



## SCREENING/SPOT/COLOUR TEST OF ANTI-CHOLINERGICS

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<p><b>Article Info</b>  <i>Received 05/10/2014</i>  <i>Revised 12/10/2014</i>  <i>Accepted 22/10/2014</i></p> <p><b>Key words:</b>          Anticholinergic,          Screening, Spot test          etc.</p>	<p><b>ABSTRACT</b>          Anticholinergic are class of drugs that blocks the neurotransmitter acetylcholine in the central and peripheral nervous system. In India, Forensic Science Laboratories run by Government under the Home ministry usually carry out this for forensic investigation. The samples have to be analyzed by the forensic toxicologist/chemists/scientist. This article deals with the screening/spot/colour test for different Anticholinergic. It attempts to simplify the standard procedures in a step-wise manner, which can be of handy reference for the forensic toxicologist/chemist. It is in continuation to toxicology manual series article-XVIII, Screening/spot test of Aphrodisiacs (Sex drugs) Vol4, No3, Jan-July 2012 published in International Journal of Medical Toxicology and Legal Medicine.</p>
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### INTRODUCTION

Anticholinergics are also known as cholinergic blocking agents or parasympatholytics. It affects the CNS and blocks the action of acetylcholine transmitter. It focuses on parasympathetic nervous system and acts as resting and reparative functions which includes digestion, excretion, cardiac and anabolism. Most of these drugs interact with muscarinic receptors in the brain, secretory gland, brain and smooth muscles. It produces the following effects:

- Decreased cardiovascular response
- Decreased respiratory tract secretions
- Decreased oral secretions, decreased sweating and relaxation of urinary bladder.
- Increased body temperature
- Ataxia, loss of co-ordination

Classification of Anticholinergics based on the receptors is given in Table 1.

**Table1. Classification of anti-cholinergics based on the receptors**

Sl.no	Anti-muscarinic agents	Anti-nicotinic agents
1	It operates on the muscarinic acetylcholine receptors.	It operates on the nicotinic acetylcholine receptors.
2	It is used in gastrointestinal(GI), genitourinary (GU) and respiratory disorders and Parkinson's disease.	It is used in increased blood pressure and in emergency situations in case of aortic dissection.
3	Examples: Atropine, hyoscine.	Examples: trimethophan, mecamlamine

We have tried to set out standard procedures for screening/spot test for alkaloids which are easily available and useful for the forensic scientific laboratory. This article covers the spot test/colour test of anti-cholinergic like adiphenine, ambutoonium bromide, atropine, atropine methobromide, atropine methonitrate, benzatropine, Benzilonium bromide, beperiden, caramiphen,

chlorphenoxamine, cyclopentolate, cycrimine, diethazine, glycopyrronium bromide, hyoscine, isopropamide iodide, lachesine chloride, mepenzolate bromide, metixene, orphenadrine, penthienate methobromide, phenglutarimide, piperidolate, poldine metilsulfate, procyclidine, Tricyclamol chloride and tropicamide [1-10].



### 1. Adiphenine

#### *Liebermann's test*

1. One to two ml of extract is taken in test tube.
2. Few drops of liebermann's reagent are added to it.
3. Brown colour is observed which indicates the presence of adiphenine.

#### *Mandelin's test*

1. One to two ml of extract is taken in test tube.
2. Few drops of mandelin's reagent are added to it.
3. Green to blue colour is observed which indicates the presence of adiphenine.

### 2. Ambutonium bromide

#### *Liebermann's test*

1. One to two ml of extract is taken in test tube.
2. Few drops of liebermann's reagent are added to it.
3. Brownish orange colour is observed which indicates the presence of ambutonium bromide.

### 3. Atropine

#### *Liebermann's test*

1. One to two ml of extract is taken in test tube.
2. Few drops of liebermann's reagent are added to it.
3. Reddish orange colour is observed which indicates the presence of atropine.

### 4. Atropine methobromide

#### *Sodium hydroxide test*

1. One to two ml of extract is taken in test tube.
2. Few drops of nitric acid are added to it.
3. Solution is heated on a water bath for 1 min.
4. The solution is cooled and then three to four times diluted with water.
5. Few drops of sodium hydroxide are added to it.
6. Solution becomes colourless which indicates the presence of atropine methobromide.

### 5. Atropine methonitrate

#### *Sodium hydroxide test*

1. One to two ml of extract is taken in test tube.
2. Few drops of nitric acid are added to it.
3. Solution is heated on a water bath for 1 min.
4. The solution is cooled and then three to four times diluted with water.
5. Few drops of sodium hydroxide are added to it.
6. Violet colour is observed which indicates the presence of atropine methonitrate.

