BHARATHIDASAN UNIVERSITY
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TAMIL NADU,INDIA
Programme : M.Sc., Biochemistry

Course Title : DETECTION METHODS OF FOOD ADULTERATION

Couse Code :BC002VAC

UNIT-I

Introduction to Bio business

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INTRODUCTION

•**Definition of Milk Adulteration**:
• The addition of harmful or cheaper substances to milk to increase quantity or reduce cost.

• **Common Adulterants**:
• Water, detergent, starch, synthetic milk, etc.

• **Importance of detecting adulteration**:
• Ensures consumer safety, maintains quality, and meets health standards.
• **DETERGENT TEST**
•**Purpose**:
• Detects the presence of detergents, commonly used in adulterating milk.
•**Procedure**:
1.Take a sample of milk in a transparent glass container.
2.Add a few drops of vinegar or acetic acid to the milk.
3.Stir the mixture gently.
4.Observe for foaming or lather formation.
•**Interpretation**:
➢ If foam or lather appears, it indicates the presence of detergent.
➢ No foam suggests clean mil

FILTER TEST

•**Purpose**:
• Detects the presence of starch or other adulterants.
•**Procedure**:
1.Place a piece of white filter paper on a clean surface.
2.Pour a small amount of milk onto the filter paper.
3.Wait for a few minutes for the milk to filter.
4.Observe the filter paper for residue.
•**Interpretation**:

- If a sticky or starchy residue is left behind, it suggests starch or other adulterants.
- Clean milk should leave no residue.
- **FLOW TEST**

- **Purpose**:

  Detects the presence of water in milk.

- **Procedure**:

  1.Hold the milk sample at an angle.

  2.Observe the milk's flow from the container.

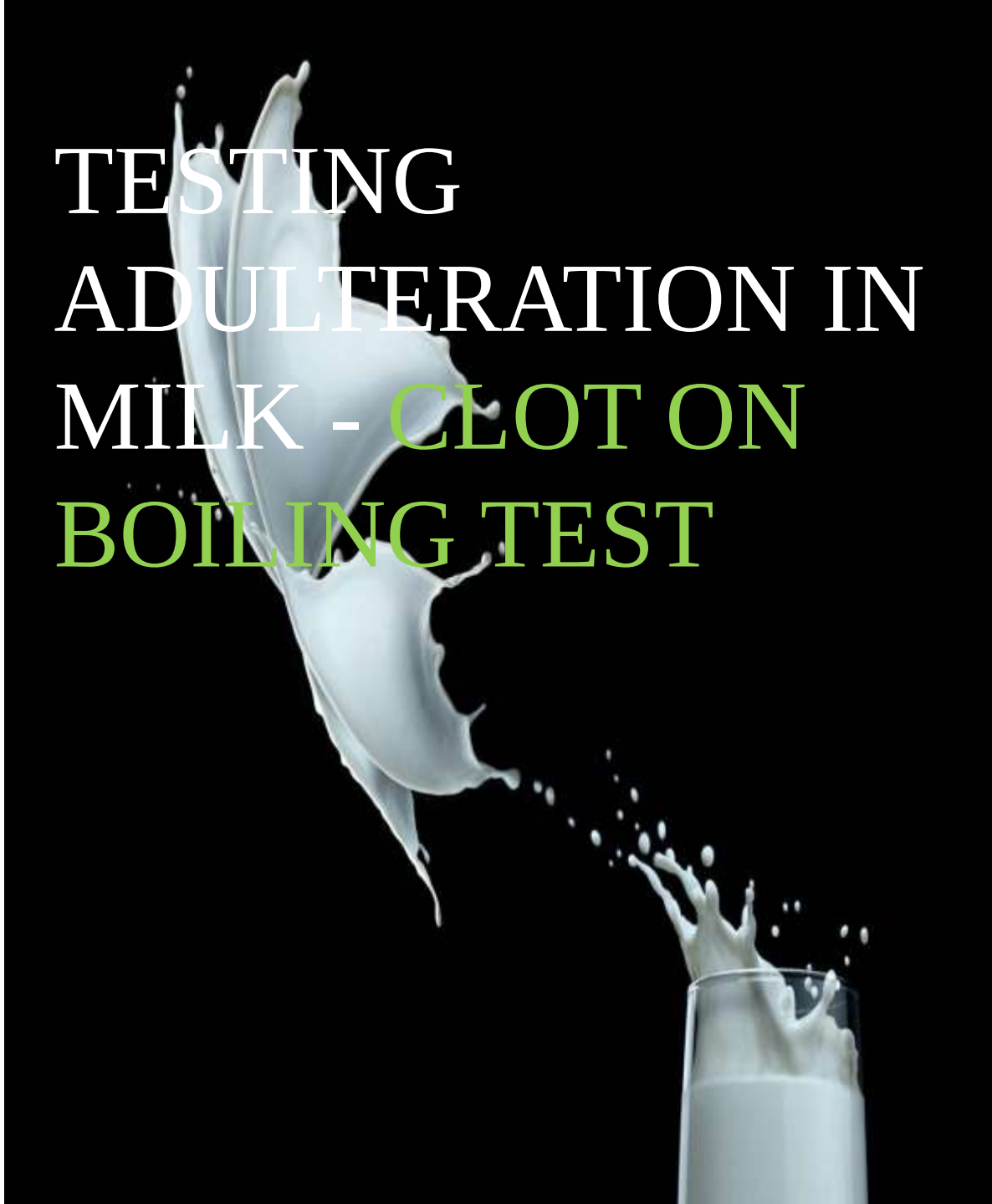
- **Interpretation**:
  - If the milk flows too quickly or looks watery, it indicates the presence of water, a common adulterant.
  - Clean milk should flow at a slower, thicker rate.

