Training Procedure for Microscopic Techniques

1.0 Purpose – With origins dating back to the late 1500's, the microscope is one of the oldest tools of science. Now in the twenty-first century, it is still a valuable tool used in Forensic Drug Chemistry. The stereomicroscope or dissecting microscope is used in Drug Chemistry primarily to identify the physical characteristics of marijuana. The polarizing light microscope is used in Drug Chemistry to help identify diluents, drugs, and optical isomers of drugs. This section will cover the microscopic analysis of marijuana and the use of microcrystalline reagents with the polarizing microscope.

2.0 Scope - This procedure applies to trainees in Drug Chemistry Sections of the State Crime Laboratory.

3.0 Procedure

3.1 Objectives

3.1.1 Successfully perform all operation and quality control procedures listed in the Drug Chemistry Technical Procedure for Polarized Light Microscopy.

3.1.2 Be able to differentiate commonly encountered sugars and other diluents dissolved in solution using the polarizing light microscope.

3.1.3 Be able to perform and describe microcrystalline tests used to identify drugs.

3.1.4 Review the polarized light microscopy exercise with the Training Coordinator (or his/her designee)

3.1.5 Review the Microcrystalline Tests portion of the Technical Procedure for Drug Chemistry Analysis and discuss with the Training Coordinator (or his/her designee).

3.1.6 Correctly identify a set of heroin/caffeine and cocaine unknowns using only microcrystalline techniques.

3.1.7 Review the Technical Procedure for the Identification of Marijuana.

3.1.7.1 Be able to explain the official definition of marijuana as it pertains to the General Statutes of North Carolina.

3.1.7.2 Be able to identify and describe the morphological characteristics of marijuana.

3.1.8 View hashish dissolved in chloroform under the polarizing microscope and record your observations. Discuss with the Training Coordinator (or his/her designee).

3.1.9 Review the "Limitations" portion for the Duquenois-Levine (Modified) color test in the Technical Procedure for Preliminary Color Tests and discuss with the Training Coordinator (or his/her designee).

3.1.10 Review the microcrystalline and plant material sections of the Drug Chemistry worksheet in FA with the Microscope Coordinator (or his/her designee).

3.1.11 Successfully complete a written exam.

3.1.12 Correctly identify a set of at least ten unknowns using all techniques introduced thus far.

3.2 Study Questions

3.2.1 List the basic components of a microscope and describe their functions.

3.2.2 What is the difference between a compound microscope and a stereomicroscope?

3.2.3 What is polarized light?

3.2.4 How does the polarizing light microscope work? What components of the microscope are necessary to enable polarized light microscopy?

3.2.5 What is a birefringent crystal?

3.2.5.1 Define the following:

-Birefringence
-Refractive index
-Interference colors
-Retardation
-Extinction

3.2.6 What is a compensator? Which one do we use for microcrystalline tests?

3.2.7 Explain what happens when a drug is mixed with a microcrystalline reagent. Explain what happens when a combination of substances is mixed with this reagent.

3.2.8 What is Kohler Illumination and why is it important? How is Kohler Illumination achieved?

3.2.9 What is required in the case notes to document microcrystalline tests when they are used as a Category C test (in conjunction with a Category A test)? (See the Technical Procedure for Drug Chemistry Analysis)

3.2.10 What is required in the case notes to document microcrystalline tests when they are used as a Category B confirmatory test (NOT used in conjunction with a Category A test)? (See the Technical Procedure for Drug Chemistry Analysis)

3.2.11 What is the definition of marijuana in Chapter 90 of the *North Carolina General Statutes*?

3.2.12 How many species are there of marijuana?

3.2.13 What is sinsemilla?

3.2.14 What is hashish (according to Chapter 90 of the *North Carolina General Statutes*) and how is it made? What is BHO (butane honey/hash oil) and how does it differ from hashish?

3.2.15 What are the weight thresholds in Chapter 90 of the *North Carolina General Statutes* in regards to the possession of hashish?

3.2.16 What are the macroscopic and microscope characteristics of marijuana?

3.2.17 Discuss the analysis scheme for the identification of the following with the training staff during the round table for this unit:

3.2.17.1 Marijuana in a plastic bag.

3.2.17.2 Very young marijuana plant – only first few leaves present.

3.2.17.3 Hashish.

3.2.17.4 Edibles suspected to contain marijuana/THC.

3.2.17.5 Large block of compressed plant material. (Convert trafficking amounts and report in pounds per policy).

3.3 Practical/Laboratory Exercises

3.3.1 Review the PowerPoint slides of marijuana and non-marijuana (see *Microsoft PowerPoint* attachment titled "Microscope images.ppt" found on the Drug Chemistry Section shared drive).

3.3.2 Using the website listed in the Required Reading below, practice achieving proper Kohler Illumination with the online interactive tool, if software allows.

3.3.3 Using the appropriate microscope and the necessary microcrystalline reagents, test a known standard set provided by the Drug Chemistry Training Coordinator and record the results.

