Technical Procedure for Polarized Light Microscopy

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

2.0 Scope - This procedure applies to all polarized light microscopy techniques used in the Drug Chemistry Section of the Raleigh location 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

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 Resource Manager section of FA.

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

5.1.1.1 Acceptable result is 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 quality control checks shall be performed 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 Resource Manager section of FA according to the Drug Chemistry Section Technical Procedure for Receipt and Quality Assurance of Supplies, Equipment, Reference Collections, Standards and Reagents.

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

5.1.4 Microcrystalline reagents may be used until depletion provided they are stored in closed containers.

5.1.5 Reagent bottles shall be labeled and checked as described in the Drug Chemistry Section Technical Procedure for Receipt and Quality Assurance of Supplies, Equipment, Reference Collections, Standards and Reagents.

5.1.6 Re-checks shall be performed on all microcrystalline reagents according to the Drug Chemistry Section Technical Procedure for Receipt and Quality Assurance of Supplies, Equipment, Reference Collections, Standards and Reagents to ensure reagent reliability.

5.2 Calibrations - N/A

5.3 Sampling - See Drug Chemistry Section Technical 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 Dry Sample Method

5.5.1.1 A small portion of sample shall be placed on a microscope slide and a drop of the reagent shall be mixed with the sample.

- Optional step indicated in some procedures uses a hydrochloric acid solution (B) which may be used to dissolve the sample before addition of solution (A).

5.5.1.2 Any crystals formed shall be observed under non-polarized and/or polarized light.

5.5.2 Criteria for Establishing Matches

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

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

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

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

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

5.5.3 Formulas for Preparing Reagents

5.5.3.1 5 % Mercuric Chloride

5.5.3.2 This reagent is used for heroin and caffeine.

5.5.3.3 5 % Mercuric Chloride (A)

5.5.3.3.1 Add 1.5 grams of mercuric chloride to 30 milliliters of distilled water (5 % weight volume solution).

5.5.3.3.2 Lot number: Eight digit format year/month/day/MerCur5/Initials of preparer. Example: 20101231MerCur5XXX

5.5.3.4 0.05 N Hydrochloric Acid (B)

5.5.3.4.1 Add 1 milliliter of concentrated hydrochloric acid to 250 milliliters of distilled water.

5.5.3.5 Application of Procedure on Evidence

5.5.3.5.1 Mix directly with reagent (A), or dilute a small portion of the sample on a microscope slide in a drop of 0.05 N hydrochloric acid (B) before mixing with a drop of the 5 % mercuric chloride (A).

5.5.3.6 QC check: Heroin forms fans/dendrites

5.5.3.7 Results: Caffeine – dendrites, but longer and less dense than heroin dendrites.

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

5.5.4.1 This reagent is used for cocaine and phencyclidine.

5.5.4.2 Gold Chloride/20 % Acetic Acid (A)

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

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

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

5.5.4.3 0.05 N Hydrochloric Acid (B)

5.5.4.3.1 Add 1 milliliter of concentrated hydrochloric acid to 250 milliliters of distilled water.

5.5.4.4 Application of Procedure on Evidence

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

5.5.4.5 QC Check: Cocaine forms cross shaped crystals.

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

5.5.5 50 % Acetic Acid and Gold Chloride in 50 % Acetic Acid

5.5.5.1 This reagent is used for enantiomer determination for propoxyphene.

5.5.5.2 50 % Acetic Acid (A)

5.5.5.2.1 Carefully add 20 milliliters glacial acetic acid to 20 milliliters of distilled water.

5.5.5.3 Gold Chloride in 50 % Acetic Acid (B)

5.5.5.3.1 Prepare a second 50 % acetic acid solution as above for solution A, and mix the contents of a one gram ampoule of gold chloride into the 50 % acetic acid solution.

5.5.5.3.2 Lot number: Eight digit format year/month/day/GdCl50/Initials of preparer. Example: 20101231GdCl50XXX

5.5.5.4 Application of Procedure on Evidence - Mixed Crystal Testing (for enantiomer determination)

5.5.5.4.1 A small portion of sample is placed on a microscope slide and a drop of the reagent is mixed with the sample. Crystals formed are compared with crystals formed from the mixture(s) in the next step**.**

5.5.5.4.2 Mix a second portion of the sample with an equal portion of the d- or l- isomer of the known compound and combine with a drop of the reagent.

5.5.5.4.3 Single isomer and mixed isomer crystals have different appearances. Compare the crystals formed from the straight unknown to the d- and l-mixed samples. Isomer determination may be made when racemic crystals are formed by one of the known isomer additions.