INTRODUCTION

The “clot on boiling test” (COB) is a test done with an inspection of the warmth stability of milk through processing. As raw milk cannot be kept at standard room temperature or even at lower temperature without preventing it from spoilage, pasteurization is needed. Hence, COB is necessary to understand whether pasteurized will be stable for additional and further processing.

CLOT ON BOILING (COB) TEST

The clot on boiling in the test if milk is retained as more at the room temperature, in there it will be enhanced on the acidity in which that is called on developed acidity. It is the acidity that is enhanced in more than 0.2%, there are coagulation due to the heat treatment, and in which the result of separation on “calcium caseinate salt”. That is why, it is essential within a heat stability on the arrival of raw milk for further clarification. So they refer to the testing on milk with an enhanced acidity on a boiling substance.



TESTING ADULTERATION IN MILK - CLOT ON BOILING TEST

IN SHORT

Clot on Boiling Test :

Apparatus:

Test Tubes, Spirit Lamp

Principle:

To detect in a rapid manner the presence of extent of developed acidity, which might render the milk unsuitable for processing and distribution.

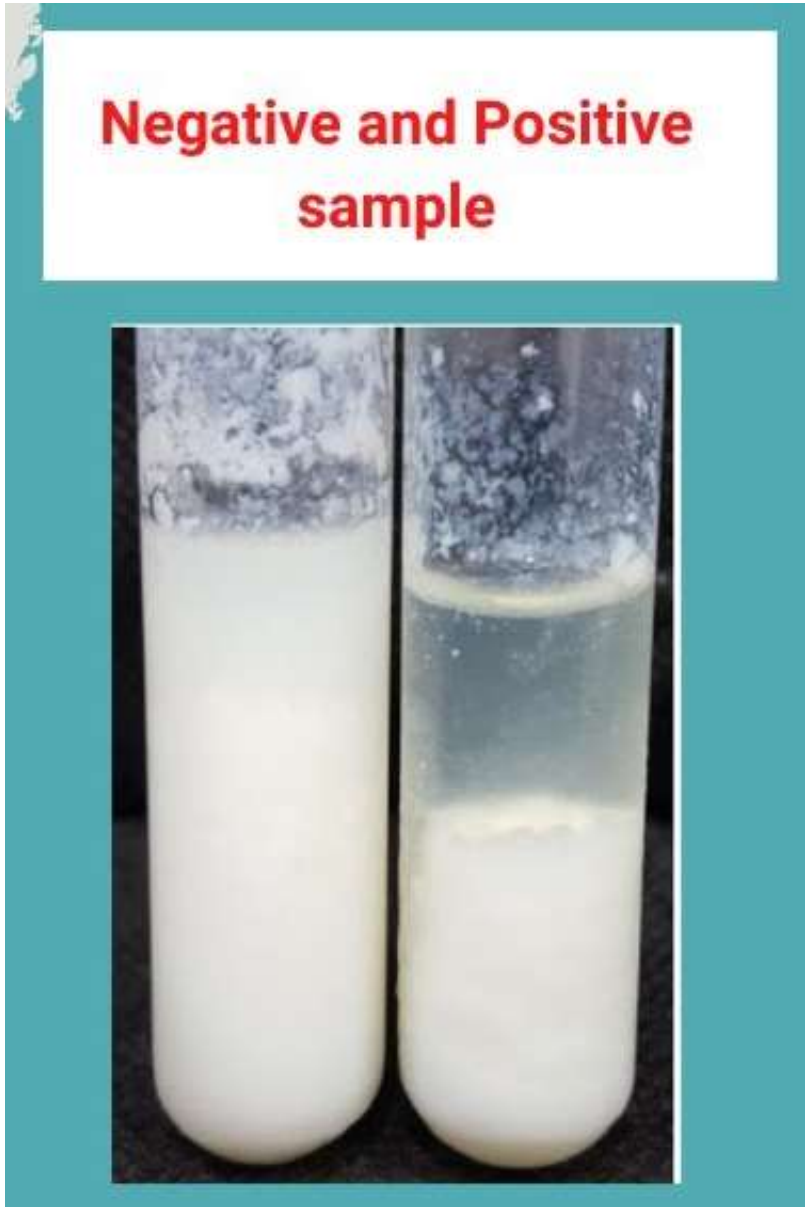
Procedure:

Conduct COB test by taking about 5 ml of milk in a test tube, boil on the flame of a spirit lamp.

Observation:

Formation of clots in the test tube indicates COB positive milk and is unacceptable.

OBSERVATION

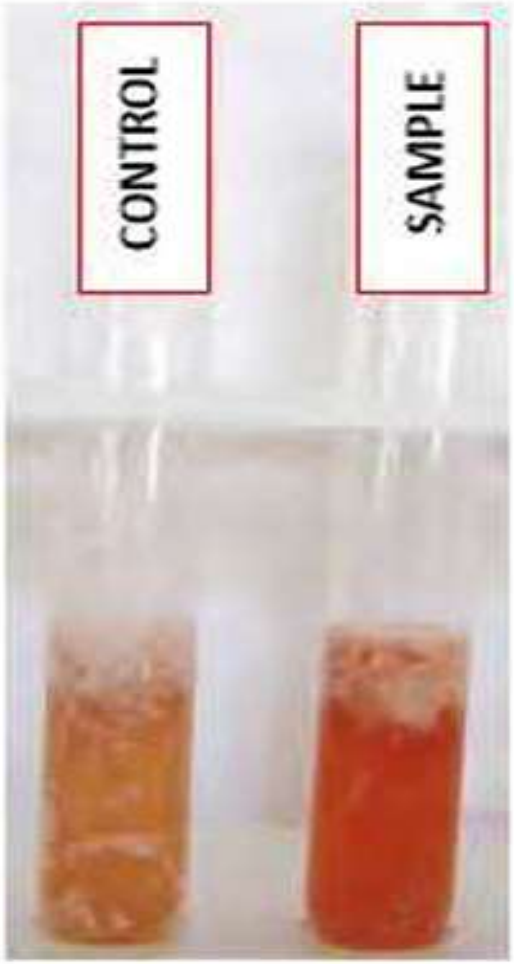


INTRODUCTION

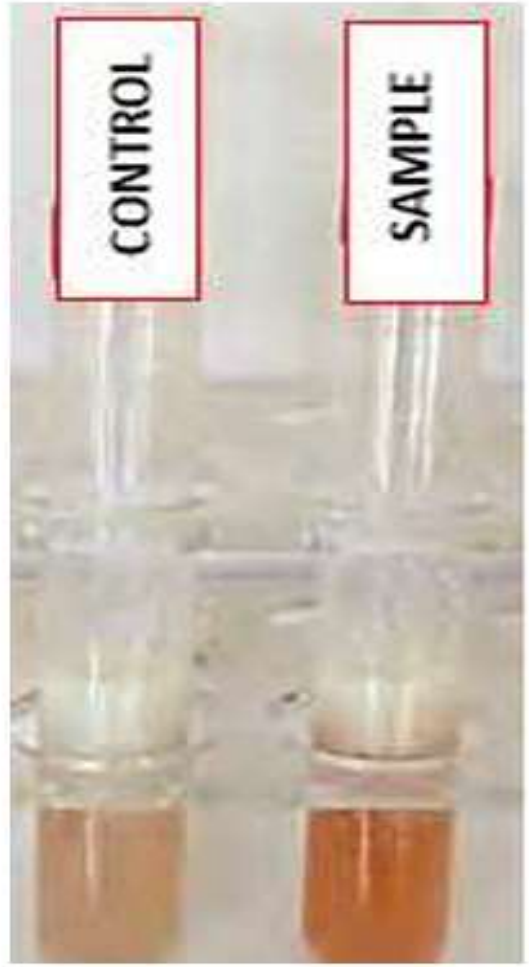
TEST FOR CANE SUGAR IN MILK AND TEST FOR BUFFALO MILK IN COW MILK

- Milk contains several vital nutrients, including proteins, carbohydrates, fats, minerals, vitamins and several biologically active substances which is vital for human to build and sustain a strong and healthy body
- Hence value and price of milk products and rising prosperity, consumption of milk and milk products is growing steadily in India.
- Sucrose is absent in milk and its presence in milk indicate adulteration.
- Adulteration of milk is a serious problem faced by purchasers, processors, sellers and consumers of milk and milk products
