



TENNESSEE BUREAU OF INVESTIGATION

Forensic Services Division

Forensic Chemistry Standard Operating Procedure Manual

Color Tests

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 (Erich'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 Refrigerated stock solutions of 4-aminophenol reagents will be stored in amber bottles with a one-year expiration date. Any non-refrigerated 4-aminophenol reagents (individual analysts' working reagents) will be stored in amber bottles with a three-month expiration date.

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>
Cobalt thiocyanate	Positive – cocaine standard	Immediate blue color
	Negative – inositol standard	No color change
Marquis	Positive – methamphetamine standard	Orange color going to brown
	Negative – inositol standard	No color change
Duquenois-Levine	Positive – THC standard	Purple color that will extract into CHCl ₃ layer
	Negative – Parsley	No color change
FPN	Positive – promethazine standard	Pink color fading rapidly



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	Negative – inositol standard	No color change
Koppanyi	Positive – pentobarbital standard	Purple color upon addition of Koppanyi reagent 2
	Negative – inositol standard	No color change
p-dimethylaminobenzyl aldehyde (Erlich's)	Positive – LSD standard	Light purple color
	Negative – inositol standard	No color change
GHB Color Test	Positive – GHB standard	Green color going to pale blue
	Negative – water	No color change
4-Aminophenol	1 st Positive – Cannabidiol standard	Pink / dark pink upon addition of reagent 2
	2 nd Positive – THC standard	Blue upon addition of reagent 2
	Negative – Parsley	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 CHCl_3



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- Marquis
Add reagent directly to sample
- 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.