

Technical Procedure for Preliminary Color Tests

1.0 Purpose - This procedure specifies the required elements for the preparation and use of preliminary color test reagents.

2.0 Scope - This procedure applies to all preliminary color tests used in the Drug Chemistry Sections of the State Crime Laboratory.

3.0 Definitions

- **Prepared reagent** – Mixture of two or more reagents or a dilution.
- **Commercial reagent** – Solvent or chemical purchased from an outside vendor.
- **Performance verification** – The initial confirmation of the reliability of a previously or externally validated method or instrument.
- **Quality control (QC) checks** – Periodic confirmation of the reliability of equipment, instrumentation, and/or reagents.
- **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.

4.0 Equipment, Materials and Reagents

4.1 Equipment

- Balance

4.2 Materials and Reagents

- Fume hood
- Eye protection
- Laboratory coat
- Gloves
- Beakers or other glass vessels (Optional)
- Culture tubes (6 X 50 mm suggested size)
- Funnel
- Glass stirring rod
- Graduated cylinder
- Pipettes with bulb
- Porcelain spot plates (Black suggested for Barium Chloride Reagent)
- Reagent bottles and stock bottles (amber-colored preferred for Duquenois reagent)
- Spatula
- Weigh boats or other weigh vessels
- Filter paper (PDMAB and Koppanyi only)
- Scissors (PDMAB and Koppanyi paper only)
- Wide mouth bottles with tops (suggested for storage of PDMAB and Koppanyi Paper)
- Commercial Reagents
- Reference materials

5.0 Procedure

5.1 Standards and Controls – Quality control checks of all reagents shall consist of a negative check and a positive check. Both checks shall be acceptable according to the procedure listed for each reagent, and shall be recorded together as a quality control check in the Resource Manager section of FA.

5.1.1 Negative quality control checks shall be performed according to the procedure listed with no sample present.

5.1.1.1 Acceptable result is no significant color formation.

5.1.1.2 If a significant color develops, steps shall be taken to ensure the spot plate is clean. Making new reagent and retesting with no sample present are further steps that can be taken to ensure no significant color develops prior to introduction of the sample.

5.1.2 Positive quality control checks shall be performed according to the procedure listed for each reagent using the specified reference material. See each procedure for acceptable results.

5.1.2.1 The result of the quality control check shall be recorded in FA with the identification of the standard used and the results of the QC check.

5.1.3 Reagents may be prepared in any amount provided that the component ratios are kept constant.

5.1.4 Labeling - All stock and use bottles shall be labeled according to the [Administrative Policy for Drug Chemistry Quality Assurance](#).

5.1.5 Storage - Stock and use solutions shall be stored in closed containers. All stock bottles shall be stored in the refrigerator, and all use bottles kept on the countertop or under the hood, unless otherwise noted in the procedure. All bottles shall be labeled as described in the [Administrative Policy for Drug Chemistry Quality Assurance](#).

5.1.5.1 Expiration Dates - Stock bottles stored in the refrigerator have a three year expiration date. They shall be labeled as such.

5.1.5.2 For use bottles, the expiration date is three years unless specifically stated in the procedure.

5.1.6 For all stock and use bottles with no expiration date, rechecks will be performed according to the [Administrative Policy for Drug Chemistry Quality Assurance](#) to ensure reagent reliability.

5.1.7 Application of Procedures on Evidence – (Specific instructions are included in the [Drug Chemistry Section Technical Procedure for Preliminary Color Tests](#) that utilize more than one reagent.).

5.1.7.1 Add 1-2 drops of the reagent to a clean spot well or a new culture tube, and ensure no significant color develops.

5.1.7.2 Add a small amount of sample to the reagent in the spot well or culture tube.

5.1.7.3 Observe any reaction or color produced.

5.1.7.4 Record results in the FA case file if test is being performed for casework or FA Resource Manager if test is being performed for quality control purposes.

5.1.8 Marquis

5.1.8.1 This color test reacts with opiates and amphetamines, as well as some non-controlled substances, to produce colored intermediates.

