

## Studies on pasteurised milk samples collected from different sources in Visakhapatnam

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### Abstract

A study was conducted to find out the physico chemical & bacteriological characteristics of milk samples in Visakhapatnam district, Andhra Pradesh. The safety of milk is an important attribute for consumers of milk and dairy products. Milk and products derived from milk of dairy cows can harbor a variety of microorganisms and can be important sources of food borne pathogens. The physicochemical assessment of milk is done by performing different tests like P<sup>H</sup>, colour, water test, clotting test, starch test, urea test, sugar test, salt test, formalin test, boric acid test, vanaspathi test, detergent test and ammonium sulphate test etc., the milk samples gave positive to the adulterants. The microbial isolation was done by streak plate method on nutrient agar and on selective media for their identification. The final identification of resulted isolates was done by their biochemical testing mentioned in accordance to the Bergey's Manual. The resulted bacterial isolates viz. *E.coli*, and *Staphylococcus*, are highly pathogenic. Poor quality of milk was recorded as the major risk factor for the dreadful diseases.

Key words: Milk, Adulterants, Quality assessment and Pathogenic bacteria

### Introduction:

Pasteurised milk or mammal milk is a white liquid produced by the mammary glands of mammals. It is the primary source of nutrition for young mammals before they are able to digest other types of food. Early-lactation milk contains colostrum, which carries the mother's antibodies to its young and can reduce the risk of many diseases. Milk contains many other nutrients and the carbohydrate lactose. As an agricultural product, milk is extracted from mammals during or soon after pregnancy and is used as food for humans. Fresh or raw milk as diet contributes to infants to adults in all over the world (**Cousin 1982**).

Worldwide, dairy farms produced about 730 million tonnes of milk in 2011, from 260 million dairy cows. India is the world's largest producer and consumer of milk, yet neither export nor imports it. New Zealand, the European Union's 28 member states, Australia, and the United States are the world's largest exporters of milk and milk products. China and Russia are the world's largest importers of milk and milk products. Throughout the world, there are more than six billion consumers of milk and milk products. Over 750 million people live within dairy farming households. Milk of cattle, buffalo, goat, sheep, camel, yak, llama, mare etc contains almost same but varying concentration of the chemical constituents. Milk differs widely in composition due to different factors including species of animal, breed, individuality, stage of lactation, frequency of milking, age, seasonal variations, feed, interval of milking, disease and abnormal conditions and administrations of drugs and hormones (Ensminger, 1993). Milk is a complex biological fluid and by its nature, a good growth medium for many micro-organisms. Because of the specific production it is impossible to avoid contamination of milk with micro-organisms therefore the microbial content of milk is major feature in determining its quality (Rogelj, 2003). It is hypothesized that differences in feeding and housing strategies of cows may influence the microbial quality of milk (Coorevits et al; 2008). Rinsing water for milking machine and milking equipment washing also involve some of the reasons for the presence of a higher number of micro-organisms including pathogens in raw milk (Bramley, 1990). The aim of the study is to evaluate the level of microbiological contamination of milk samples taken in Visakhapatnam area.

## **MATERIAL AND METHODS**

### **Physiochemical analysis of milk:**

To assess the physical and chemical changes in processed milk samples the following tests were carried out.

#### **pH:**

The pH value of milk was determined by using a digital pH meter. Prior to use, the pH meter was standardized with standard buffer solution of pH 4 and 7.

#### **Clot-on-boiling test:**

Five ml of milk was placed in a test tube and it was placed in a boiling water bath for five minutes. Then, the test tube was carefully removed from the water bath and examined for the presence of floccules.

### **Water:**

The presence of water can be detected by putting a drop of milk on a polished slanting surface. The drop of pure milk flows slowly leaving a white trail behind it, whereas milk adulterated with water will flow immediately without leaving a mark.

### **Starch:**

Add a few drops of tincture of iodine or iodine solution. Formation of blue colour indicates the presence of starch. (Iodine solution is easily available in the medical stores.

### **Urea:**

Take a teaspoon of milk in a test tube. Add  $\frac{1}{2}$  teaspoon of soybean or arhar powder. Mix up the contents thoroughly by shaking the test tube. After 5 minutes, dip a red litmus paper in it. Remove the paper after  $\frac{1}{2}$  a minute. A change in color from red to blue indicates the presence of urea in the milk.

### **Detergent:**

Shake 5-10 ml of sample with an equal amount of water. Lather indicates the presence of detergent.

### **Synthetic Milk test for protein:**

The milk can easily be tested by urease strips (available in the medical store. Color chart of the urease strip test given below will show the quantity of urea present in milk.

### **Test for glucose / Invert Sugar:**

Take a strip of diacetic strip and dip it in the milk for 30 sec – 1min if the strip changes color, than it shows that the sample of milk contains glucose. If there is no change in color of the strip, than glucose is absence.

