



# TENNESSEE BUREAU OF INVESTIGATION

## Forensic Services Division

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### Forensic Chemistry Standard Operating Procedure Manual

#### Color Tests

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## 16.0 CHEMICAL COLOR TESTS

### 16.1 Application

Color tests produce characteristic color reactions that can indicate the presence of particular functional group(s). These tests are presumptive and should be used in combination with Category A tests for identification of legally significant substances

### 16.2 Sampling Equipment

Spot plates (both disposable and porcelain), disposable test tubes, or weigh boats can be utilized when conducting chemical color tests. Porcelain spot plates will be thoroughly cleaned using a soap and water wash followed by a methanol or ethanol rinse.

### 16.3 Reagent Preparation

16.3.1 The following color test reagents will have a one year expiration date from the date it is prepared. These reagents will be prepared using the instructions listed below:

- Cobalt Thiocyanate  
Dissolve 6.8 grams of cobalt chloride and 4.3 grams of ammonium thiocyanate in 100 mL of high purity/RO water.
- Dillie-Koppanyi  
Reagent 1: Dissolve 0.1 grams cobalt acetate in 100 mL of methanol plus 0.2 mL of glacial acetic acid.  
Reagent 2: Mix 5 mL of isopropylamine in 95 mL of methanol.
- para-Dimethylaminobenzaldehyde (p-DMAB)  
Dissolve 1 gram of p-dimethylaminobenzaldehyde in 100 mL of ethanol. Add concentrated hydrochloric acid to sample + p-DMAB at time of test.  
*For p-DMAB spray (Elrich's reagent) – add 100 mL of concentrated hydrochloric acid to the above solution. Solution must be made fresh.*
- Duquenois-Levine  
Dissolve 0.3 mL of acetaldehyde and 2 grams of vanillin in 100 mL of 95% reagent alcohol.
- FPN  
Mix 5 mL of aqueous 5% ferric chloride solution with 45 mL of 70% perchloric acid: high purity/RO water (1:5) and 50 mL of nitric acid: high purity/RO water (1:1).
- GHB color test reagent  
Mix 50 mL of concentrated sulfuric acid and 50 mL of high purity/RO water. Add 0.25 gram of chromium trioxide and mix thoroughly.



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#### 16.3.2 4-Aminophenol color test

16.3.2.1 The two 4-Aminophenol reagents will have an expiration date of 3 months from the date of preparation.

#### 16.3.2.2 Reagent Preparation

Reagent 1: Mix 300 mg of 4-aminophenol with 5 mL of 2N HCl and 995 mL of ethanol

Reagent 2: Mix 30 g of NaOH pellets with 300 mL of high purity water and 700 mL of ethanol

#### 16.3.3 Marquis color test

16.3.3.1 Marquis can be prepared and used for up to one (1) month provided it is stored in accordance with the guidelines outlined in the most recent edition of *Clarke's Analysis of Drugs and Poisons*. However, it may be prepared and discarded more frequently at the unit supervisor's discretion.

16.3.3.2 Mix one (1) part of formaldehyde solution with nine (9) equal parts of sulfuric acid.

#### 16.4 Color Test Reagent Quality Assurance

Positive and negative controls will be performed before a chemical color test reagent is placed into stock. The stock and any color test reagents remaining at the analysts' work stations will be re-verified quarterly. Verifications will be documented in the appropriate logbook. Reagents that do not produce the expected results will be discarded.

Positive and negative control verifications are outlined in the following table.

<u>Reagent</u>	<u>Controls</u>	<u>Expected Action</u>
<b>Cobalt thiocyanate</b>	Positive – cocaine standard	Immediate blue color
	Negative – inositol standard	No color change
<b>Marquis</b>	Positive – methamphetamine standard	Orange color going to brown
	Negative – inositol standard	No color change
<b>Duquenois-Levine</b>	Positive – THC standard	Purple color that will extract into CHCl <sub>3</sub> layer
	Negative – reagent blank	No color change
<b>FPN</b>	Positive – promethazine standard	Pink color fading rapidly
	Negative – inositol standard	No color change



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<b>Koppanyi</b>	Positive – pentobarbital standard	Purple color upon addition of Koppanyi reagent 2
	Negative – inositol standard	No color change
<b>p-dimethylaminobenzyl aldehyde (Erlich's)</b>	Positive – LSD standard	Light purple color
	Negative – inositol standard	No color change
<b>GHB Color Test</b>	Positive – GHB standard	Green color going to pale blue
	Negative – water	No color change
<b>4-Aminophenol</b>	1 <sup>st</sup> Positive – Cannabidiol standard	Pink / dark pink upon addition of reagent 2
	2 <sup>nd</sup> Positive – THC standard	Blue upon addition of reagent 2
	Negative – reagent blank	No color change

Color test procedural blanks will be performed daily by the analyst before use in casework. The results of the blank will be documented in the case notes by describing the color or the lack thereof.

#### 16.5 Testing Procedures

The proper testing protocols for the color test reagents in use are listed below.

- Cobalt Thiocyanate  
Add reagent directly to sample  
If cocaine base is suspected: Add 0.1 N HCl to sample, then add reagent.
- Dillie-Koppanyi  
Add equal amounts of reagent 1 and reagent 2 directly to sample
- para-Dimethylaminobenzaldehyde  
Add reagent followed by concentrated HCl to sample
- Duquenois-Levine (modified)  
Add equal amounts of reagent and concentrated HCl to sample, then add  $\text{CHCl}_3$
- Marquis  
Add reagent directly to sample



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- FPN  
Add reagent directly to sample
- GHB color test reagent  
Add reagent directly to sample
- 4-Aminophenol  
Add equal amounts of reagent 1 and reagent 2 directly to sample. Evaluate color change within one minute.

#### **16.6 Interpretation and Documentation**

- 16.6.1 Only positive test results can be used to meet the minimum requirements for the identification of a controlled substance. Negative results will be documented in the case notes as well.
- 16.6.2 The most recent edition of *Clarke's Analysis of Drugs and Poisons* (formerly known as *Clarke's Isolation and Identification of Drugs*), other reputable literature sources, and/or in-house color tests performed on primary reference standards will be used as references for test results.
- 16.6.3 If the resulting color is atypical, the analyst should consult other scientific literature, a fellow scientist, and/or the TBI FCU Technical Leader for assistance.
- 16.6.4 Refer to the Documentation chapter for preparation and casework documentation requirements.