#### *Liebermann's test*

1. One to two ml of extract is taken in test tube.
2. Few drops of liebermann's reagent are added to it.
3. Orange colour is observed which indicates the presence of atropine methonitrate.

### 6. Benzatropine

#### *Mandelin's test*

1. One to two ml of extract is taken in test tube.
2. Few drops of mandelin's reagent are added to it.
3. Yellow colour is observed which indicates the presence of benzatropine.

#### *Marquis test*

1. One to two ml of extract is taken in test tube.
2. Few drops of marquis reagent are added to it.
3. Yellow colour is observed which indicates the presence of benzatropine.

### 7. Benzilonium bromide

#### *Sulphuric acid test*

1. Few drops of extract are taken on a white tile.
2. Few drops of sulphuric acid are added to it.
3. Orange colour is observed which indicates the presence of benzilonium bromide.

#### *Marquis Reagent test*

1. One to two ml of extract is taken in test tube.
2. Few drops of marquis reagent are added to it.
3. Play of colour from orange to green and finally to blue is observed which indicates the presence of benzilonium bromide.

#### *Liebermann's test*

1. One to two ml of extract is taken in test tube.
2. Few drops of liebermann's reagent are added to it.
3. Brown colour is observed which indicates the presence of benzilonium bromide.

### 8. Beperiden

#### *Marquis Reagent test*

1. One to two ml of extract is taken in test tube.
2. Few drops of marquis reagent are added to it.
3. Reddish brown colour is observed which indicates the presence of beperiden.

#### *Sulphuric acid test*

1. Few drops of extract are taken on a white tile.
2. Few drops of sulphuric acid are added to it.
3. Orange to brown colour is observed which indicates the presence of beperiden.

#### *Liebermann's test*

1. One to two ml of extract is taken in test tube.
2. Few drops of liebermann's reagent are added to it.
3. Brown colour is observed which indicates the presence of beperiden.

### 9. Caramiphen

#### *Sulphuric acid test*

1. Few drops of extract are taken on a white tile.
2. Few drops of sulphuric acid are added to it.
3. Red colour is observed which indicates the presence of caramiphen.



#### **Marquis Reagent test**

1. One to two ml of extract is taken in test tube.
2. Few drops of marquis reagent are added to it.
3. Yellow colour is observed which indicates the presence of caramiphen.

#### **Liebermann's test**

1. One to two ml of extract is taken in test tube.
2. Few drops of liebermann's reagent are added to it.
3. Reddish orange colour is observed which indicates the presence of caramiphen.

#### **10. Chlorphenoxamine**

##### **Marquis Reagent test**

1. One to two ml of extract is taken in test tube.
2. Few drops of marquis reagent are added to it.
3. Yellow to green colour is observed which indicates the presence of chlorphenoxamine.

#### **11. Cyclopentolate**

##### **Mandelin's test**

1. One to two ml of extract is taken in test tube.
2. Few drops of mandelin's reagent are added to it.
3. Brown colour is observed which indicates the presence of cyclopentolate.

#### **12. Cycrimine**

##### **Mandelin's test**

1. One to two ml of extract is taken in test tube.
2. Few drops of mandelin's reagent are added to it.
3. Reddish brown colour is observed which indicates the presence of cycrimine.

##### **Marquis Reagent test**

1. One to two ml of extract is taken in test tube.
2. Few drops of marquis reagent are added to it.
3. Reddish brown colour is observed which indicates the presence of cycrimine.

#### **13. Diethazine**

##### **Forrest test**

1. One to two ml of extract is taken in test tube.
2. Few drops of forrest reagent are added to it.
3. Red colour is observed which indicates the presence of diethazine.

##### **Formaldehyde-sulphuric acid Reagent test**

1. One to two ml of extract is taken in test tube.
2. Few drops of Formaldehyde-sulphuric acid reagent are added to it.
3. Reddish violet colour is observed which indicates the presence of diethazine.

##### **FPN test**

1. One to two ml of extract is taken in test tube.
2. Few drops of FPN reagent are added to it.
3. Orange colour is observed which indicates the presence of diethazine.

#### **14. Glycopyrronium bromide**

##### **Liebermann's test**

1. One to two ml of extract is taken in test tube.
2. Few drops of liebermann's reagent are added to it.
3. Black colour is observed which indicates the presence of glycopyrronium bromide.

#### **15. Hyoscine**

##### **Liebermann's test**

1. One to two ml of extract is taken in test tube.
2. Few drops of liebermann's reagent are added to it.
3. Reddish orange colour is observed which indicates the presence of hyoscine.