5.1.8.2 Add 8-10 drops of (40 %) formaldehyde solution to 10 milliliters of concentrated sulfuric acid, with stirring.

5.1.8.3 The expiration date for this reagent shall be one month after preparation.

5.1.8.4 Lot number: Eight digit format year/month/day/Mq/initials of preparer.
Example: 20101231MqXXX

5.1.8.5 QC check: Guaifenesin produces a purple color.

5.1.8.6 Results: Opiates (heroin, oxycodone) – purple
Phenethylamines (meth)amphetamine – orange/brown
MDA/MDMA – purple/black
Aspirin – slow cherry red

5.1.9 Duquenois-Levine (Modified)

5.1.9.1 This color test reacts with marijuana, hashish, and cannabinoids to produce a violet blue color that transfers to the chloroform layer.

5.1.9.2 **Duquenois (A)** - Dissolve 2.0 grams of vanillin and 2.5 milliliters of acetaldehyde in 100 milliliters of ethanol.

5.1.9.2.1 Amber-colored use bottles shall be used to protect this reagent from light.

5.1.9.2.2 Use bottles have a three month expiration date.

5.1.9.2.3 Lot number: Eight digit format year/month/day/Duq/initials of preparer. Example: 20101231DuqXXX

5.1.9.3 Concentrated Hydrochloric Acid (B)

5.1.9.3.1 Prepare a (dropper) bottle of concentrated hydrochloric acid.

5.1.9.4 Chloroform (C)

5.1.9.4.1 Prepare a (dropper) bottle of chloroform.

5.1.9.5 Application of Procedure on Evidence

- 5.1.9.5.1 Place a small amount of sample in a culture tube or spot plate.
- 5.1.9.5.2 Add two to three drops of the Duquenois reagent (A).
- 5.1.9.5.3 Add at least an equal volume of concentrated hydrochloric acid (B) and observe any color changes.
- 5.1.9.5.4 Add at least two to three drops of chloroform (C) and agitate.
- 5.1.9.5.5 Allow phases to separate and observe the color in the (bottom) chloroform layer.
- 5.1.9.5.6 Record results in the FA case file if performing casework, or in the Resource Manager section of FA if performing a QC check.
- 5.1.9.5.7 QC check: Marijuana produces a violet blue color after addition of the hydrochloric acid. For a positive result, this color shall transfer to the chloroform layer with shaking.
- 5.1.9.5.8 Results: Marijuana, hashish, cannabinoids – violet blue color after addition of the hydrochloric acid, which extracts into the chloroform layer with shaking.

5.1.9.6 Limitations

- 5.1.9.6.1 For wet or fresh plant material, the color development may be hindered. In these cases, wash the wet or new plant material with the Duquenois reagent quickly and decant the reagent to a new culture tube. Proceed with addition of acid and chloroform as described in the procedure.
- 5.1.9.6.2 For old plant material, the color development may be hindered. In these cases, place the material in a culture tube. Cover with petroleum ether and let sit for approximately two minutes. Decant the petroleum ether to a clean culture tube. Evaporate petroleum ether on hot plate. (Set tube in a beaker for support if needed.) Proceed with addition of Duquenois reagent, acid, and chloroform, to the residue left from the petroleum ether wash.
- 5.1.9.6.3 If color formation is slow, a small amount of heat may be added to the plant material and Duquenois reagent to facilitate color development. (Careful use of a heat gun is suggested.)
- 5.1.9.6.4 For smoking devices and/or paraphernalia, the item may be washed with chloroform to remove the marijuana residue. Duquenois-Levine reagent, and acid are then added to a portion of the chloroform wash as described in the procedure.

5.1.10 Cobalt Thiocyanate

- 5.1.10.1 This color test reacts with secondary and tertiary amines, as well as some alkaloids, to produce a blue color.

