Chapter 7: Technical Procedures for Color Tests

1. Purpose / Scope - This procedure provides direction for the preparation and the use of color test in the Drug Chemistry Unit of the Wake County Bureau of Forensic Services Laboratory.

2. Definitions

2.1. Prepared reagent – Mixture of two or more reagents or a dilution.
2.2. Commercial reagent – A purchased solvent or chemical.

2.3. Performance verification – The initial confirmation of the reliability of a previously or externally validated method or instrument.

2.4. Quality control check – Periodic confirmation of the reliability of equipment, instrumentation, and/or reagents.

2.5. Reference material – Material sufficiently homogenous and stable with reference to specified properties, which has been established to be fit for its intended use in measurement or in examination of nominal properties.

2.6. Stock container – a container of reagent prepared to serve as a reserve source of the reagent from which Use Containers are prepared. Stock containers shall not be used directly for analysis.

2.7. Use container – a container of reagent used directly for analysis.

3. Equipment, Materials and Reagents

3.1. Equipment

3.1.1. Balance

3.2. Materials

3.2.1. Beakers or other glass vessels
3.2.2. Test tubes
3.2.3. Funnel

3.2.4. Glass stirring rod
3.2.5. Graduated cylinder, class A
3.2.6. Pipettes with bulb
3.2.7. Spot plates
3.2.8. Reagent bottles and stock bottles, including amber
3.2.9. Spatula
3.2.10. Weigh boats, paper or other weigh vessels
3.2.11. Filter paper
3.2.12. Scissors
3.2.13. Deionized water

3.3. Commercial Reagents

3.3.1. Sulfuric acid, ACS
3.3.2. Formaldehyde, approximately 40% aqueous, ACS
3.3.3. Cobalt (II) Thiocyanate
3.3.4. Ferric Chloride, anhydrous
3.3.5. Colbalt(II) Acetate, tetrahydrate
3.3.6. Methanol
3.3.7. Isopropylamine, 99%
3.3.8. *Para*-Dimethylaminobenzaldehyde, ACS
3.3.9. Hydrochloric acid, ACS
3.3.10. Glacial acetic acid, ACS grade

3.4. Reference materials

3.4.1. Heroin
3.4.2. Cocaine hydrochloride
3.4.3. Phenobarbital
3.4.4. Lysergic acid diethylamide (LSD)
3.4.5. *Gamma*-hydroxybutyric acid (GHB)

4. Standards and Controls

4.1. Reagents shall be prepared, labeled and stored in accordance with the Drug Chemistry Unit Technical Procedure for Quality Assurance.

4.2. Perform positive and negative quality control checks on all use containers of color test reagents prior to use for analysis. The quality control checks must have acceptable results prior to the use of the reagent for analysis.

4.3. Perform negative quality control checks (NQCC) according to the procedure listed for each color test with no sample present.

4.3.1. Acceptable result is no significant color formation, i.e., Negative.

4.3.2. If a significant color develops, take steps to ensure that the spot well is clean or use a new spot well or use a new culture tube.

4.3.3. If the significant color formation persists, dispose of the reagent and prepare a new lot of reagent.

4.4. Perform positive quality control checks (PQCC) according to the procedure listed for each color test using the specified reference material.

4.4.1. Refer to each color test for acceptable results.

4.4.1.1. If acceptable results are not observed, take steps to ensure that the spot well is clean or use a new spot well or use a new culture tube and repeat the PQCC. If the problem persists, dispose of the reagent and prepare a new lot of reagent.

4.4.2. Record any observations, the reference material identification, and the results of the positive quality control check in the Reagent Log.

5. Color Tests

5.1. Marquis

5.1.1. Useful for general screening mostly with opium alkaloids, opioids, methylenedioxy substituted cathinones, and amphetamines.

