine what cation or anion is present following the General Bridging Procedure outlined in Part I.

Results

Each inorganic ion will produce a characteristic crystal both in shape and color, which can be visualized under plane polarized light, cross-polarized light, and with the first-order waveplate. **Table 1** describes the reagents used for each inorganic ion, as well the crystals that will form from a positive reaction.

The longer the reaction time, the more crystals will form, therefore if crystals are not seen immediately it is recommended to let the reaction sit for 5-10 minutes before searching for crystals under the microscope. Crystals form first along the edges of the channel and are the ideal place to start locating crystals.

TABLE 1 Description reagents used to test for inorganic ions, along with the crystal morphologies expected for a positive reaction as seen under plane polarized light.

Reagent	Reacts with	Positive reaction crystal descriptions
Silver Nitrate ($AgNO_3$)	Zinc Powder (Zn)	Black dendrites, pine tree branch-like spikes
	Aluminum Sulfate ($Al_2(SO_4)_3$)	Clear rods
	Cupric Chloride ($CuCl_2$)	Clear needles and rods, fans and tufts created from rods
	Sodium Nitrite ($NaNO_2$)	Clear rods, interference colors seen under XPL
	Potassium Chromate (K_2CrO_4)	Red stars and crosses
Potassium Mercuric Thiocyanate ($K_2Hg(SCN)_4$)	Zinc Acetate ($C_4H_6O_4Zn$	Clear Feather like fans, interference colors seen under XPL
	Copper Acetate ($Cu(CH_3COO)_2$)	Yellow feather like fans
	Cobalt (Co)	Blue needles, rosettes, and clusters
Potassium Dichromate ($K_2Cr_2O_7$)	Lead (II) Nitrate ($PbNO_3$)	Yellow needles, blue/green under XPL
Dimethylglyoxime (DMG)	Nickel (II) Nitrate ($Ni(NO_3)_2$)	Pink needles, blue under XPL
Chloroplatinic Acid (H_2PtCl_6)	Potassium Chloride (KCl)	Yellow plates and prisms, typically square or rhombus shaped

FIGURES 5–15 depict the crystals that are formed for each inorganic ion under the microscope at varying levels of magnification. The following are in the same order as TABLE 1.

Dendrite Crystals

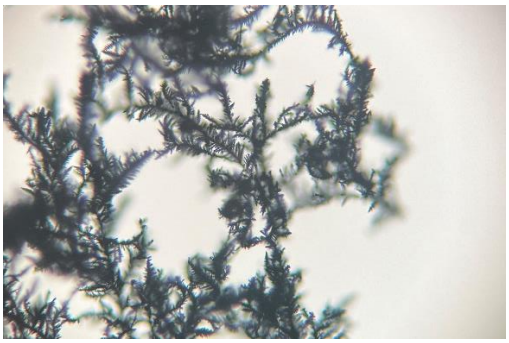

FIGURE 5 Black dendrite crystals as a result of Silver Nitrate reacting with Zinc Powder, seen under plane polarized light at 400x magnification.

Alumimum Sulfate Crystals

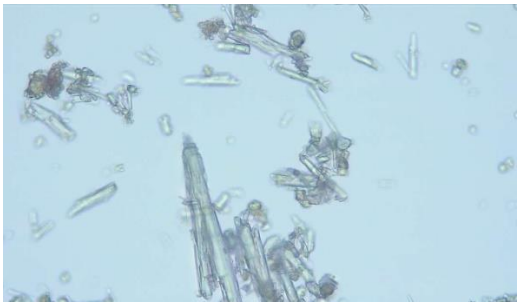

FIGURE 6 Clear rod crystals as a result of Aluminum Sulfate reacting with Silver Nitrate, seen under plane polarized light at 400x magnification.

Cupric Chloride Cystals

FIGURE 7 Clear rod and tuft crystals as a result of Cupric Chloride reacting with Silver Nitrate, seen under plane polarized light at 400x magnification.

Sodium Nitrite Crystals

FIGURE 8 *Clear rod crystals as a result of Sodium Nitrite reacting with Silver Nitrate, seen under plane polarized light at 400x magnification.*

Potassium Chromate Crystals

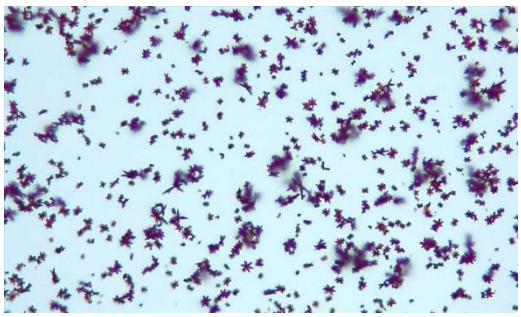

FIGURE 9 Red star crystals as a result of Potassium Chromate reacting with Silver Nitrate, seen under plane polarized light at 400x magnification.