- 5.1.10.2 Dissolve 0.38 grams of ammonium thiocyanate in 25 ml water, and then slowly add 0.63 gram cobaltous acetate with stirring.
- 5.1.10.3 Alternate recipe: Dissolve 1.5 grams of cobalt thiocyanate in 29.0 milliliters of water.
- 5.1.10.4 Lot number: Eight digit format year/month/day/Cobalt/initials of preparer. Example: 20101231CobaltXXX
- 5.1.10.5 QC check: Cocaine produces a blue color.
- 5.1.10.6 Results: Cocaine – blue
PCP - blue

5.1.11 Ferric Chloride

- 5.1.11.1 This color test reacts with phenols, enols, and GHB to produce colored intermediates.
- 5.1.11.2 Dissolve 1.5 grams of ferric chloride in 29.0 milliliters of water.
- 5.1.11.3 Lot number: Eight digit format year/month/day/FerChl/initials of preparer. Example: 20101231FerChlXXX
- 5.1.11.4 QC check: Acetaminophen produces a violet-purple color.
- 5.1.11.5 Results: Acetaminophen – violet-purple
GHB - red/brown

5.1.12 Koppányi

- 5.1.12.1 This color test reacts with barbiturates to produce colored intermediates.
- 5.1.12.2 **Koppányi Paper (A)**
 - 5.1.12.2.1 Dissolve 0.1 gram cobalt acetate in 100 milliliters of methanol.
 - 5.1.12.2.2 Add 0.2 milliliter glacial acetic acid.
 - 5.1.12.2.3 Soak filter paper in the solution and allow to dry completely.
 - 5.1.12.2.4 Cut filter paper into small pieces for use. (Approximate one inch squares suggested.)
 - 5.1.12.2.5 Store filter paper in a wide mouth bottle with top.
 - 5.1.12.2.6 Lot number: Eight digit format year/month/day/Kopp/initials of preparer. Example: 20101231KoppXXX
- 5.1.12.3 **5% Isopropylamine (B)**
 - 5.1.12.3.1 Mix 5 milliliters isopropylamine and 95 milliliters methanol. (Approximate 1:20 isopropylamine:methanol ratio.)

- 5.1.12.3.2 Lot number: Eight digit format
year/month/day/KoppSoln/initials of preparer.
Example: 20101231KoppSolnXXX

5.1.12.4 Application of Procedure on Evidence

- 5.1.12.4.1 Place a small amount of sample on a piece of the Koppanyi paper (A).
- 5.1.12.4.2 Press the sample onto the paper with a spatula (optional).
- 5.1.12.4.3 Place a drop of the 5 % Isopropylamine solution (B) on the edge of the Koppanyi paper and tilt to allow the drop to meet the sample.
- 5.1.12.4.4 Record results in the case file if test is being performed for case work or Resource Manager in FA if test is being performed for quality control purposes.
- 5.1.12.4.5 QC check: Barbiturates produce a red-violet color upon addition of the 5 % Isopropylamine reagent to the Koppanyi paper.
- 5.1.12.4.6 Results: Barbiturates – red-violet
 Oxymorphone HCl – violet
 Pseudoephedrine HCl – light green
 Psilocybin – blue
 Theophylline - violet

5.1.13 Potassium Permanganate

- 5.1.13.1 This color test reacts with compounds containing reactive double bonds to produce a brown color.
- 5.1.13.2 Dissolve 0.3 gram potassium permanganate in 30 milliliters of water.
- 5.1.13.3 Lot number: Eight digit format year/month/day/KMNO4/initials of preparer.
Example: 20101231KMNO4XXX
- 5.1.13.4 **Application of Procedure on Evidence**
- 5.1.13.4.1 A culture tube instead of a spot well is suggested for use of this reagent.
- 5.1.13.4.2 QC check: Opiates produce a brown color.
- 5.1.13.4.3 Results: Opiates produce a brown color.

5.1.14 para-Dimethylaminobenzaldehyde (PDMAB)

5.1.14.1 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.

