



# TENNESSEE BUREAU OF INVESTIGATION

## Forensic Services Division

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### Forensic Chemistry Standard Operating Procedure Manual

#### Cannabis Analysis

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## 25.0 CANNABIS ANALYSIS

### 25.1 Visual Examination

25.1.1 Macroscopic and microscopic examinations of marijuana exhibits will be considered as uncorrelated techniques from Category B when observations include documented details of botanical features.

25.1.2 The macroscopic exam details the overall appearance of the substance, such as the leaf color, plant material, seeds, and stems. A minimum of one of the above listed characteristics is sufficient for a positive macroscopic technique.

25.1.3 Cystolithic hairs **must** be present for a positive microscopic test. These hairs have a short bear claw-like appearance with a calcium carbonate base. The microscopic examination may also include, but is not limited to, the following morphological characteristics:

- Bracts - unserrated leaflets enclosing the pistil (female flower)
- Cover hairs - curved, fine tipped hairs, found primarily on the lower leaf surface
- Glandular hairs and/or resin beads – coarse hairs with easily detached bead-like storage structures at the distal end, found primarily on the lower leaf surface
- Styles and/or stigmas – pale green to reddish brown, fuzzy, filamentous pollen receptor surfaces
- Ribbed stem tissue indicating fiber bundles
- Seeds – immature oval structures that may exhibit a mottled appearance

25.1.4 If these other morphological characteristics are observed, they should be documented in the case record. The location, size, density, or color of these characteristics may vary according to the age, reproductive stage, and storage condition of the plant material.

25.1.5 The analyst's descriptions and/or drawings of the physical characteristics of the material are very important for making conclusions. Case notes must include these descriptions instead of using positive and negative for the results.

### 25.2 Color Tests

#### 25.2.1 Duquenois-Levine

25.2.1.1 Duquenois-Levine tests will be run to determine if cannabinoids are present in plant material exhibits after cystolithic hairs are identified.

25.2.1.2 A positive Modified Duquenois-Levine test consists of a blue/purple color change preceding the addition of chloroform. This purple color must then transfer into the chloroform layer. Detailed descriptions of the color changes during the Duquenois-Levine test are important because a positive result is dependent upon those changes in both steps of the test.



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#### **25.2.2 4-Aminophenol**

25.2.2.1 4-Aminophenol will be run on plant material exhibits that have cystolithic hairs and give a positive Duquenois-Levine color test.

25.2.2.2 This color test gives different colored results based on the ratio of cannabidiol (CBD) versus tetrahydrocannabinol (THC). Plant material samples that have higher ratios of THC to CBD will give a blue result, while plant material samples with a higher ratio of CBD to THC will give a pink to dark pink result.

25.2.2.3 Color changes typically will develop quickly with this test. However, THC false positives can occur if the tested material is immersed within the reagents for an extended time period. Evaluation of results within the first minute of analysis is recommended.

#### **25.3 Instrumental Analysis**

25.3.1 Plant material exhibits that lack cystolithic hairs or produce a negative Duquenois-Levine test will be analyzed using GCMS to determine if cannabinoids are present.

#### **25.4 Quality Assurance**

25.4.1 Blanks of the Modified Duquenois-Levine and 4-aminophenol tests will be performed daily at minimum and documented in the case record.

25.4.2 If a purple color develops in either layer while performing a Duquenois-Levine blank, the reagent will be disposed of and replaced.

25.4.3 If any color develops while performing a 4-aminophenol blank, each reagent will be disposed of and replaced. Please note the blank may change to a light yellow color if allowed to sit for an extended time period. Blanks will be evaluated in the same manner as the exhibit for this color test.

25.4.4 Analysts should note that materials other than marijuana and THC can produce false positive results with the Duquenois-Levine and 4-aminophenol test.

