

# TEST YOURSELF – DETECT ADULTERATION IN MILK

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Although many known methods for detection of adulteration in milk, exists, the methods compiled below are not only simple and rapid but also very sensitive to detect milk adulteration. These tests can be carried out easily by consumers using simple laboratory apparatus, common chemicals and the milk adulteration test reagent kit developed. The testing protocol is as given below.

### REAGENTS REQUIRED.

1. Concentrated Hydrochloric acid. (1:1)
2. Concentrated Sulphuric acid. (1:1)
3. Concentrated Nitric acid. (1:1)
4. Citric acid. (Concentrated Solution)
5. Ammonia Solution: (1:1)
6. Phosphomolybdic acid. 1% (w/v) water.
7. Resorcinol (White flakes)
8. (N/10) hydrochloric acid standard.
9. Rosolic Acid: 1% (w/v) in alcohol.
10. Phenolphthalein Indicator: 1% (w/v) alcohol.
11. Paraphenylene diamine indicator: 1% (w/v) alcohol.
12. Iodine solution: 1% iodine in 10% Potassium Iodide Solution (w/v)
13. Vanadium Pentoxide Reagent: 1% (w/v) in 6% (w/v) sulphuric acid solution.
14. Barford Reagent: Dissolve 24 gm of Copper acetate in 450 ml of boiling distilled water. Add 25 ml of 8.5 % (w/v) acetic acid solution, shake, cool to room temperature and make upto 500 ml. After sedimentation filter the reagent and store in dark coloured bottle.
15. Para-dimethyl amino benzaldehyde reagent: 16% (w/v) in 10% (w/v) hydrochloric acid.
16. Urease solution: (20 mg / ml).
17. Bromothymol blue solution: 0.5% (w/v) in water.
18. Barium Chloride: 5% (w/v) solution in water.
19. Sodium hydroxide: 2% (w/v) solution in water.
20. Sodium hypochlorite: 2% (w/v) solution in water.
21. Phenol solution: 5% (w/v) solution in water.
22. Silver nitrate reagent : 0.8% (w/v) in water
23. Potassium dichromate: 1% (w/v) in water.
24. Bromocresol purple solution: 0.5% (w/v) in water.
25. Ferric Chloride: 0.5% solution (w/v) in water.
26. Turmeric Paper.
27. Lactometer, test tubes, droppers, gas burner, measuring cylinders, beakers, bottles and other simple laboratory equipments.

### I. DETECTION OF NEUTRALIZERS IN MILK

Prohibited neutralizers like hydrated lime, sodium hydroxide, sodium carbonate or sodium bicarbonate are added to milk to prevent spoilage.

#### Rosolic acid test (Soda Test)

- Take 5 ml of milk in a test tube and add 5 ml alcohol followed by 2-3 drops of rosolic acid. If the colour of milk changes to pinkish red, it is inferred that the milk is adulterated with sodium carbonate / sodium bicarbonate and so unfit for human consumption. (Please note that this test will be effective only if the neutralizers are present in milk. In case the added neutralizers get

nullified by the naturally developed acidity in milk, then this test will be negative and one needs to test, the alkaline condition of the milk for the presence of soda ash.)

### **Alkalinity Test**

- Take 20 ml of milk in a silica crucible and evaporate the water. The contents are then burnt in a muffle furnace at 550°C. The ash is dispersed in 10 ml distilled water and titrated against decinormal (N/10) hydrochloric acid using phenolphthalein indicator. If the titre value exceeds 1.2 ml, it can be construed that the milk is adulterated with neutralizers.

### **II. TEST FOR DETECTION OF HYDROGEN PEROXIDE**

- Take 5 ml milk in a test tube. Add 3 drops of paraphenylene diamine and shake well. Change in colour of the milk to blue confirms that the milk is adulterated with hydrogen peroxide.
- To 10 ml of milk sample in a test tube add 10-15 drops of Vanadium Pentoxide reagent and mix. The development of pink or red colour indicates presence of hydrogen peroxide.

### **III. TEST FOR DETECTION OF FORMALIN**

Formalin (40%) although poisonous, can preserve milk for a long time.

- Take 10 ml of milk in a test tube. Add 5 ml conc. sulphuric acid through the sides of the test tube without shaking. If a violet or blue ring appears at the intersection of the two layers, it shows the presence of formalin. Note violet coloration usually does not appear when relatively large quantities of formaldehyde are present.

### **IV. TEST FOR DETECTION OF CANE SUGAR IN MILK**

Generally cane sugar is mixed in milk to increase the percentage solids content of milk i.e., to increase the lactometer reading of milk, that was already diluted with water.

- Take 10 ml of milk in a test tube. Add 5 ml of hydrochloric acid along with 0.1 g of resorcinol. Shake the test tube well and place it in a boiling water bath for 5 min. Appearance of red colour indicates the presence of added cane sugar in milk.

### **V. TEST FOR DETECTION OF STARCH**

Addition of starch increases the SNF content of milk. Wheat flour, arrowroot, rice flour, etc., can also be added for increasing the SNF content.

- Take 3 ml milk in a test tube and boil it thoroughly. Cool the milk to room temperature. Add 2 to 3 drops of 1% iodine solution. Change of colour to blue indicates that the milk is adulterated with starch.

### **VI. TEST FOR DETECTION OF GLUCOSE**

Poor quality glucose is sometimes added to milk to increase the lactometer reading.

- Take 3 ml of milk in a test tube. Add 3 ml Barford's reagent and mix it thoroughly. Keep the test tube in a boiling water bath for 3 min and then cool it for 2 min by immersing it in tap water without disturbance. Add 1 ml of phosphomolybdic acid and shake. If blue colour is visible, then glucose is present in the milk sample.