5.1.2. Selected Characteristic Results:

Opiates, some opioids (morphine, codeine, heroin, buprenorphine) - purple
Guaifenesin - purple
(Meth)amphetamine - orange
MDA/MDMA - purple/black
Aspirin - slow cherry red
Bufotenine and psilocin - green-brown

Methylenedioxy substituted cathinones - bright yellow
Fentanyl - orange

5.1.3. Preparation: Add 10 drops of 40% aqueous formaldehyde solution to 10 ml of concentrated sulfuric acid.

5.1.3.1. Storage: Amber glass.

5.1.3.2. Expiration: Stock container: NA
Use container: One month.

5.1.3.3. Lot number: Eight-digit format year/month/day/Mq/initials of preparer.
Example: 20120131MqXXX

5.1.3.4. PQCC

5.1.3.4.1. Reference material: Heroin
5.1.3.4.2. Acceptable result: Purple color observed

5.1.4. Procedure:

5.1.4.1. Add 1-2 drops of the reagent to a clean spot well or a new test tube and observe any reaction or color produced.

5.1.4.1.1. If a significant color develops, take steps to ensure that the spot well is clean or use a new spot well or use a new test tube.

5.1.4.1.2. If the significant color formation persists, prepare a new lot of reagent.

5.1.4.2. Add a small amount of sample to the reagent.

5.1.4.3. Observe any reaction or color produced.

5.1.4.4. Record observations.

5.2. Cobalt Thiocyanate

5.2.1. Reacts with secondary and tertiary amines as well as some alkaloids to produce a blue color.

5.2.2. Selected characteristic results: Cocaine – blue
PCP – blue
Ketamine - blue

5.2.3. Preparation: Dissolve 2.0 g cobalt (II) thiocyanate in 100 ml of deionized water.

5.2.3.1. Storage: Glass

5.2.3.2. Expiration: Stock container: Three years.
Use container: Three months

5.2.3.3. Lot number: Eight-digit format year/month/day/CoSCN2/initials of preparer.

Example: 20120131CoSCN2XXX

5.2.3.4. Positive Quality Control Check (PQCC):

5.2.3.4.1. Reference Material: Cocaine hydrochloride.

5.2.3.4.2. Acceptable result: A blue color is observed, i.e., Positive.

5.2.4. Procedure

5.2.4.1. Add 1-2 drops of the reagent to a clean spot well or a new test tube and observe any reaction or color produced.

5.2.4.1.1. If a significant color develops, take steps to ensure that the spot well is clean or use a new spot well or use a new test tube.

5.2.4.1.2. If the significant color formation persists prepare a new lot of reagent.

5.2.4.2. Add a small amount of sample to the reagent.

5.2.4.3. Observe any reaction or color produced.

5.2.4.4. Record observations.

5.3. Ferric Chloride

5.3.1. Reacts with phenols, enols, and GHB to produce color.

5.3.2. Selected characteristic results: GHB - red/brown

5.3.3. Preparation: Dissolve 1.5 grams of ferric chloride in 29.0 milliliters of deionized water to produce a 5% w/v solution.

5.3.3.1. Storage: Glass

5.3.3.2. Expiration: Stock container: Three years
Use container: Three months

5.3.3.3. Lot number: Eight-digit format year/month/day/FeCl3/initials of preparer.

Example: 20120131FeCl3XXX

5.3.3.4. Positive Quality Control Check (PQCC):

5.3.3.4.1. Reference material: GHB

5.3.3.4.2. Acceptable result: A red/brown color is observed.

5.3.4. Procedure

5.3.4.1. Add 1-2 drops of the reagent to a clean spot well or a new test tube and observe any reaction or color produced.

5.3.4.1.1. If a significant color develops, take steps to ensure that the spot well is clean or use a new spot well or use a new test tube.