3.3.3.1 Marijuana (See the Drug Chemistry Technical Procedure for the Identification of Marijuana.)

- Identify and describe all macroscopic characteristics.
- Using the stereomicroscope, identify and describe all microscopic characteristics.
- Using the polarizing light microscope and chloroform, be able to identify the cystolithic hairs of marijuana/hashish.

3.3.3.2 Examples of standards to test using the stereomicroscope

- Mixture of marijuana and non-marijuana
- Hashish

- Thyme
- Parsley
- Sage
- Basil
- Celery seed
- Catnip
- Alfalfa
- Khat

3.3.3.3 Examples of standards to test using the polarizing light microscope and water

- Inositol
- Mannitol
- Lactose
- Sucrose
- Starch
- Caffeine

3.3.3.4 Examples of standards to test using the polarizing light microscope and the appropriate gold chloride reagent

- Cocaine
- PCP
- Mix of drugs (i.e., cocaine and currently encountered diluents)

3.3.4 Examples of standards to test using the polarizing light microscope and 5 % mercuric chloride reagent

- Heroin
- Caffeine
- Mix of drugs (i.e., heroin and procaine)

3.3.5 Using the appropriate microcrystalline reagents, identify a set of unknowns including heroin/caffeine and cocaine.

3.3.6 Using color tests, tablet identification resources, UV and IR spectroscopy, microscopic techniques, and extraction techniques, successfully identify a set of unknowns provided by the Training Coordinator.

3.3.6.1 Record the results of all tests using Proficiency Lab case records in the FA system.

4.0 Required Reading

Microsoft PowerPoint – "Microscope images.ppt" found on the Drug Chemistry Section shared drive.

http://www.microscopyu.com/tutorials/java/kohler/ - Interactive website that can be used to practice microscope alignment.

https://www.olympus-lifescience.com/en/microscope-resource/primer/anatomy/kohler/

Easy Kohler Illumination Method – Academic Skills Center – Trent University

Essentials of Polarized Light Microscopy- John Gustav Delly- Chapter 6 – Set up of Kohler Illumination

https://micro.magnet.fsu.edu/primer/anatomy/anatomy.html

5.0 References

U.S. Treasury Department Bureau of Narcotics. *Marihuana Its Identification.* Washington, D.C: United States Printing Office, 1948.

North Carolina General Statutes §90-87 (16) and §90-95(d)(4).

6.0 Records

- Drug Chemistry - Training Checklist
- Section Completion Summary

7.0 Attachments – N/A

Revision History		
Effective Date	Version Number	Reason
09/17/2012	1	Original Document
05/03/2013	2	**Scope** – Changed to match wording of other ISO Documents. **3.1.7 – 3.1.9** – Added new objectives. **3.1.11** – Reworded to clarify use of all techniques introduced thus far. (Original 3.1.8) **3.2.9** – Added question on Kohler Illumination. **3.2.10 and 3.2.11** – Reworded Original question 3.2.9 and added an additional question – both regarding use of microcrystalline tests for casework. **3.2.16 -** "hashish" corrected to "marijuana" **3.2.17** – Added new study question for discussion at round table. **3.3.2** – Added new practical exercise to practice Kohler Illumination skills with online interactive tool. **3.3.3.4** – Reworded for currently encountered diluents and removed Methamphetamine volatility test. **3.3.5.1** – Clarified hard copies of section worksheet to be used for unknowns. **3.4.2** – Added interactive website for microscope alignment.
12/06/2013	3	Added issuing authority to header
10/19/2015	4	**Header –** Revised issuing authority **Objectives** – Rearranged original, added new objectives **3.2** - Edited study questions **3.3.2** – Added "if software allows" for online practical exercise. **3.3.3.3** – Added caffeine as example to test with water **3.3.5** – Added practical exercise present in objectives for heroin/caffeine and propoxyphene enantiomer determination **3.3.6** – Edited tablet resource information and use of FA Proficiency lab in lieu of hardcopy worksheets.
08/17/2018	5	**Objectives –** rearranged original, added and removed objectives **3.1.4 –** added new practical exercise **3.1.5** – removed question, exercise being removed from training block **3.1.7** – removed d/l-propoxyphene from unknown exercise and added cocaine as an example **3.1.11** – removed, will be covered in a previous procedure **3.1.12** – removed, FA will be covered in each training section **3.1.13** – added new objective to reflect updated checklist **Study Questions –** rearranged original, added and removed objectives **3.2.1** – added "and describe their functions"

		3.2.4 – added "What components of the microscope are necessary to enable polarized light microscopy?" reworded answer and fixed typos **3.2.5.1** – added new study question to expand the terms to define **3.2.6** – added new study question **3.2.7 and 3.2.8** – combined these questions **3.2.9 –** added "How is Kohler Illumination achieved?" **3.2.14** – added BHO into the question **3.2.18.4** – removed Marijuana smoking device with burned material present and added edibles suspected to contain marijuana/THC **3.3.3.2** – added Khat **3.3.3.4** – removed propoxyphene, will not be included in training **3.3.5** – removed propoxyphene and added cocaine **Required Reading** – added three new references