- Qualitative tests used for detection of common adulterants in milk possesses several advantages like better specificity, better sensitivity, better reliability and affordability

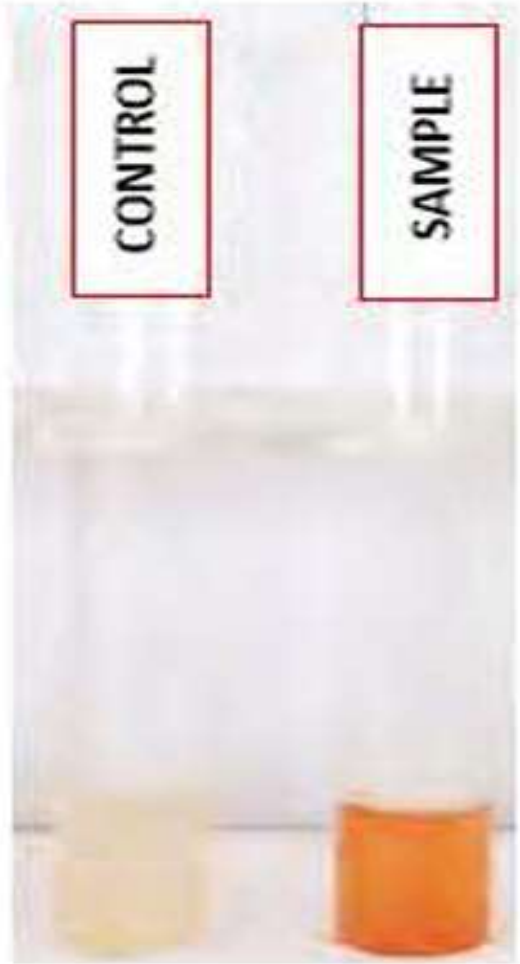
- Sucrose is a disaccharide containing glucose and fructose [a ketose sugar]
- Seliwanoff test is used for detection of ketoses
- The dilute hydrochloric acid used in Seliwanoff reagent along with heat leads to hydrolysis of sucrose and subsequent dehydration of fructose
- Further keto group more actively attacks resorcinol in comparison to aldehyde group
- The dehydration product 5-hydroxymethylfurfural [HMF] condenses with resorcinol forming cherry red colour
- Ketoses react rapidly in comparison to aldoses because dehydration of aldoses to 5-hydroxymethylfurfural proceeds in a much slower way than the reaction of ketoses



Seliwanoff Test
Srivastava (2010)



