Technical Procedure for Polarized Light Microscopy

1.0 Purpose - This procedure specifies the required elements for the preparation and use of microcrystalline reagents.

2.0 Scope - This procedure applies to all polarized light microscopy techniques used in the Drug Chemistry Sections of the State Crime Laboratory.

3.0 Definitions

- **Quality control (QC) check -** Periodic confirmation of the reliability of equipment, instrumentation, and/or reagents.
- **Reference Material** – Material sufficiently homogeneous and stable with reference to specified properties, which has been established to be fit for its intended use in measurement or in examination of nominal properties.

4.0 Equipment, Materials and Reagents

4.1 Equipment

- Polarized light microscope

4.2 Materials and Reagents

- Fume hood
- Eye protection
- Laboratory coat
- Gloves
- Balance
- Beakers or other glass vessels
- Graduated cylinder
- Glass stirring rod
- Reagent bottle(s)
- Microscope slides
- Objective centering screws
- Spatula
- Weigh boats or other weigh vessels
- Reference materials
- Commercial reagents (ACS grade or higher, where applicable)

5.0 Procedure

5.1 Standards and Controls - Quality control checks of all reagents shall consist of a negative check and a positive check. Both checks shall be acceptable according to the procedure listed for each reagent, and shall be recorded together as a quality control check in the Forensic Advantage (FA) Resource Manager.

5.1.1 Negative quality control checks shall be performed according to the procedure listed with no sample present.

5.1.1.1 Acceptable results are: no crystal formation.

5.1.1.2 If crystals do form, steps will be taken until no crystals are formed. This includes retesting with a new microscope slide, re-cleaning any utensils used, or making a new reagent.

5.1.2 Positive and negative quality control checks shall be performed at six month intervals according to the procedure listed for each reagent using the reference material listed. See each procedure for acceptable results.

5.1.2.1 The result of the quality control check shall be recorded in the FA Resource Manager according to the Administrative Policy for Drug Chemistry Quality Assurance.

5.1.3 Microcrystalline reagents may be prepared in any amount provided that the component ratios are kept constant.

5.1.4 Microcrystalline reagents shall expire three years from date of preparation.

5.1.5 Reagent bottles shall be labeled and checked as described in the Administrative Policy for Drug Chemistry Quality Assurance.

5.2 Calibrations - N/A

5.3 Sampling - See Drug Chemistry Section Administrative Procedure for Sampling.

5.4 Operation of the Polarized Light Microscope

5.4.1 Switch on the light source. (Refer to the operator manual for location/description of specific parts mentioned below.)

5.4.2 Place the specimen slide on the stage.

5.4.3 Adjust the desired light intensity with the control lever.

5.4.4 Make sure the field diaphragm is open to the edge of the field view.

5.4.5 Focus with the coarse and fine adjustments for the desired objective.

5.4.6 Move the microscope slide around to view the entire specimen, adjusting the focus accordingly.

5.4.7 Push the filter in to view the specimen with polars crossed, or pull it out to view with uncrossed polars.

5.4.8 If the objective is changed:

5.4.8.1 Adjust the fine focus adjustment.

5.4.8.2 Set the field diaphragm to just inside the field of view.

5.4.8.3 Adjust the aperture diaphragm for optimum contrast and resolution.

5.5 Application of Procedures on Evidence

5.5.1 Criteria for Establishing Matches

5.5.1.1 Test samples that have evaporated to dryness may not be used for evaluation of crystals.

5.5.1.2 Crystals are identified by morphology (i.e., shape).

5.5.1.3 Crystals shall be compared to those of standards tested in the same manner.

5.5.1.4 Final comparison shall be with actual crystals given by the standards listed.

5.5.1.5 For frequently observed crystals (e.g., cocaine) standards are not required to be run with each test sample.

5.5.1.6 Any crystals formed shall be observed under non-filtered and/or filtered light.

5.5.1.7 NOTE: The sole formation of "modified" crystals is not considered a positive test, and therefore shall require additional testing to be performed.

5.6 Formulas for Preparing Reagents

5.6.1 Gold Chloride in 20 % Acetic Acid With Optional 0.05 N Hydrochloric Acid

5.6.1.1 This reagent is used for cocaine and phencyclidine.

5.6.1.2 Gold Chloride/20 % Acetic Acid (A)

5.6.1.2.1 Add 10 milliliters glacial acetic acid to 40 milliliters of water.

5.6.1.2.2 Dissolve 1.0 gram of gold chloride in the 50 milliliters of 20 % acetic acid, with stirring.

5.6.1.2.3 Lot number: Eight digit format year/month/day/GdCl20/Initials of preparer. Example: 20101231GdCl20XXX

5.6.1.3 0.05 N Hydrochloric Acid (B)

5.6.1.3.1 Add 1 milliliter of concentrated hydrochloric acid to 250 milliliters of distilled water. Check the pH of the solution to ensure acidity.

