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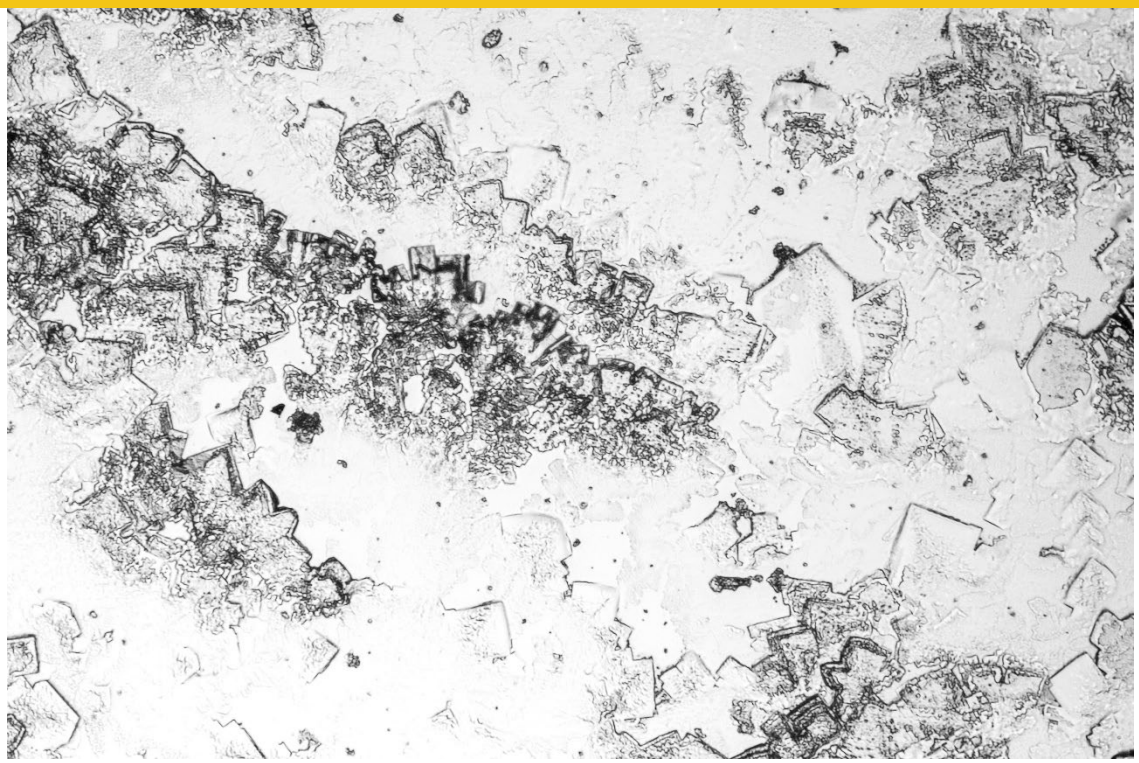
The Forensic Laboratory Needs Technology Working Group (FLN-TWG), formed by the National Institute of Justice (NIJ) in partnership with the Forensic Technology Center of Excellence (FTCOE) at RTI International, created this document in support of NIJ's mission to improve knowledge and understanding of federal, state, local, and tribal forensic science service providers' (FSSPs') technology needs.



TECHNICAL NOTE
Microcrystal Tests

Introduction

Microcrystal tests[1] provide a rapid and inexpensive alternative to instrumental analysis techniques through the development of unique crystal formations between a given analyte and a specific reagent. The unique microcrystals can be compared against a reference/control using a polarized light microscope (PLM) to assess crystal shape, size, color, and spatial arrangement.[1-13] The inclusion of specific optical properties such as refractive index, birefringence, and sign of elongation adds additional support for the presence of a controlled substance(s) in a seized drug sample.[8] Refractive index is the ratio of the velocity of light in a vacuum to the velocity in a given medium (i.e., the degree to which light is bent through interaction with a given medium). Birefringence, or double refraction of light, is an optical property of a material that is manifested due to the existence of orientation-dependent differences in refractive index. Sign of elongation is a measure of the relationship between the crystal orientation and the magnitude of the refractive indices for each dimension.