#### **16. Isopropamide iodide**

##### **Sodium Hydroxide test**

1. One to two ml of extract is taken in test tube.
2. Few drops of nitric acid are added to it.
3. Solution is heated on a water bath for 1 min.
4. The solution is cooled and then three to four times diluted with water.
5. Few drops of sodium hydroxide are added to it.
6. Solution becomes colourless which indicates the presence of isopropamide iodide.

##### **Liebermann's test**

1. One to two ml of extract is taken in test tube.
2. Few drops of liebermann's reagent are added to it.
3. Grey colour is observed which indicates the presence of isopropamide iodide.

#### **17. Lachesine Chloride**

##### **Mandelin's test**

1. One to two ml of extract is taken in test tube.
2. Few drops of mandelin's reagent are added to it.
3. Orange to green colour is observed which indicates the presence of lachesine chloride.

##### **Marquis Reagent test**

1. One to two ml of extract is taken in test tube.
2. Few drops of marquis reagent are added to it.
3. Blue colour is observed which indicates the presence of lachesine chloride.

#### **18. Mepenzolate bromide**

##### **Liebermann's test**

1. One to two ml of extract is taken in test tube.
2. Few drops of liebermann's reagent are added to it.
3. Brown colour is observed which indicates the presence of mepenzolate bromide.

##### **Marquis Reagent test**

1. One to two ml of extract is taken in test tube.
2. Few drops of marquis reagent are added to it.
3. Orange colour is observed which indicates the presence of mepenzolate bromide.



### 19. Methanthelinium bromide

#### *Mandelin's Reagent test*

1. One to two ml of extract is taken in test tube.
2. Few drops of mandelin's reagent are added to it.
3. Orange colour is observed which indicates the presence of methanthelinium bromide.

#### *Marquis test*

1. One to two ml of extract is taken in test tube.
2. Few drops of marquis reagent are added to it.
3. Orange colour is observed which indicates the presence of methanthelinium bromide.

### 20. Metixene

#### *Sulphuric acid test*

1. Few drops of extract are taken on a white tile.
2. Few drops of sulphuric acid are added to it.
3. Orange colour is observed which indicates the presence of metixene.

#### *Formaldehyde-sulphuric acid Reagent test*

1. One to two ml of extract is taken in test tube.
2. Few drops of Formaldehyde-sulphuric acid reagent are added to it.
3. Orange colour is observed which indicates the presence of metixene.

#### *Liebermann's test*

1. One to two ml of extract is taken in test tube.
2. Few drops of liebermann's reagent are added to it.
3. Reddish orange colour is observed which indicates the presence of metixene.

#### *Mandelin's test*

1. One to two ml of extract is taken in test tube.
2. Few drops of mandelin's reagent are added to it.
3. Orange colour is observed which indicates the presence of metixene.

### 21. Orphenadrine

#### *Marquis Reagent test*

1. One to two ml of extract is taken in test tube.
2. Few drops of marquis reagent are added to it.
3. Yellow to orange colour is observed which indicates the presence of orphenadrine.

#### *Sulphuric acid test*

1. Few drops of extract are taken on a white tile.
2. Few drops of sulphuric acid are added to it.
3. Orange colour is observed which indicates the presence of orphenadrine.

#### *Mandelin's test*

1. One to two ml of extract is taken in test tube.
2. Few drops of mandelin's reagent are added to it.
3. Orange colour is observed which indicates the presence of orphenadrine.

### 22. Penthienate methobromide

#### *Liebermann's test*

1. One to two ml of extract is taken in test tube.
2. Few drops of liebermann's reagent are added to it.
3. Violet colour is observed which indicates the presence of penthienate methobromide.

#### *Marquis test*

1. One to two ml of extract is taken in test tube.
2. Few drops of marquis reagent are added to it.
3. Violet colour is observed which indicates the presence of penthienate methobromide.

#### *Sulphuric acid test*

1. Few drops of extract are taken on a white tile.
2. Few drops of sulphuric acid are added to it.
3. Orange colour is observed which indicates the presence of penthienate methobromide.

#### *Mandelin's test*

1. One to two ml of extract is taken in test tube.
2. Few drops of mandelin's reagent are added to it.
3. Violet colour is observed which indicates the presence of penthienate methobromide.

### 23. Phenglutarimide

#### *Koppanyi - Zwikker test*

1. The residue is extracted in 1 ml ethanol in a test tube.
2. One drop of 1% solution of cobalt nitrate in ethanol is added to it.
3. One drop of pyrrolidine is added to it.
4. Mixture is agitated for 2 mins.
5. Violet colour is observed which shows the presence of phenglutarimide.