5.6.1.4 Application of Procedure on Evidence

5.6.1.4.1 A drop of the gold chloride/20% acetic acid (A) reagent shall be placed on a microscope slide and viewed to ensure no crystals have formed.

5.6.1.4.1.1 If crystals form after the reagent is added to the slide, see **5.1.1.2.**

5.6.1.4.2 A small portion of sample shall be mixed with the reagent.

5.6.1.4.3 Optional step: Dilute a small portion of the sample on a microscope slide in a drop of 0.05N hydrochloric acid (B) before mixing with a drop of gold chloride/20% acetic acid (A).

5.6.1.5 QC Check: Cocaine forms cross shaped crystals.

5.6.1.6 Results: Cocaine forms cross-shaped crystals.
Phencyclidine forms squares with diagonal markings, often elongated along one axis.

5.6.1.7 Record results in the FA case file if test is being performed for casework or FA Resource Manager if test is being performed for quality control purposes.

5.6.1.8 Limitations: This technique does not distinguish between the salt and free base forms or the d-/l- forms of cocaine. It can however distinguish cocaine from its diastereoisomers.

5.7 New Polarized Light Microscopes

5.7.1 New microscopes shall be installed by a certified engineer according to the manufacturer's instructions.

5.7.2 Kohler illumination adjustments shall be performed as needed.

5.7.3 Polarized light microscopes shall be added to the FA Resource Manager, to include serial number and/or asset number.

5.8 Maintenance

5.8.1 Polarized light microscopes shall be serviced yearly by a certified vendor. Service records shall be maintained in the FA Resource Manager.

5.8.2 When a polarized light microscope has been placed out of service (e.g., maintenance, malfunction, leaving the direct control of the Laboratory), correct operation shall be demonstrated by a quality control check utilizing Gold Chloride in 20 % Acetic Acid With Optional 0.05 N Hydrochloric Acid as outlined in **5.6.1**.

5.8.2.1 Results of the QC check shall be documented in the corresponding microscope resource of the FA Resource Manager.

5.8.3 If the amount of light passing through the optics decreases significantly so that a sample cannot be seen, steps shall be taken to correct this. This may include, but is not limited to, checking the Kohler illumination parameters.

5.9 Calculations - N/A

5.10 Uncertainty of Measurement - N/A

6.0 Limitations

6.1 Diluents may interfere with crystal formation in some cases. Extraction or solvent washes may be needed to remove unwanted components before microcrystalline reagents are used.

6.2 Concentration of samples may need to be increased or decreased to aid in crystal formation.

7.0 Safety - Caution shall be taken when working with acids, bases, or other chemicals listed throughout the procedure. Refer to Appendix 1 for chemical hygiene and safety precautions.

8.0 References

Allen, A.C., Cooper, D.A., Kiser, W.O., Cottrell, R.C. "The Cocaine Diastereoisomers", *Journal of Forensic Sciences*, JFSCA, Vol. 26, No. 1, Jan. 1981, pp. 12-26.

Brinsko, K.M, Golemis, D., et. Al. *A modern compendium of microcrystal tests for illicit drugs and diverted pharmaceuticals*, McCrone Research Institute, Chicago; 155 pages; 2016.

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Instruction Manual for Olympus Microscope, Model BHSP.

Butler, William P. *Methods of Analysis for Alkaloids, Opiates, Marihuana, Barbiturates, and Miscellaneous Drug,. Publication #341*. Washington, D.C.: U.S. Treasury Department, Internal Revenue Service December, 1966: 55, 114.

Clarke, E.G.C., and R.G. Todd, **eds**. *Isolation and Identification of Drugs*. 1st Edition. London: Pharmaceutical Press, 1969: 135-141,801.

Fulton, C.C. *Modern Microcrystal Tests for Drugs – The Identification of Organic Compounds by Microcrystalline Chemistry*. New York: Wiley-Interscience, 1969.

Sobol, S.P. and R.A. Moore. *Analytical Manual*, J.W. Gunn, ed. Bureau of Narcotics and Dangerous Drugs, Washington, D.C.: U.S. Government Printing Office 0-506-836, 1970: 105.

Smith, F.P., ed. *Handbook of Forensic Drug Analysis*. Boston, Massachusetts: Elsevier Academic Press, 2005: 238.

ASTM International Standard E1968, 2011, "Standard Guide for Microcrystal Testing in Forensic Analysis of Cocaine." ASTM International: West Conshohocken, PA, 2011, www.astm.org.