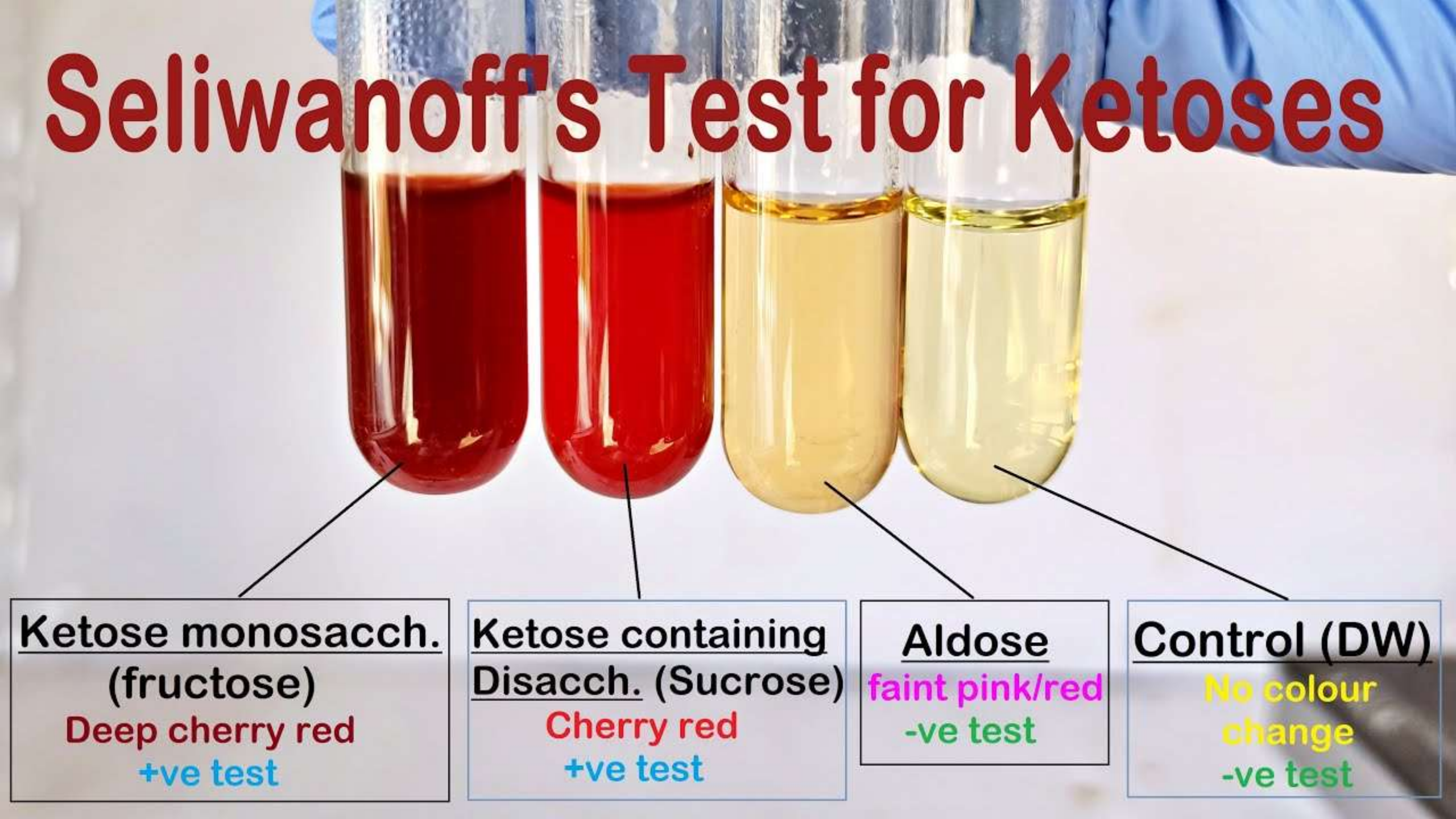
Seliwanoff Test (Milk)
Modified- AAU (2018)



Seliwanoff Test
(Whey)
Modified- AAU (2018)

Fig. 4: Test for Sucrose

Seliwanoff's Test for Ketoses



Ketose monosacch.
(fructose)
Deep cherry red
+ve test

Ketose containing Disacch. (Sucrose)
Cherry red
+ve test

Aldose
faint pink/red
-ve test

Control (DW)
No colour change
-ve test

Rapid PCR Based Detection Of Buffalo Milk in Cow Milk

- 12S rRNA gene in mitochondria is targeted in both cow and buffalo which slightly differs
- for this reverse primer with 220 bp specific for buffalo and 346 bp specific for cow is taken and common forward primer is taken
- firstly, purity of DNA samples tested by OD260:280 [ratio between 1.8 and 2.0 -pure]
- also runed through gel electrophoresis
- then subjected to PCR amplification
- with suitable temperature of 63 degree celcius [mastercycle gradient]
- to evaluate specificity of primers both cow and buffalo specific primers are allowed to pair with respective DNAs

Sr. No.	Cow Milk (ml)	Buffalo Milk (ml)	Total Mix (ml)	Mix %	Remark
1	19.9	0.1	20	0.5%	Buffalo milk mixed in Cow milk
2	19.8	0.2	20	1.0%	
3	18	2	20	10.0%	
4	14	6	20	30.0%	
5	10	10	20	50.0%	
6	10	-	10	100.0%	Pure Cow milk
7	-	10	10	100.0%	Pure Buffalo milk

Table : pure buffalo milk mixed in pure cow milk at different levels

HANSA TEST

- 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)

KIT

- Rapid test kit also commercially available to detect buffalo milk fraud with cow milk