5.1.14.2 PDMAB Paper (A)

5.1.14.2.1 Dissolve 1.0 gram of para-dimethylaminobenzaldehyde (PDMAB) in 100 milliliters of methanol.

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

5.1.14.2.3 Cut filter paper into small pieces for use. (Suggested 1-inch squares.)

5.1.14.2.4 Store PDMAB paper in wide mouth bottle with top for ease of use.

5.1.14.2.5 Lot number: Eight digit format
year/month/day/PDMABpp/initials of preparer.
Example: 20101231PDMABppXXX

5.1.14.3 Methanol (B)

5.1.14.3.1 Prepare a (dropper) bottle of methanol.

5.1.14.4 Hydrochloric Acid (concentrated) (C)

5.1.14.4.1 Prepare a (dropper) bottle of concentrated hydrochloric acid.

5.1.14.5 Application of Procedure on Evidence

5.1.14.5.1 Place a small amount of sample on a piece of the PDMAB paper.

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

5.1.14.5.3 Place a drop of methanol (B) on top of the sample to help it dissolve into the paper.

5.1.14.5.4 Add a drop of hydrochloric acid (C) to the filter paper by one of the following methods:

- Adding the drop directly on the methanol spot.
- Adding the acid drop to the edge of the paper and allowing the acid and methanol spots to meet (e.g., LSD and Psilocin.)
- Allowing the fumes of the acid to contact the paper (e.g., procaine and benzocaine)

5.1.14.5.5 Record results in the case file if test is being performed for casework or Resource Manager in FA if test is being performed for quality control purposes.

5.1.14.5.6 QC check: Procaine produces a yellow-orange color upon addition of the hydrochloric acid to the PDMAB paper.

5.1.14.5.7 Results: Carbamate – yellow
LSD – purple
Psilocin – dark purple
Procaine, Benzocaine – orange/yellow

5.1.15 Froehde

5.1.15.1 This color test reacts with a wide range of aromatic compounds to produce colored intermediates.

5.1.15.2 Dissolve 50 milligrams of molybdic acid (or sodium molybdate) in sulfuric acid with heating and stirring.

5.1.15.3 The expiration date for this reagent shall be one month after preparation.

5.1.15.4 Lot Number: Eight digit format year/month/day/Fro/initials of preparer.
Example: 20101231FroXXX

5.1.15.5 QC check: Guafenesin produces a purple color.

5.1.15.6 Results: Acetaminophen – blue
Bufotenine – yellow/brown
Heroin HCl – purple
MDMA – yellow/green to dark blue
MDA – green to olive to blue
Morphine – purple

5.1.16 Mecke

5.1.16.1 This color test reacts with a wide range of aromatic compounds to produce colored intermediates.

5.1.16.2 Add 0.25 gram selenious acid to 25 milliliters of concentrated sulfuric acid with stirring.

5.1.16.3 The expiration date for this reagent shall be one month after preparation.

5.1.16.4 Lot number: Eight digit format year/month/day/Meck/initials of preparer.
Example: 20101231MeckXXX

5.1.16.5 QC Check: Guafenesin produces a green to red color.

5.1.16.6 Results: Bufotenine – brown to black/purple
Diphenhydramine HCl - yellow
Heroin HCl – green/blue
Hydrocodone bitartrate – dark blue
Methadone – green/brown
MDMA – green to dark blue
MDA – green to blue

5.1.17 Silver Nitrate

- 5.1.17.1 This color test reacts with halide ions to produce a white precipitate.
- 5.1.17.2 Dissolve 1 gram of silver nitrate in 20 milliliters of water (5 % solution).
- 5.1.17.3 Lot number: Eight digit format year/month/day/SilNit/initials of preparer.
Example: 20101231SilNitXXX
- 5.1.17.4 **Application of Procedure on Evidence** - A culture tube or black spot plate is suggested for use of this reagent.
 - 5.1.17.4.1 QC Check: Sodium chloride produces a white precipitate.
 - 5.1.17.4.2 Results: All halide salts – white precipitate.

