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.

Consumers can easily carry out these tests using simple laboratory apparatus, common chemicals available in the market. CGSI's milk adulteration testing kit (launched in 2006) uses these methods.

Please find below the method to make the milk adulteration testing reagent kit and testing protocol.

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. Para phenylene diamine: 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 up to 500 ml. After sedimentation filter the reagent and store in dark colored 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: 0.5% (w/v) in water.
18. Barium Chloride: 5% (w/v) in water.
19. Sodium hydroxide: 2% (w/v) in water.
20. Sodium hypochlorite: 2% (w/v) 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: 0.5% (w/v) in water.
25. Ferric Chloride: 0.5% (w/v) in water.
26. Turmeric Paper.
27. Lactometer, test tubes, droppers, gas burner, measuring cylinders, beakers, bottles, etc.

I. DETECTION OF NEUTRALIZERS

Adulterators add prohibited neutralizers like hydrated lime, sodium hydroxide, sodium carbonate or sodium bicarbonate 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 color of milk changes to pinkish red, we infer adulteration of milk 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 nullify due the naturally developed acidity in milk, then this test will be negative. One needs to test, the alkalinity of milk for presence of soda ash.)

Alkalinity Test

Take 20 ml of milk in a silica crucible and evaporate the water. Burn the contents in a muffle furnace at 550°C. Disperse the ash in 10 ml distilled water and titrate it against decinormal (N/10) hydrochloric acid using phenolphthalein indicator. If the titer value exceeds 1.2 ml, it can conclude that the milk contains neutralizers and adulterated.

II. TEST FOR DETECTION OF HYDROGEN PEROXIDE

- Take 5 ml milk in a test tube. Add 3 drops of Para phenylene diamine and shake well. Change in color of the milk to blue confirms adulteration of milk 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 color indicates presence of hydrogen peroxide.

III. TEST FOR DETECTION OF FORMALIN

Poisonous Formalin (40%) preserves milk for a long time.

Take 10 ml of milk in a test tube. Add 5 ml conc. sulphuric acid (containing traces of Ferric

Chloride) 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, mixing cane sugar in milk increases the percentage solids content of milk i.e., to increase the lactometer reading of milk, 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 color indicates the presence of added cane sugar.

V. TEST FOR DETECTION OF STARCH

Addition of starch increases the SNF (Solid–Not–Fat) content of milk. Adding wheat flour, arrowroot, rice flour, etc., also increases SNF.
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 color to blue indicates addition of starch and adulteration of milk with starch.

VI. TEST FOR DETECTION OF GLUCOSE

Adding (poor quality) glucose as an adulterant to milk increases 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 color is visible, then glucose is present in milk sample.

VII. TEST FOR DETECTION OF UREA

Adulterators add urea in the preparation of synthetic milk to raise the SNF value.

- Mix 5 ml of milk with 5 ml Para dimethyl amino benzaldehyde reagent. If the solution turns distinct yellow in color, then the given sample of milk contains urea. Control, normal milk may show a faint yellow color 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 color 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.

- Take 5 ml of hot milk in a test tube. Add a suitable acid for e.g. citric acid and separate the whey obtained by filtration. Take the whey in another test tube and add 0.5 ml of 5% barium chloride. 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 color 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 increase the corrected lactometer reading.

Take 5 ml of silver nitrate reagent 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 color, then milk contains salt. If it turns to chocolate or reddish brown in color, the milk sample is free from salt.

X. TEST FOR DETECTION OF 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 color indicates soap adulteration of milk.

XI. TEST 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 color indicates the presence

of detergent in milk. Unadulterated milk samples will show a very faint violet coloration.

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.

Although we can check the adulteration of milk with water by lactometer reading, other adulterations above too affect the lactometer reading. Hence, we usually adopt the freezing point (FP) depression method, recognized by AOAC as a confirmatory test.

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

Normal freezing point of milk is – 0.55°C with a tolerance level of 3%, i.e., equivalent to specifying a minimum freezing point depression for authentic milk of – 0.55°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 color, it indicates adulteration of milk with skim milk powder. Samples without skim milk powder shows yellow color.

XIV. TEST FOR DETECTION OF BENZOIC AND SALICYLIC ACID

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 color indicates presence of benzoic acid and violet color 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°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 color 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 equipment's and only an expert analyst can conduct the same. We mention it here only for 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

Hansa test detects the presence of buffalo milk in cow milk. It is based on immunological assay. Dilute 1 ml of milk with 4 ml of water and then treat it with 1 ml of antiserum. The characteristic precipitation reaction indicates the presence of buffalo milk in the sample taken. (We develop the antiserum by injecting buffalo milk proteins into rabbits).