5.5.5.4.4 QC Check: d,l-Propoxyphene forms small, curved, irregular needles almost at once.

5.5.5.4.5 Results: single isomer propoxyphene (d- and l-) gives large, straight needles which are very slow to form.

5.5.6 Microscopic Examination of Hashish Using Chloroform

5.5.6.1 This reagent is used to identify plant particles of marijuana.

5.5.6.2 Reagent grade chloroform shall be used.

5.5.6.3 Application of Procedure on Evidence

5.5.6.3.1 Place a small sample of suspected hashish or marijuana on a microscope slide and add a drop of chloroform to the material.

5.5.6.3.2 Observe under a relatively low magnification (approximately 10x).

5.5.6.3.3 QC Check: Marijuana or hashish cystolithic hairs look like bear claws.

5.5.6.3.4 Results: Marijuana or hashish cystolithic hairs look like bear claws.

5.6 New Microscopes

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

5.6.2 Kohler illumination adjustments shall be performed as needed.

5.7 Maintenance

5.7.1 Polarizing microscopes shall be serviced yearly by a certified vendor.

5.7.2 When a 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 performance verification of ensuring proper Kohler illumination adjustments have been made according to the procedure listed below in **5.7.5**.

5.7.3 Laboratory personnel shall examine the effect(s) if any, of a malfunction on analysis and implement the Laboratory Procedure for Corrective Action as required.

5.7.4 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.

5.7.5 Instructions for Kohler illumination for microscopes with a built-in illuminator:

5.7.5.1 Focus on any slide preparation of small dry particles using the coarse and fine adjustment knobs.

5.7.5.2 Adjust the observation tube.

5.7.5.2.1 For one ocular tube:

- Focus specimen using the fine adjustment knob.
- Focus ocular cross hairs by rotating the ocular top lens.

5.7.5.2.2 For binocular tubes:

- Adjust the interpupillary distance by sliding the knurled dovetail slides of the right and left eyepiece tubes, until perfect binocular vision is obtained.

- Looking through the right eyepiece (with cross hairs in view) with your right eye, rotate the upper helicoid ring of the eyepiece until the cross hairs are sharply focused.
- Focus on the specimen with the coarse and fine adjustment knobs so that the sharp images of the specimen and cross hairs can be obtained simultaneously.
- Now look at the image through the left eyepiece with your left eye and rotate the diopter adjustment ring to focus on the specimen without using the coarse and find adjustment knobs.

5.7.5.3 Center the stage rotation (with the objective centering screws) for the highest dry objective so the specimen rotates evenly about the x and y axis of the cross hairs.

5.7.5.4 Close the field diaphragm.

5.7.5.5 Ensure the image of the field diaphragm is in the center of view by adjusting the condenser using the attached condenser centering screws.

5.7.5.6 Focus the image of the field diaphragm by turning the substage condenser knob up or down.

5.7.5.7 Repeat Step **5.7.5.5** until the image is clear.

5.7.5.8 Open the field diaphragm to the edge of the field of view.

5.7.5.9 If the microscope has an adjustable lamp, perform the following Steps **5.7.5.9.1**-**5.7.5.9.4**; if not, proceed to Step **5.7.5.10**.

5.7.5.9.1 Introduce the Bertrand lens to check the focus and centration of the filament, and remove the ocular and observe the objective back focal plane.

5.7.5.9.2 Focus image of the filament in the objective back focal plane (adjustment on lamp).

5.7.5.9.3 Center image of the filament (adjustment on lamp).

5.7.5.9.4 Turn Bertrand lens out.

5.7.5.10 Swing in the next highest objective and center it with the objective centering screws on that objective mount. Repeat this step for any remaining objectives.

5.7.5.11 Adjust the substage aperture diaphragm for optimum contrast and adjust the field diaphragm image size to the edge of the field of view for each objective.

5.8 Calculations - N/A

5.9 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 - See State Crime Laboratory Safety Manual.

8.0 References

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9.0 Records - N/A

10.0 Attachments – N/A

Revision History		
Effective Date	Version Number	Reason
09/17/2012	1	Original Document for Conversion to ISO Standards. Technical Procedures B-1, B-2, B-4, B-11, and B-14 were combined to make this procedure. Technical Procedures B-3, B-5 through B-10, B-12, B-13, and B-15 were rescinded. They may be reinstated by the Forensic Scientist Manager of the Drug Chemistry Section.