Zinc Crystals

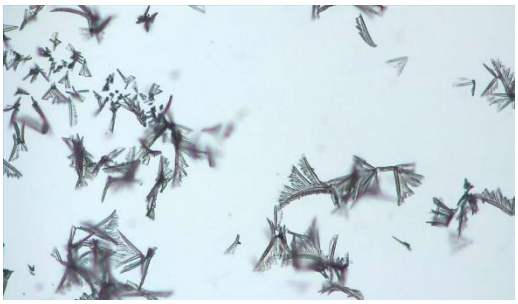

FIGURE 10 Clear feather like fan crystals as a result of Zinc Acetate reacting with Potassium Mercuric Thiocyanate, seen under plane polarized light at 100x magnification.

Copper Acetate Crystals

FIGURE 11 Yellow feather like fans as a result of Copper Acetate reacting with Potassium Mercuric Thiocyanate, seen under plane polarized light at 400x magnification.

Cobalt Crystals

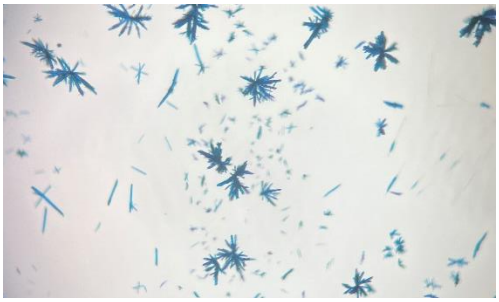

FIGURE 12 Blue needle, rosette, and cluster crystals as a result of Cobalt reacting with Potassium Mercuric Thiocyanate, seen under plane polarized light at 100x magnification.

Lead Nitrate Crystals

FIGURE 13 Yellow needle crystals as a result of Lead Nitrate reacting with Potassium Dichromate, seen under plane polarized light at 400x magnification.

Nickel Nitrate Crystals

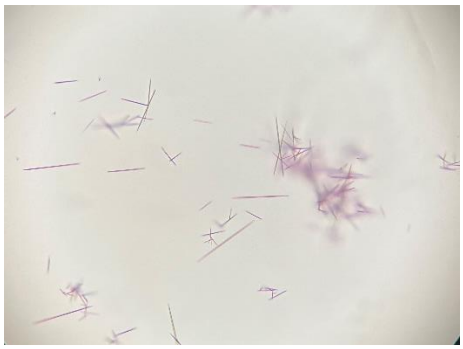

FIGURE 14 Pink needle crystals as a result of Nickel Nitrate reacting with Dimethylglyoxime, seen under plane polarized light at 400x magnification.

Potassium Chloride Crystals

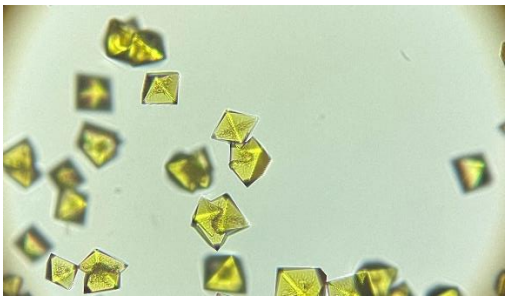

FIGURE 15 Yellow prism and plate crystals as a result of Potassium Chloride reacting with Chloroplatinic Acid, seen under plane polarized light at 400x magnification.

Discussion and Conclusion

Microchemical crystal tests act as a quick and easy presumptive test for the presence of particular elements, ions, or compounds. In this laboratory procedure, the ideal conditions for both sample and reagent preparation were determined. Utilizing Chamot's bridge method to carry out the reaction between reagents and inorganic ions, microscopic crystal morphologies were found and described as seen under plane polarized light of a polarizing light microscope.

Although the theories behind microchemical testing are well known and accepted within the scientific community, many other procedures make the experiment more difficult and require more preparation due to the use of alternate reagent mixing methods. The bridge method, simple dissolving, or a hanging drop can be used. In this procedure, the bridge method was used for all reaction except one, with the one other reaction being simple dissolving of a solid sample into a reagent. The hanging drop method requires an additional piece of equipment, a glass tubing spacer, usually 6mm diameter glass tubing. In this method, a drop of sample is placed on a microscope slide and then surrounded by placing the glass spacer on top of it. A coverslip with a drop of reagent is then placed on top of the spacer, effectively "hanging" the reagent over the sample drop. This creates a gaseous reaction between the sample and the reagent. Crystals are then formed on the coverslip, which then must be inverted and placed onto another microscope slide to be able to visualize under the polarizing light microscope (5). Handling the coverslip is both dangerous and difficult to do properly without disturbing the crystals. This method is often referenced in other procedures but does not provide a more efficient or effective reaction as compared to the bridge method.

Limitations of this procedure include the lack of exact measurements of solid samples. Such a minute amount is needed to carry out the reaction, but for standardization purposes, the weight of solid sample in grams is ideal.

Microchemical crystal testing provides an easy screening tool to indicate the presence of compounds which can be utilized in drug or trace forensic disciplines. A more advanced extension of this laboratory procedure would utilize controlled substances as samples and their resulting crystals following this same procedure using Chamot's bridge method.

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