## **VII. TEST FOR DETECTION OF UREA**

Urea is generally added in the preparation of synthetic milk to raise the SNF value.

- 5 ml of milk is mixed well with 5 ml paradimethyl amino benzaldehyde reagent. If the solution turns distinct yellow in colour, then the given sample of milk contains urea. Control, normal milk may show a faint yellow colour due to presence of natural urea.
- Take 5 ml of milk in a test tube. Add 0.2 ml of fresh urease (20 mg / ml). Shake well at room temperature. Add 0.1 ml of bromothymol blue solution. Appearance of blue colour after 10 – 15 min indicates the adulteration milk with urea.

## **VIII. TEST FOR DETECTION OF AMMONIUM SULPHATE**

The presence of sulphate in milk increases the lactometer reading.

- 5 ml of hot milk is taken in a test tube. A suitable acid for e.g. citric acid is added and the whey obtained is separated and filtered. The whey is taken in another test tube and 0.5 ml of 5% barium chloride is added. Appearance of precipitate indicates the presence of ammonium sulphate.
- Take 5 ml of milk add 2.5 ml of 2% sodium hydroxide, 2.5 ml of 2% sodium hypochlorite and 2.5 ml of 5% phenol solution. Heat for 20 seconds in boiling water bath. If bluish colour turns to deep blue it indicates the presence of ammonium sulphate, however in case it turns to pink it shows that the sample is free from Ammonium sulphate.

## **IX. TEST FOR DETECTION OF SALT**

Addition of salt in milk is mainly resorted to with the aim of increasing the corrected lactometer reading.

- 5 ml of silver nitrate reagent is taken in a test tube. Add 2-3 drops of potassium dichromate reagent. Add 1 ml of milk in the above test tube and mix thoroughly. If the contents of the test tube turn yellow in colour, then milk contains salt. If it turns to chocolate or reddish brown in colour, the milk sample is free from salt.

## **X. TEST FOR DETECTION OF PULVERIZED SOAP**

- Take 10 ml of milk in a test tube and dilute it with equal quantity of hot water. Add 1 – 2 drops of phenolphthalein indicator. Development of pink colour indicates that the milk is adulterated with soap.

## **XI. DETECTION OF DETERGENTS IN MILK**

- Take 5 ml of milk in a test tube and add 1-2 drops of bromocresol purple solution. Mix well. Appearance of violet colour indicates the presence of detergent in milk. Unadulterated milk samples will show a very faint violet colouration.

## **XII. DETECTION OF WATER IN MILK**

Lactometer reading detects adulteration of milk with water.

- Take raw milk in a long stemmed wide mouth bottle or a measuring cylinder. Place the lactometer in it taking care to see that the lactometer does not touch the sides of the bottle or the measuring cylinder. Note down the reading at the surface of milk sample taken. Also note the temperature of the milk sample.

Though the adulteration of milk with water can be checked by lactometer reading, other adulterations too affect the lactometer reading. Hence freezing point depression, recognized by AOAC, is usually adopted.

$$\text{Percentage of water added} = \frac{\text{Normal freezing point} - \text{Observed freezing point}}{\text{Normal freezing point}} \times 100$$

Normal freezing point of milk is taken as  $-0.55^{\circ}\text{C}$ . A tolerance level of 3% is given which is equivalent to specifying a minimum freezing point depression for authentic milk of  $-0.55^{\circ}\text{C}$ .

### **XIII. DETECTION OF SKIM MILK POWDER IN MILK**

- If the addition of nitric acid drop by drop in to the test milk sample results in the development of orange colour, it indicates the milk is adulterated with skim milk powder. Samples with out skim milk powder shows yellow colour.

### **XIV. DETECTION OF BENZOIC AND SALICYLIC ACID IN MILK**

- Take 5 ml of milk in a test tube. Add 3-4 drops of concentrated sulphuric acid. Add 0.5% ferric chloride solution drop by drop and mix well. Development of buff colour indicates presence of benzoic acid and violet colour indicates presence of salicylic acid.

### **XV. DETECTION OF BORAX AND BORIC ACID IN MILK**

- Take 5 ml milk in a test tube. Add 1 ml of concentrated hydrochloric acid and mix well. Dip the tip of turmeric paper into the acidified milk and dry in a watch glass at  $100^{\circ}\text{C}$  or over a small flame. If the turmeric paper turns red, it indicates the presence of borax or boric acid. Add a drop of ammonia solution on the turmeric paper and if the red colour changes to green, it confirms the presence of boric acid.

**The following two adulteration tests are difficult to carry out by regular consumers as they require sophisticated equipments and so can be conducted only by qualified analysts. It is mentioned here in as additional information and for understanding.**

### **XVI. DETECTION OF VEGETABLE FAT IN MILK**

- The characteristic feature of milk is in its fatty acid composition, which mainly consists of short chain fatty acids such as butyric, caproic, caprylic acid; whereas the vegetable fats consist mainly of long chain fatty acids and hence adulteration of vegetable fat in milk can be easily found out by analyzing the fatty acid profile by gas chromatography.

### **XVII. DETECTION OF BUFFALO MILK IN COW MILK**

- The presence of buffalo milk in cow milk is tested by Hansa test. It is based on immunological assay. One ml of milk is diluted with 4 ml of water. It is then treated with 1 ml of antiserum. The characteristic precipitation reaction indicates the presence of buffalo milk in the sample taken. (The antiserum is developed by injecting buffalo milk proteins into rabbits).