25.4.5 Refer to the GCMS chapter for quality assurance guidelines if used in analysis.



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#### 25.5 Interpretation and Reporting

25.5.1 Exhibits that demonstrate all of the following results will be identified and reported as below:

- Presence of cystolithic hairs
- Positive Duquenois-Levine
- Positive for higher THC content with 4-aminophenol (blue color)

EXHIBIT(S):

001-a Plant material

<u>RESULTS:</u>	<u>Controlled Substance</u>	<u>Schedule</u>	<u>Amount</u>
001-a	Cannabis	-	26.75 grams

*Presumptive testing indicates the exhibit is marijuana.*

*Testing Method: Macroscopic*

*Testing Method: Microscopic*

*Testing Method: Color test*

25.5.2 Exhibits that demonstrate all of the following results will be identified and reported as below:

- Presence of cystolithic hairs
- Positive Duquenois-Levine
- Positive for higher CBD content with 4-aminophenol (pink color)

EXHIBIT(S):

001-a Plant material

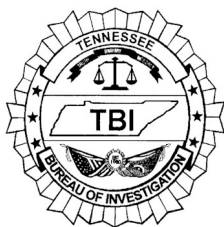
<u>RESULTS:</u>	<u>Controlled Substance</u>	<u>Schedule</u>	<u>Amount</u>
001-a	Cannabis	-	26.75 grams

*Presumptive testing indicates the exhibit is hemp.*

*Testing Method: Macroscopic*

*Testing Method: Microscopic*

*Testing Method: Color test*



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25.5.3 Exhibits that do not have microscopic characteristics and require a GCMS will report the identifiable cannabinoids present and/or other legally significant substances as below:

<u>EXHIBIT(S):</u>			
001-a	Plant material		
<u>RESULTS:</u>	<u>Controlled Substance</u>	<u>Schedule</u>	<u>Amount</u>
001-a	delta-9-tetrahydrocannabinol Cannabinol	-	26.75 grams

25.5.4 Exhibits that produce an inconclusive 4-aminophenol (purple) test will be reported as below.

<u>EXHIBIT(S):</u>			
001-a	Plant material		
<u>RESULTS:</u>	<u>Controlled Substance</u>	<u>Schedule</u>	<u>Amount</u>
001-a	Cannabis	-	26.75 grams
<i>Testing did not produce sufficient results to make a presumptive determination. Please contact the TBI Crime Laboratory if further testing is necessary.</i>			
<i>Testing Method: Macroscopic</i>			
<i>Testing Method: Microscopic</i>			
<i>Testing Method: Color test</i>			

25.5.5 Exhibits that have complete presumptive testing as well as a GCMS will be reported as follows

<u>EXHIBIT(S):</u>			
001-a	Plant material		
<u>RESULTS:</u>	<u>Controlled Substance</u>	<u>Schedule</u>	<u>Amount</u>
001-a	Cannabis	-	26.75 grams
<i>Presumptive testing indicates the exhibit is marijuana.</i>			
delta-9-tetrahydrocannabinol			
Cannabidiol			
<i>Testing Method: Macroscopic</i>			
<i>Testing Method: Microscopic</i>			
<i>Testing Method: Color test</i>			
<i>Testing Method: GC/MS</i>			



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25.5.6 Testing methods will be included on the report according to the Reporting Chapter.

25.5.7 Analytical requirements and reporting for cannabis plant materials in death investigations or other atypical cases are discussed in the Death Investigations Chapter.

25.5.8 Reporting guidelines for THC concentration screening and full quantitation are discussed in their respective chapters.

25.5.9 *Reporting for edible exhibits (excluding gummies)*

Other edible exhibits (e.g. cookies and brownies) present great difficulty for quantitation due to the complex matrices present. These exhibits will be reported with the cannabinoids present. The remark *Edible exhibits, with the exception of gummy candy, cannot be quantitated by the TBI Crime Laboratory* will be included on the report as demonstrated below.

<u>EXHIBIT(S):</u>			
001-a	Cookie		
<u>RESULTS:</u>	<u>Controlled Substance</u>	<u>Schedule</u>	<u>Amount</u>
001-a	delta-9-tetrahydrocannabinol	-	32.58 grams
	<i>Edible exhibits, with the exception of gummy candy, cannot be quantitated by the TBI Crime Laboratory.</i>		