Microcrystal tests are classified as a Category B technique according to both the SWGDRUG guidelines[14] and the ASTM E2329-17 Standard Practice for Identification of Seized Drugs[15] because of the ability to provide selectivity/discriminating power through

[1] The term "microcrystalline tests" is also used to describe this methodology.

chemical and physical characteristics. According to SWGDRUG recommendations and ASTM E2329-17, the most appropriate incorporation of a Category B technique is in combination with a Category A technique or with at least two other techniques, with one of the two additional techniques belonging to Category B.[14-15] Although the application of microcrystal tests to the analysis of seized drugs is not novel, the evolution of microcrystal tests and their application to the analysis of seized drugs is important given the current challenges of seized drug analysis. In particular, the ability to rapidly identify even minute quantities of relatively common seized drugs provides great value to forensic laboratories. Even though microcrystal tests have a relatively limited scope of analysis (i.e., common seized drugs), they are quick, sensitive, and inexpensive alternatives to advanced analytical techniques such as gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS). Analyzing common drugs of abuse with microcrystal tests enables laboratories to dedicate more advanced instrumentation to more challenging seized drug samples. Microcrystal tests may also be incorporated into a validated analytical scheme to confirm the presence of controlled substances identified with alternative techniques.[15] Exhibit 1 summarizes the approximate initial cost, cost per analysis, and analysis time for microcrystal tests.

Exhibit 1. Summary of microcrystal tests cost and analysis time considerations.

Initial Cost	Cost/Analysis	Analysis Time
$30,000	$10–$20	8–10 minutes/sample

These figures were provided as an estimate by a representative of the McCrone Research Institute.

Capabilities

Microcrystal testing is accessible to many laboratories that have microscopes for macroscopic and microscopic examinations and trained microscopists. The combination of analyst expertise and a PLM can readily detect the presence of specific drugs and do so relatively inexpensively. Using microcrystal testing for screening several of the most abused drugs of interest, such as cocaine,[16-18] methamphetamine,[19-21] and heroin,[22] can reduce the burden on GC-MS and LC-MS instrumentation, freeing this instrumentation for more difficult analyses and secondary/confirmatory analysis. In addition, using microcrystal tests may address recommendations, such as those by SWGDRUG, for the use of multiple techniques that exploit different chemical or physical properties of the analyte. The inclusion of specific optical properties such as refractive index, birefringence, and sign of elongation adds additional weight to the identification of a specific drug beyond qualitatively measuring crystal shape, type, color, and size [8]. Finally, microcrystal tests can be used to differentiate between racemic mixtures and pure stereoisomers for compounds such as amphetamine and methamphetamine, which aids in differentiating between clandestine synthesis and pharmaceutical diversion.[3]

Limitations

As of the September 2021 revision of the McCrone Modern Compendium of Microcrystal Tests,[8] 19 controlled substances have established microcrystal tests. Since completing additional research in 2020, the McCrone Research Institute has published *New Microcrystal Tests for Controlled Drugs, Diverted Pharmaceuticals, and Bath Salts (Synthetic Cathinones)*,[23] which contains newly developed microcrystal tests and reagents for nine additional drugs: alprazolam,[24] butylone ,[24] MDPV,[25] 4-MEC,[25] mephedrone,[26] methylone,[26] α-PVP,[27] tramadol, [23] and zolpidem.[23] Even with an expanding scope of controlled substances, microcrystal testing is still limited to a relatively small subset of controlled substances. The existing validated microcrystal tests are designed to be an inexpensive and rapid alternative to analytical instrumentation to help address the analysis of common drugs of abuse, rather than to provide the specificity required to address challenges associated with isomer differentiation with novel psychoactive substances (NPS).

Microcrystal testing has proved most effective when other tests indicate the likely presence of one of the commonly abused drugs that have known microcrystal tests, which includes cocaine,[16-18] oxycodone,[28] heroin,[22] methamphetamine,[19-21] and MDMA,[12,22] among others. [29-44] If other drugs are mixed with the sample, any drugs beyond those for which tests exist cannot be identified via microcrystal testing although the targeted drug may still be

identifiable. Extensive cutting agents have been found to interfere with crystal formation such that the drug of interest is no longer able to be identified. Some laboratories have moved away from testing certain drugs like heroin because of the frequent inclusion of fentanyl, for which they do not have a valid microcrystal test, and difficulties with crystal formation caused by extensive cutting agents.

Another limitation is that it may be difficult to obtain reviewable data, as capturing photographs of sufficient quality to include in the case file has proven difficult even with microscopes with included cameras, requiring a second examiner to verify the crystal formations.

Pharmaceuticals in various formulations and delivery mechanisms, including tablets, oral solutions, and transdermal patches can be analyzed with microcrystal tests but require additional preparation such as microscale extraction and concentration to yield successful microcrystal tests.