#### *Liebermann's test*

1. One to two ml of extract is taken in test tube.
2. Few drops of liebermann's reagent are added to it.
3. Violet colour is observed which indicates the presence of phenglutarimide.

### 24. Piperidolate

#### *Marquis Reagent test*

1. One to two ml of extract is taken in test tube.
2. Few drops of marquis reagent are added to it.
3. Orange colour is observed which indicates the presence of piperidolate.

#### *Mandelin's test*

1. One to two ml of extract is taken in test tube.
2. Few drops of mandelin's reagent are added to it.
3. Brown colour is observed which indicates the presence of piperidolate.

#### *Liebermann's test*

1. One to two ml of extract is taken in test tube.
2. Few drops of liebermann's reagent are added to it.



3. Orange to brown colour is observed which indicates the presence of piperidolate.

### 25. Poldine metilsulfate

#### *Marquis Reagent test*

1. One to two ml of extract is taken in test tube.
2. Few drops of marquis reagent are added to it.
3. Blue colour is observed which indicates the presence of poldine metilsulfate.

#### *Mandelin's test*

1. One to two ml of extract is taken in test tube.
2. Few drops of mandelin's reagent are added to it.
3. Orange colour is observed which indicates the presence of poldine metilsulfate.

### 26. Procyclidine

#### *Sulphuric acid test*

1. Few drops of extract are taken on a white tile.
2. Few drops of sulphuric acid are added to it.
3. Yellow colour is observed which indicates the presence of procyclidine.

#### *Marquis Reagent test*

1. One to two ml of extract is taken in test tube.
2. Few drops of marquis reagent are added to it.
3. Violet colour is observed which indicates the presence of procyclidine.

#### *Mandelin's test*

1. One to two ml of extract is taken in test tube.
2. Few drops of mandelin's reagent are added to it.
3. Black colour is observed which indicates the presence of procyclidine.

### 27. Tricyclamol chloride

#### *Marquis test*

1. One to two ml of extract is taken in test tube.
2. Few drops of marquis reagent are added to it.
3. Violet colour is observed which indicates the presence of Tricyclamol chloride.

#### *Mandelin's test*

1. One to two ml of extract is taken in test tube.
2. Few drops of mandelin's reagent are added to it.
3. Grey to violet colour is observed which indicates the presence of Tricyclamol chloride.

### 28. Tropicamide

#### *Cyanogen bromide test*

1. One to two ml of extract is taken in test tube.
2. Few drops of cyanogens bromide reagent are added to it.
3. Violet to Pink colour is observed which indicates the presence of tropicamide.

#### *Liebermann's test*

1. One to two ml of extract is taken in test tube.
2. Few drops of liebermann's reagent are added to it.
3. Yellow colour is observed which indicates the presence of tropicamide.

### PREPARATION OF SOLUTIONS/ REAGENTS

#### 1. **Cyanogen bromide:**

Solution (I): Decolourisation of bromine water is done by the addition of solid potassium cyanide and then more bromine solution is added until the solution becomes pale yellow. Solution(II): Saturated solution of aniline in water. solution (I) and (II) are mixed.

2. **Formaldehyde-sulphuric acid:** Four volumes of sulphuric acid and six volumes of formaldehyde solution are mixed.

3. **Forrest reagent:** Equal volumes of a 0.2 % (w/v) solution of potassium dichromate, 30 % (w/v) solution of sulphuric acid, 20 % (w/v) solution of perchloric acid and 50 % solution of nitric acid are mixed.

4. **FPN reagent:** 5 ml of 5 % (w/v) ferric chloride solution, 45 ml of 20 % (w/w) solution of perchloric acid and 50 ml of 50 % (v/v) solution of nitric acid are mixed.

5. **Liebermann's reagent:** 1 gm of sodium or potassium nitrite is mixed in 10 ml of sulphuric acid with cooling and swirling to absorb the brown fumes.

6. **Mandelin's reagent:** 1 g of ammonium vanadate is dissolved in 1.5 ml of water and diluted to 100 ml with concentrated sulphuric acid.

7. **Marquis reagent:** 100 ml of concentrated sulphuric acid is mixed with 1 ml of 40% (v/v) formaldehyde solution.

### CONCLUSION

In any analysis of poison, screening/spot test is very useful for knowing the presence of the anti-cholinergic which can be confirmed by the more confirmatory tests. It saves time for the toxicologist in ruling out the poisons which can be confirmed by the more confirmatory tests. It saves time for the toxicologist in ruling out the poisons and gives a quick clue to the doctors for patient management in emergency poisoning cases. The result of the analytical methods depends on the amount and purity of the sample extracted. The techniques are being improved every time. It is important for the forensic toxicologists to know the best available method and help to detect the poison in the crime investigations.

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