5.1.18 Zwikker

- 5.1.18.1 This color test reacts with barbiturates to produce a purple color that transfers to the organic layer of the reagent.
- 5.1.18.2 **0.5% Cupric Sulfate (A)**
 - 5.1.18.2.1 Dissolve 0.12 gram cupric sulfate in 25 milliliters of water.
 - 5.1.18.2.2 Lot number: Eight digit format year/month/day/Zwik/initials of preparer. Example: 20101231ZwikXXX
- 5.1.18.3 **5% Pyridine (B)**
 - 5.1.18.3.1 Add 1 milliliter of pyridine to 24 milliliters of chloroform.
 - 5.1.18.3.2 Lot number: Eight digit format year/month/day/ZwikPyr/initials of preparer. Example: 20101231ZwikPyrXXX
- 5.1.18.4 **Application of Procedure on Evidence**
 - 5.1.18.4.1 Place a small amount of sample in a culture tube.
 - 5.1.18.4.2 Add a drop of 0.5 % cupric sulfate (A) and observe any reaction or color change.
 - 5.1.18.4.3 Add a drop of 5 % pyridine (B) and observe any reaction or color change.
 - 5.1.18.4.4 Record results in the FA case file if test is being performed for case work or Resource Manager in FA if test is being performed for quality control purposes.
 - 5.1.18.4.5 QC check: Phenobarbital produces a purple color that transfers to the organic layer.

5.1.18.4.6 Results: Barbiturates – purple or bright green color that transfers to the organic layer.

5.1.19 Barium Chloride

- 5.1.19.1** This color test reacts with sulfates to produce a white precipitate.
- 5.1.19.2** Dissolve 3.0 grams of barium chloride in 27 milliliters of water. (10 % solution)
- 5.1.19.3** Lot number: Eight digit format year/month/day/BaCl/initials of preparer. Example: 20101231BaClXXX
- 5.1.19.4** Application of Procedure on Evidence – A culture tube or black spot plate is suggested for use of this reagent.
- 5.1.19.5** QC check: Sodium sulfate produces a white precipitate.
- 5.1.19.6** Results: Sulfate compounds produce a white precipitate.

5.1.20 Cupric Sulfate (Secondary Amine #1)

- 5.1.20.1** This color test reacts with secondary amines to produce a yellow/brown color in the organic phase.
- 5.1.20.2 Cupric Sulfate (A)**
 - 5.1.20.2.1** Dissolve 0.12 gram cupric sulfate in 25 milliliters of water. (1 % w/v)
 - 5.1.20.2.2** Lot number: Eight digit format year/month/day/SA1/initials of preparer. Example: 20101231SA1XXX
- 5.1.20.3 Concentrated Ammonium Hydroxide (B)**
 - 5.1.20.3.1** Prepare a sealed bottle of concentrated ammonium hydroxide, or take an aliquot from a stock bottle for use.
- 5.1.20.4 Toluene/Carbon Disulfide (C)**
 - 5.1.20.4.1** Mix 20 milliliters of toluene with 5 milliliters of carbon disulfide.
- 5.1.20.5 Application of Procedure on Evidence**
 - 5.1.20.5.1** Dissolve a small amount of sample with 2-3 drops of cupric sulfate (A) in a new culture tube.
 - 5.1.20.5.2** Add 2-3 drops of concentrated ammonium hydroxide (B) and then add 2-3 drops of toluene/carbon disulfide (C) and agitate the culture tube.

- 5.1.20.5.3 Observe any reaction or color produced.
- 5.1.20.5.4 Record the results in the FA case file if test is being performed for case work or Resource Manager section of FA if test is being performed for quality control purposes.
- 5.1.20.5.5 QC check: Methamphetamine produces a yellow/brown color in the organic phase after addition of the toluene/carbon disulfide solution.
- 5.1.20.5.6 Results: Methamphetamine, BZP, and secondary amines – yellow/brown

5.1.21 Simon's Test (Modified Sodium Nitroprusside) (Secondary Amine #2)

- 5.1.21.1 This color test reacts with secondary amines to produce a blue-violet color and it reacts with primary amines to produce a slow pink to cherry red color.

5.1.21.2 1 % Sodium Nitroprusside/10 % by Volume of Acetaldehyde (A)

- 5.1.21.2.1 Dissolve 1 gram of sodium nitroprusside in 100 milliliters of water, then discard 10 milliliters of this solution.
- 5.1.21.2.2 Add 10 milliliters of acetaldehyde to the remaining 90 milliliters of sodium nitroprusside/water.
- 5.1.21.2.3 The expiration date for this reagent shall be one month after preparation.
- 5.1.21.2.4 This reagent shall be refrigerated.
- 5.1.21.2.5 Lot number: Eight digit format year/month/day/SA2/initials of preparer. Example: 20101231SA2XXX

5.1.21.3 2 % Sodium Carbonate (B)

- 5.1.21.3.1 Dissolve 2 grams of sodium carbonate in 100 milliliters of water.