Installation needs

Installation requires a laboratory workspace and bench area to operate the microscope. The microscopy bench falls into one of three categories: ordinary desks and tables, those custom made for the purpose, and those commercially available from laboratory manufacturers. The basic bench consists of any available table (e.g., a single 63" × 31.5" office desk provides more than adequate area for setting up a single PLM and associated accessories). Microscopes typically weigh less than 25 lbs., and they can be relocated or transported by hand when grasped properly. Wall-mounted shelves are useful for storing reagents and supplies. A comfortable chair with adjustable seat height and without armrests is preferable. A nearby voltage supply and outlet (120 V AC) is required.

Vendor considerations

Name-brand polarized light microscopes are available from the leading global manufacturers of precision instruments and are sold and distributed by their national and international dealers. PLMs with Köhler or Köhler-type Illumination and the following accessories are desirable for microcrystal testing: 10×, 20×, and 40× objectives; 10× binocular head (or trinocular for photography image capture) for oculars/eyepieces, including one cross-line reticle preferably "keyed" to eyepiece tube; 360-degree circular, rotatable stage; substage condenser with aperture diaphragm; polarizer and analyzer that can each be positioned and set with linear vibration directions at 90 degrees to one another (i.e., crossed polars) and can be aligned to the cross-line reticle in the ocular/eyepiece; accessory slot for wave plates and compensators; and first order (Red I compensator) retardation wave plate with approximately 550 nm retardation and known slow and fast vibration directions. In addition, the following accessories are required for photograph documentation: camera/image capture device and any related software or storage and a microscope to camera coupler for mechanical attachment of the camera to the eyepiece/tube or trinocular head.

Requirements of the space

Commercial polarized light microscopes, similar to most microscopes, are designed for indoor use at altitudes up to 2,000 meters and ambient temperatures between 5 °C and 40 °C. Most microscopes are electrical voltage rated at 120 V AC and consume fewer than 150 W. With a sturdy desk, table, or bench, vibration isolation is usually not required in most laboratory environments, and no external gases or liquids are required for routine microscope operation.

User training and skill level

Training courses, both in person and online, exist for users at various skill levels. At a minimum, analysts must be proficient with micro-manipulation and PLM use and be familiar with relevant microcrystalline literature. According to the McCrone Research Institute, analysts require ~60 hours of classroom and laboratory training and ~170 hours of practice to maximize the utility of microcrystalline testing. Laboratories that have implemented microcrystal testing, such as those listed in Exhibit 2, may be able to provide guidance as to the time required for sufficient training and practice.

Implementation needs

Microscopes (stereo, compound, or polarized light) are among standard laboratory equipment in most forensic science laboratories. Anyone properly trained in microscope use should be permitted to set up, operate, and perform routine maintenance. For example, analysts trained to perform microscopical analysis of *Cannabis sativa* L. will

already possess the relevant microscopical skills required for microcrystal tests.

Validated methods

The McCrone Research Institute has published a compendium of numerous methods for 19 commonly encountered drugs of abuse[8] and new microcrystal tests for controlled drugs, diverted pharmaceuticals, and bath salts (synthetic cathinones) for nine additional drugs.[23]

Reporting and testimony

Per the SWGDRUG recommendations and ASTM E2329-17 guidelines, [14-15] all Category A and B techniques shall have reviewable data, which includes either contemporaneous documented peer-reviewed notes or photographs/digital images for microcrystal testing.

Consumables

Chemical reagents and microscopy laboratory supplies and equipment used for microcrystal tests are easy to make, relatively inexpensive, and readily available. The resources developed through the McCrone Research Institute, such as the *Modern Compendium of Microcrystal Tests*[8] and *New Microcrystal Tests for Controlled Drugs, Diverted Pharmaceuticals, and Bath Salts (Synthetic Cathinones)*,[23] provide in-depth information about necessary consumables for microcrystal testing.

Early adopting laboratories

Exhibit 2. Point of contact information from early adopting laboratories that have implemented microcrystal tests into casework. This list may not be exhaustive but is intended to highlight points of contact for those interested in the implementation of microcrystal tests.

Laboratory	POC	Email	Phone Number
Oakland Police Department Criminalistics Laboratory	Dr. Sandra Sachs	SSachs@oaklandca.gov	(510) 238-3386
Minnesota Bureau of Criminal Apprehension	Rebecca Willis	Rebecca.willis@state.mn.us	(651) 793-2876

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Relevant standards and guidelines

ASTM E1968-19 Standard Practice for Microcrystal Testing in Forensic Analysis for Cocaine
ASTM E1969-19 Standard Practice for Microcrystal Testing in Forensic Analysis for Methamphetamine and Amphetamine
ASTM E2125-19 Standard Practice for Microcrystal Testing in Forensic Analysis for Phencyclidine and its Analogues
ASTM E2329-17 Standard Practice for the Identification of Seized Drugs
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