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Review Article

Screening/Spot Colour Test of Analgesics

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Abstract

Analgesics give relief pain without blocking the conduction of nerve impulse or altering the function of the sensory apparatus. In forensic autopsy case, the forensic pathologist may require a complete toxicological investigation for different poisons including analgesics. The samples have to be analyzed by the Forensic Toxicologist/Chemists/ Scientist. This article deals with the screening/spot test for analgesics. It attempts to simplify the standard procedures in a step-wise manner, which can be of handy reference for the forensic toxicologist.This article is in continuation of Screening/spot/colour test of anti-depresents, Journal of Forensic Chemistry and Toxicology. Volume 2 Number 1,January – june 2016.

Keywords: Analgesics; Screening; Spot Test; Color Test; etc.

Introduction

Analgesia simply means the absence of pain without losing consciousness. Analgesics are the drugs that selectively inhibit the perception (sensation) of pain. There are basically two kinds of analgesics: non-narcotics and narcotics (Table 1). The pain relief induced by analgesics occurs by blocking pain signals going to the brain or by interfering with the brain's interpretation of the signals, without producing anesthesia or loss of consciousness. The common side effects of analgesics are nausea, vomiting, drowsiness, dry mouth, orthostatic hypotension urinary retention and constipation [1-5]. WHO analgesic ladder are

- Patients with mild to moderate pain should be treated with non-opioid analgesic.
- Patients who have limited opioid exposure and present with moderate to severe pain or who fail to achieve adequate relief after a trial of a non-opioid analgesic should be treated with an opioid conventionally used for moderate pain.
- Patients who present with severe pain or who fail to achieve adequate relief following appropriate administration of drugs on the second step of the analgesic ladder should receive an opioid conventionally used for severe pain.

We have tried to set out standard procedures for screening/spot test for analgesics poisons. This article covers different analgesics which include acetanilide, aletamine, alphaprodine, aminophenazone, aspirin, azapropazone, benorilate, butorphanol, cinchnophen, dextromoramide, dextropropoxyphene, diclofenac, diflunisal, dipyrone, etenzamide, fenbufe, fenoprofen, feprazone, ibuprofen, indometacin, ketoprofen, mefenamic acid, marazone, naproxen, nofopam, nifenazone, paracetamol, phenazone, piroxican, salicylamide, sulindac, tolmetin, zomepirac [6-10] etc.

Table 1: Classification of analgesics

S. No.	Narcotic (Opioids)	Non-narcotic (non-opioids)
1.	It relieves pain by acting directly on CNS.	They are principally analgesic and also has anti-inflammatory actions.
2.	They bind to the opioid receptors which are present in many regions of the nervous system and are involved in pain and are classified under controlled substance.	They do not bind to opioids receptors and are not classified under controlled substance.
3.	It can cause addiction to an individual.	It does not cause addiction to an individual.
4.	It produces CNS effect.	It does not produce CNS effect.
5.	It has no anti-inflammatory effect.	It has anti-inflammatory effect.
6.	Examples are butorphanol, dextropropoxyphene etc	Examples are Aspirin, ibuprofen, acetaminophen etc

- **Acetanilide**

Libermann's Test

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

- **Aletamine**

Libermann's Test

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

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 Aletamine.

- **Alphaprodine**

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. Colour changes from brown to red are observed which indicates the presence of Alphaprodine.

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. Red colour is observed which indicates the presence of Alphaprodine.

- **Aminophenazone**

Ferric Chloride Test

1. One to two ml of extract is taken in a test tube.
2. Few drops of ferric chloride are added to it.
3. Bluish violet colour is observed which indicates the presence of aminophenazone.

Libermann's Test

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

Nitrous Acid Test

1. One to two ml of extract is taken in test tube.
2. Solid sodium nitrite is added in one to two ml of

water.

3. Few drops of hydrochloric acid are added to it.
4. Violet colour is observed which indicates the presence of aminophenazone.

- **Aspirin**

McNally's Test

1. One to two ml of extract in acetone is taken in test tube.
2. One to two ml of water is added to it.
3. Two to three drops of copper sulfate solution is added.
4. Few drops of sodium nitrite solution are added.
5. Red colour is observed which indicates the presence of aspirin.

Jorrisen's Test

1. One to two ml of extract is taken in a test tube.
2. Two ml of distilled water is added to it.
3. Few drops of 10 % potassium nitrite followed by few drops of glacial acetic acid are added to it.
4. Few drops of 10 % copper sulphate solution are added.
5. Red colour is observed which indicates the presence of aspirin.

- **Azapropazone**

Libermann's Test

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

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. Greyish green colour is observed which indicates the presence of azapropazone.

- **Benorilate**

Libermann's Test

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

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 colour is observed which indicates the presence of benorilate.

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 benorilate.

- **Butorphanol**

Libermann's Test

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

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. Grey colour is observed which indicates the presence of butorphanol.

- **Cinchnophen**

Libermann's Test

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

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 cinchnophen.