5.3.4.1.2. If the significant color formation persists prepare a new lot of reagent.

5.3.4.2. Add a small amount of sample to the reagent.

5.3.4.3. Observe any reaction or color produced.

5.3.4.4. Record observations.

5.4. Dille-Koppanyi (modified)

5.4.1. This color test reacts with barbiturates to produce a red-violet color.

5.4.2. Selected characteristic results: Barbiturates - red-violet.

5.4.3. Preparation

5.4.3.1. Dille-Koppanyi Paper

5.4.3.1.1. Dissolve 0.1 gram cobalt (II) acetate in 100 milliliters of methanol.

5.4.3.1.2. Add 0.2 milliliter glacial acetic acid.

5.4.3.1.3. Soak filter paper in the solution and allow to dry completely.

5.4.3.1.4. Cut filter paper into small pieces for use. (Approximate one inch squares suggested.)

5.4.3.1.5. Store filter paper in a wide mouth bottle with top.

5.4.3.1.6. Storage: Amber glass

5.4.3.1.7. Expiration: Use container: Three years

5.4.3.1.8. Lot number: Eight-digit format year/month/day/DKPap/initials of preparer.

Example: 20120131DKPapXXX

5.4.3.1.9. Positive Quality Control Check (PQCC)

5.4.3.1.9.1. Reference material: Phenobarbital

5.4.3.1.9.2. Acceptable result: Red-violet color produced

5.4.3.2. 5% Isopropylamine

5.4.3.2.1. Mix 5 milliliters isopropylamine and 95 milliliters methanol.

5.4.3.2.2. Storage: Amber glass.

5.4.3.2.3. Expiration: Stock container: Three years
Use container: Three months

5.4.3.2.4. Lot number: Eight-digit format year/month/day//initials of preparer.

Example: 20101231IPAm5%XXX

5.4.3.2.5. Positive Quality Control Check (PQCC)

5.4.3.2.5.1. Reference material: Phenobarbital
5.4.3.2.5.2. Acceptable result: Red-violet color produced

5.4.4. Procedure

5.4.4.1. Place a small amount of sample on a piece of the Dille-Koppanyi paper.

5.4.4.2. Press the sample onto the paper with a spatula (optional).

5.4.4.3. Place a drop of the 5 % Isopropylamine solution on the edge of the Koppanyi paper and tilt to allow the drop to meet the sample.

5.4.4.4. Record observations.

5.5. *para*-Dimethylaminobenzaldehyde (*p*DMAB)

5.5.1. This color test uses a filter paper soaked with the reagent. This test reacts with indoles (e.g., LSD), primary aromatic amines (e.g., procaine), and carbamates to produce colored intermediates.

5.5.2. Selected Characteristic Results: Carbamate - yellow
LSD - purple
Psilocin - dark purple
Procaine, Benzocaine - orange/yellow
Dimethyltryptamine - purple

5.5.3. Preparation: *p*DMAB Paper

5.5.3.1. Dissolve 1.0 gram of *p*DMAB in 100 milliliters of methanol.

5.5.3.2. Soak the filter paper in the solution and allow it to dry completely.

5.5.3.2.1. Cut filter paper into small pieces for use.

5.5.3.2.2. Storage: Amber glass

5.5.3.2.3. Expiration: Use container: Three years

5.5.3.2.4. Lot number: Eight-digit format year/month/day/*p*DMAB/initials of preparer.

Example: 20120131*p*DMABXXX

5.5.3.3. Positive Quality Control Check (PQCC)

5.5.3.3.1. Reference material: LSD

5.5.3.3.2. Acceptable results: Purple color produced

5.5.4. Procedure

5.5.4.1. Place a small amount of sample on a piece of the *p*DMAB paper.

5.5.4.2. Press the sample onto the paper with a spatula. (optional)

5.5.4.3. Place a drop of methanol on top of the sample to help it dissolve into the paper.

5.5.4.4. Add a drop of concentrated hydrochloric acid to the filter paper by one of the following methods:

5.5.4.4.1. Adding the drop directly on the methanol spot.

5.5.4.4.2. Adding the acid drop to the edge of the paper and allowing the acid and methanol spots to meet (e.g., LSD and Psilocin.)

5.5.4.4.3. Allowing the fumes of the acid to contact the paper (e.g., procaine and benzocaine)

5.5.4.5. Heated air may be applied.

5.5.4.6. Record observations.

5.6. Additional Color Tests

5.6.1. Additional color tests may be used if necessary.

6. Limitations

6.1. The size, color or form (i.e. liquid) of the material may inhibit the color result observed.

7. Safety - Refer to the MSDS of any chemicals used prior to use.

8. References

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9. Records

9.1. Prepared reagent log

Document Revision History		
Revision Date	Prepared By	Revision
11/7/2024	A. Abernethy	Document revised to reflect the agency name change from Raleigh/Wake City-County Bureau of Identification to Wake County Bureau of Forensic Services, effective December 1, 2024. Changed header and revision history format. No change to procedure content.