ASTM International Standard E-2125, 2007, "Standard Guide for Microcrystal Testing in the Forensic Analysis of Phencyclidine and Its Analogues." ASTM International: West Conshohocken, PA, 2007, www.astm.org.

User manual for appropriate polarizing light microscope model(s).

9.0 **Records -** Entries in Resource Manager of the Forensic Advantage (FA) System

Laboratory Safety Manual- Chemical Hygiene Plan and Hazardous Communication Program

10.0 **Attachments** – Appendix 1.

Revision History		
Effective Date	Version Number	Reason
09/21/2020	8	Removed all references to hairs/hash/THC/marijuana. **4.2** – Added commercial reagents (ACS grade or higher). **5.1 & 5.1.2.1** – Reworded. **5.1.1.2 –** Removed "obtaining new solvent." **5.3** – Changed "Technical" to "Administrative". **5.5.1. –** Removed "Dry Sample Method", reflected elsewhere. Moved old **5.5.1.2** to **5.5.1.6**. **5.5.1.7** – Added. **Original 5.6.1, 5.6.3, and 5.6.4 –** Removed; original 5.6.2 becomes 5.6.1. **5.6.1.3.1** – Added pH check. **5.6.1.4 (Old 5.6.2.4) –** Updated steps. **5.6.1.7** – Added. **5.7** and **5.8** – Clarified polarized light microscopes and updated quality control check and FA Resource Manager requirements. **7.0** – Removed reference to Safety Manual, and replaced completely with current. **8.0** - Removed duplicate reference and added user manuals. **9.0 –** Added resource manager to Records. **10.0** – Added Appendix 1.

Appendix 1: Chemical Hygiene and Safety Precautions

Hydrochloric Acid **DANGER: HIGH RISK CHEMICAL**	
	**HEALTH 3** **FLAMMABILITY 0** **REACTIVITY 1**
Detection of Release	Light yellow liquid; pungent odor
Signs/Symptoms of Exposure	Severe skin burns and eye damage; respiratory irritation
PEL	OSHA Table Z-1: 5 ppm/7 mg/m3
Associated Hazards	Severe skin burns; Severe eye damage; respiratory irritation
Controls	Use under fume hood. Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the product. Use eye protection. Handle with gloves. Wear lab coat. Gloves: nitrile (break through time 16 minutes).
Safe handling, storage, disposal	Avoid contact with skin and eyes. Avoid inhalation of vapor or mist. Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage. Store in cool dry area. Dispose in Hazardous Waste.
Emergency Procedures	**Eye Contact:** Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician. Continue rinsing eyes during transport to hospital. **Inhalation Exposure:** If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician. **Ingestion:** Do NOT induce vomiting. Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician. **Skin Contact:** Take off contaminated clothing and shoes immediately. Wash off with soap and plenty of water. Consult a physician. **Spills:** Avoid breathing vapors, mist or gas. Ensure adequate ventilation. Evacuate personnel to safe areas. Small contained spill: wearing appropriate PPE, soak up with inert absorbent material, and place in container. Dispose in Hazardous Waste. Large spills: Evacuate area and call 911 (Haz Mat).

Glacial Acetic Acid **DANGER: HIGH RISK SUBSTANCE** *	
	**HEALTH 3** **FLAMMABILITY 2** **REACTIVITY 0**
Detection of Release	Colorless liquid; stinging odor
Signs/Symptoms of Exposure	Severe skin burns and eye damage; respiratory irritation.
PEL	ACGIH TWA – 10 ppm; ACGIH STEL – 15 ppm (Inhalation)
Associated Hazards	Causes severe skin burns and eye damage. Flammable liquid and vapor.
Controls	Use under fume hood. Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the product. Use eye protection. Handle with gloves. Wear lab coat. Gloves: nitrile (break through time 32 minutes)
Safe handling, storage, disposal	Avoid contact with skin and eyes. Avoid inhalation of vapor or mist. Keep away from sources of ignition. Take measures to prevent the build-up of electrostatic charge. Keep in a tightly closed container. Containers which are opened must be carefully resealed and kept upright to prevent leakage. Dispose of in Hazardous Chemical Waste.
Emergency Procedures	**Eye Contact:** Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician. Continue rinsing eyes during transport to hospital. **Inhalation Exposure:** If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician. **Ingestion:** Do NOT induce vomiting. Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician. **Skin Contact:** Take off contaminated clothing and shoes immediately. Wash off with soap and plenty of water. Consult a physician. **Spills:** Avoid breathing vapors, mist or gas. Ensure adequate ventilation. Evacuate personnel to safe areas. Small contained spill: wearing appropriate PPE, soak up with inert absorbent material, and place in container. Dispose in Hazardous Waste. Large spills: Evacuate area and call 911 (Haz Mat).