Sulphuric Acid TEST

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

- **Dextromoramide**

Libermann's Test

1. One to two ml of extract is taken in test tube.
2. Few drops of libermann's reagent are added to it.
3. The solution is heated at 100°C for few minutes.
4. Green colour is observed which indicates the presence of dextromoramide.

- **Dextropropoxyphene**

Libermann's Test

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

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 colour is observed which indicates the presence of dextropropoxyphene.

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. Colour changes to black to violet and finally to green colour is observed which indicates the presence of dextropropoxyphene.

- **Diclofenac**

Libermann's Test

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

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 diclofenac.

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. A colour change slowly to green is observed which indicates the presence of diclofenac.

- **Diflunisal**

Ferric Chloride Test

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

McNally's Test

1. One to two ml of extract in acetone is taken in test tube.
2. One to two ml of water is added to it.
3. Two to three drops of copper sulfate solution is added.
4. Few drops of sodium nitrite solution are added.
5. Violet colour is observed which indicates the presence of diflunisal.

Folin-Ciocalteau Test

1. Two ml of extract is taken in a test tube.
2. Few drops Folin-ciocalteau reagent followed by purified water are added to it.
3. One ml of sodium hydroxide solution is added to it.
4. The solution is vortex for 5 seconds.
5. Blue colour is observed which indicates the presence of diflunisal.

- **Dipyrone**

Libermann's Test

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

3. The solution is heated at 100°C for few minutes.
4. Blue colour is observed which indicates the presence of dipyrone.

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 dipyrone.

Nitrous Acid Test

1. One to two ml of extract is taken in test tube.
2. Solid sodium nitrite is added in one to two ml of water.
3. Few drops of hydrochloric acid are added to it.
4. Transient blue colour is observed which indicates the presence of dipyrone.

Ferric Chloride Test

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

Folin-Ciocalteau Test

1. Two ml of extract is taken in a test tube.
2. Few drops Folin-ciocalteau reagent followed by purified water are added to it.
3. One ml of sodium hydroxide solution is added to it.
4. The solution is vortex for 5 seconds.
5. Blue colour is observed which indicates the presence of dipyrone.

- **Etenzamide**

Libermann's Test

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

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 colour is observed which indicates the presence of etenzamide.

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. Red colour is observed which indicates the presence of etenzamide.

Nessler's Test

1. Two to three drops of extract is taken in a porcelain basin.
2. Two to three drops of nessler's reagent is added to it.
3. Agitate & heat the mixture to 100! in water bath.
4. Orange to brown colour is observed which indicates the presence of etenzamide.

- **Fenbufen**

Libermann's Test

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

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 fenbufen.

- **Fenoprofen**

Libermann's Test

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

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. Pink colour is observed which indicates the presence of fenoprofen.

- **Feprazone**

P-Dimethylaminobenzaldehyde

1. Two ml of extract is taken in test tube.
2. Few drops of p-dimethylaminobenzaldehyde reagent are added to it.
3. Red colour is observed which indicates the presence of feprazone.

Libermann's Test

1. One to two ml of extract is taken in test tube.
2. Few drops of libermann's reagent are added to it.
3. The solution is heated at 100°C for few minutes.
4. Brown colour is observed which indicates the presence of feprazone.

- **Ibuprofen**

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. The solution is heated at 100°C for few minutes.
4. Orange colour is observed which indicates the presence of ibuprofen.

Libermann's Test

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

- **Indometacin**

Libermann's Test

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

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 indometacin.

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 colour is observed which indicates the presence of indometacin.

- **Ketoprofen**

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. 10 µl of pyrrolidine is added to it.
4. Mixture is agitated for 2 mins.
5. Violet colour is observed which shows the presence of ketoprofen.

- **Mefenamic Acid**

Libermann's Test

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

- **Morazone**

Libermann's Test

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

- **Naproxen**

Libermann's Test

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

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 naproxen

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. The solution is heated at 100°C for few minutes.
4. Brown colour is observed which indicates the presence of naproxen.

- **Nefopam**

Libermann's Test

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

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 nefopam.

- **Nifenazone**

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. Orange colour is observed which indicates the presence of nifenazone.

Ferric Chloride Test

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

Libermann's Test

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

- **Paracetamol**

Ferric Chloride Test

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

Libermann's Test

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

Nessler's Test

1. Two to three drops of extract is taken in a porcelain basin.
2. Two to three drops of nessler's reagent is added to it.
3. Agitate & heat the mixture to 100! in water bath.
4. Orange to brown colour is observed which indicates the presence of paracetamol.

Folin-Ciocalteau Test

1. Two ml of extract is taken in a test tube.
2. Few drops Folin-ciocalteau reagent followed by purified water are added to it.
3. One ml of sodium hydroxide solution is added to it.
4. The solution is vortex for 5 seconds.
5. Blue colour is observed which indicates the presence of paracetamol.

- **Phenazone**

P-Dimethylaminobenzaldehyde

1. Two ml of extract is taken in test tube.
2. Few drops of p-dimethylaminobenzaldehyde reagent are added to it.
3. Red colour is observed which indicates the presence of phenazone.