5.1.21.4 Application of Procedure on Evidence

- 5.1.21.4.1 Place a small amount of sample in a culture tube or clean spot well and add one drop of Reagent A, then add 2 drops of Reagent B.
- 5.1.21.4.2 Observe any reaction or color produced.
- 5.1.21.4.3 Record results in the case file if the test is being performed for case work or Resource Manager of FA if test is being performed for quality control purposes.
- 5.1.21.4.4 QC check: Methamphetamine produces a blue-violet color.

5.1.21.4.5 Results: Methamphetamine and secondary amines – blue-violet
Amphetamine and primary amines – slow pink to cherry red

5.1.22 Liebermann's Reagent

5.1.22.1 This color test reacts with methcathinone, analogs of methcathinone, and most of the synthetic cannabinoids with an indole substructure. When a synthetic cannabinoid is present on plant material, an extraction with methylene chloride/acetonitrile shall be conducted prior to performing the color test.

5.1.22.2 Lieberman's Reagent (A)

5.1.22.2.1 Add 5 grams of sodium nitrite to 50 milliliters of concentrated sulfuric acid, with stirring.

5.1.22.2.2 Lot number: Eight digit format year/month/day/LB/initials of preparer. Example: 20131231LBXXX

5.1.22.3 Methylene chloride-acetonitrile solution (B)

5.1.22.3.1 Mix equal amounts of methylene chloride and acetonitrile.

5.1.22.4 Application of Procedure on Evidence – Methcathinone and analogs of methcathinone

5.1.22.4.1 See 5.1.7 for all powder samples.

5.1.22.4.2 QC check: 3,4-MDPV turns yellow-green

5.1.22.4.3 Results:

- 3,4-MDMC turns bright orange
- MDMA turns brown

5.1.22.5 Application of Procedure on Evidence – Synthetic cannabinoids present on plant material

5.1.22.5.1 Add a small amount of plant material to test tube (NOT a small culture tube) and cover with Reagent B.

5.1.22.5.2 Shake the test tube and immediately pipette the liquid to a new test tube.

5.1.22.5.3 Add approximately five drops of Reagent A to the liquid extract and mix thoroughly.

5.1.22.5.4 Observe any reaction or color produced.

5.1.22.5.5 QC check: See 5.1.22.4.2

5.1.22.5.6 Results:

- AM-2201 turns yellow-brown
- JWH-073 and JWH-018 turn yellow-brown
- JWH-122 turns yellow-brown
- JWH-019/JWH-081 turns yellow-brown
- Many synthetic cannabinoids turn yellow/brown/orange colors

5.2 **Sampling** - See Drug Chemistry Section Technical Procedure for Sampling.

5.3 **Calculations** - N/A

5.4 **Uncertainty of Measurement** - N/A

6.0 **Limitations** - See specific procedures listed above.

7.0 **Safety** - See [State Crime Laboratory Safety Manual](#).

8.0 **References**

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9.0 Records - Entries in Resource Manager in the FA System

10.0 Attachments - N/A

Revision History		
Effective Date	Version Number	Reason
09/17/2012	1	Original Document for Conversion to ISO Standards. Technical Procedures A-1 through A-15 were combined.
02/15/2013	2	2.0 – Scope changed to reflect all three laboratories. 5.1.4, 5.1.5, and 5.1.6 Updated reference to Administrative Policy for Drug Chemistry Quality Assurance . 5.1.9.6.4 –Duquenois-Levine reagent added. 5.1.10.2 – Recipe changed to accommodate reagents on hand. 5.1.10.3 – Original recipe moved to "Alternate recipe" 5.1.22 – Liebermanns' Reagent added References – Poyner and Toole references added for Liebermann's Reagent.