Ferric Chloride Test

1. One to two ml of extract is taken in a test tube.

2. Few drops of ferric chloride are added to it.
3. Red colour is observed which indicates the presence of phenazone.

Nitrous Acid Test

1. One to two ml of extract is taken in test tube.
2. Solid sodium nitrite is added in one to two ml of water.
3. Few drops of hydrochloric acid are added to it.
4. Green colour is observed which indicates the presence of phenazone.

- **Piroxican**

Libermann's Test

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

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. 10 μl of pyrrolidine is added to it.
4. Mixture is agitated for 2 mins.
5. Orange colour is observed which shows the presence of piroxican.

- **Salicylamide**

McNally's Test

1. One to two ml of extract in acetone is taken in test tube.
2. One to two ml of water is added to it.
3. Two to three drops of copper sulfate solution is added.
4. Few drops of sodium nitrite solution are added.
5. Orange colour is observed which indicates the presence of salicylamide.

Nessler's Test

1. Two to three drops of extract is taken in a porcelain basin.
2. Two to three drops of nessler's reagent is added to it.
3. Agitate & heat the mixture to 100! in water bath.
4. Brown to orange colour is observed which indicates the presence of salicylamide.

Ferric Chloride Test

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

- **Sulindac**

Libermann's Test

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

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. Brown colour is observed which indicates the presence of sulindac.

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. The solution is heated at 100°C for few minutes.
4. Green colour is observed which indicates the presence of sulindac.

- **Tolmetin**

Formaldehyde-Sulphuric Acid Test

1. Two ml of extract is taken in test tube.
2. Few drops of Formaldehyde-sulphuric acid (6 drops: 4 drops) reagent are added to it.
3. Brown colour is observed which changes to red indicate the presence of tolmetin.

Libermann's Test

1. One to two ml of extract is taken in test tube.

2. Few drops of libermann's reagent are added to it.
3. Red colour is observed which indicates the presence of tolmetin.

- **Zomepirac**

Formaldehyde-Sulphuric Acid Test

1. Two ml of extract is taken in test tube.
2. Few drops of Formaldehyde-sulphuric acid (6 drops: 4 drops) reagent are added to it.
3. Red colour is observed which indicates the presence of zomepirac

Libermann's Test

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

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. The solution is heated at 100°C for few minutes.
4. Yellow colour is observed which indicates the presence of zomepirac.

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 zomepirac.

Conclusion

In any analysis of poison, screening/spot test is very useful for knowing the presence of the anlagesics which can be confirmed by confirmatory tests. It saves time for the toxicologist in ruling out the poisons. The result of the analytical methods depends on the amount and purity of the sample extracted. Screening/spot test has been developed after repeated trial and testing. The techniques are being improved every time. It is important for the forensic toxicologists to know the best available method so as to help them in detecting the type of poison administered in the process of crime investigations.

- *Preparation of Solutions/ Reagents*

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.

Ferric Chloride Solution

10 gm of ferric chloride is dissolved in 100 ml distilled water.

Folin-Ciocalteau Reagent

100 gm of sodium tungstate and 25 gm of sodium molybdate are mixed in 800 ml of water. 50 ml of concentrated orthophosphoric acid and 100 ml of concentrated hydrochloric acid are added and refluxed for 10 hours. After cooling 150 g of lithium sulfate, 50 ml of water and 0.5 ml of elemental bromine are added and allowed to stand for 2 hours and then boiled for 15 minutes to remove excess bromine. Content is cooled and filtered if necessary, and diluted to 1 litre with water. This solution is yellow and could be stable for 4 months if stored at 4°C.

Formaldehyde-Sulphuric Acid

Four volumes of sulphuric acid and six volumes of formaldehyde solution are mixed.

Koppanyi- Zwikker reagent

A 1% (w/v) solution of cobalt nitrate in ethanol.

Libermann'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.

Mandelin's Reagent

1 gm of ammonium vanadate is dissolved in 1.5 ml of water and diluted to 100 ml with concentrated sulphuric acid.

Marquis Reagent

100 ml of concentrated sulphuric acid is mixed

with 1 ml of 40% (v/v) formaldehyde solution.

McNally's Reagent

Solution (I) (0.5 % solution of copper sulphate in 10 % acetic acid. Solution

Solution(II) Freshly prepared 2% solution of sodium nitrite.

Solution (I) is mixed with solution (II).

Nessler's Reagent

Solution (I) 50 gm of mercuric chloride and 35 g of potassium iodide are dissolved in 200 ml of water and cool.

Solution (II) 50 gm of sodium hydroxide is dissolved in 250 ml of water and cool.

Cold solution (II) is mixed with cold solution (I) and made up to 500 ml with water. Mixture is allowed to stand and decanted the clear supernatant for use. Stored in dark brown bottles away from light.

Nitrous Acid

Small amount of solid sodium nitrite is added to 2M hydrochloric acid.

P-Dimethylaminobenzaldehyde

1 g of p-dimethylaminobenzaldehyde is dissolved in 100 ml of ethanol. The solution is acidified with 10 ml of dilute hydrochloric acid.

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