### **Sugar:**

Take 3 ml of milk in a test tube. Add 2 ml of the hydrochloric acid. Heat the test tube after adding 50 mg of resorcinol. The red colouration indicates use of sugar in the milk.

### **Boric Acid:**

Take 3 ml of milk in a test tube. Add 20 drop of hydrochloric acid and shake the test tube or mix up the content thoroughly. Dip a yellow paper strip, and remove the same after 1 min. A change in color yellow to red, followed by the change from red to green, by addition of 1 drop of ammonia solution, indicates that the boric present in milk.

### **Vanaspathi:**

Take 3 ml of milk in a test tube. Add 10 drops of hydro chloric acid. Mix 1 teaspoon full of sugar. After 5 min , examine the mixture. The red coloration indicates the presence of vanaspathi in the milk.

### **Formalin:**

Take 10ml of milk in a test tube and add 5 ml of concentrated sulphuric acid from the side of the wall without shaking if a violet or blue ring appears at the intersection of two layers than it shows presence of formalin.

### **Ammonia Sulphate:**

Take 5 ml of hot milk in a test tube. Add a suitable acid, Ex: Citric Acid. The Whey obtained is separated & filtered. Take the Whey in another test tube & add 0.5ml of 5% barium chloride. Appearance of participate indicates the presence of ammonium sulphate. Take 5 ml of milk in a test tube. Add 2.5ml of 2% sodium hydroxide, 2.5 ml of 2% sodium hypo chlorite And 2.5 ml of 5% phenol solution. Heat the solution for 20 sec in boiling water bath. If bluish color turns to deep blue , it indicates the presence of ammonium sulphate. However in case it turns pink, it shows that the sample is free from ammonium sulphate.

### **Salt:**

Take 5 ml of silver nitrate reagent in a test tube. Add 2-3 drop 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, then milk contains salt. If it turns to chocolate the pH meter was standardized with standard buffer solution of pH 4 and 7. color or reddish brown, the milk sample is free from salt.

**Colour:** The colour will be observed by appearance of the milk.

**Microbiological Analysis:**

**MILK PHOSPHATE TEST:**

This is a statutory test. The test can be used to assess the quality of milk i.e, the extent of microbial contamination and as a measure for the total inactivation and of the enzyme along with the pathogenic microorganism at 145°F for 30 minutes or 160°F for 15 seconds as in the recognized methods of pasteurization. The presence of the enzyme is detected based on its ability to catalase the liberation of phenol from disodium phenol phosphate. The phenol is estimated Calorimetrically with FC reagent, which yields a blue coloured complex. The amount of phenol liberated is proportional to enzyme present in it which inturn indicates the extent of microbial population which inturn indicates the extent of microbial population.

**Methylene Blue Test (MBRT):**

Methylene blue test for the assessment of mastitis was performed according to procedure described by method followed by Awan, J.A and S.U.Rehman. The test is used to diagnose mastitis, the ability of bacteria to reduce the colour of methylene blue dye from the milk sample. Dye reduction time is inversely proportion to the presence of total number of bacteria in sample, hence greater the bacterial population shorter the dye reduction time.

**Determination of the most probable number of bacteria coliform:**

**(MPN):**

This is done to Milk confirm whether contains lactose-fermenting gas producing bacteria. It is used to determine the most probable number (MPN) of coliforms in a sample of milk besides their properties of fermenting lactose and producing gas. If after inoculation and incubation of lactose-broth, gas is produced, it is presumed that coliforms are present in the sample.

**Total Bacterial Count (TBC):**

Total bacterial count in different milk samples was determined by method followed by Esron et al. Count the microbial population in sample as number of microscopic fields present in 1 cm square prescribed areas of microscope glass slides.

**Determination of Coliforms:**

Coliform counts were determined by pour plate method on Eosin Methylene Blue Agar, prepared according to the manufacture's instructions. All plates were incubated at 37<sup>0</sup>C for 24 hours.

## RESULTS AND DISCUSSION

### Physiochemical analysis of milk:

An adulterant is a substance found within other substances (e.g. food, beverages, fuels), although not allowed for legal or other reasons. The addition of adulterants is called adulteration. An adulterant is distinct from, for example, permitted food additives. There can be a fine line between adulterant and additive; chicory may be added to coffee to reduce the cost—this is adulteration if not declared, but may be stated on the label. The term "contamination" is usually used for the inclusion of unwanted substances due to accident or negligence rather than intent. Adulterants added to reduce the amount of expensive product in illicit drugs are called cutting agents. Deliberate addition of toxic adulterants to food or other products for human consumption is poisoning.

It is sad to note that most Indians are resigned to drinking milk diluted with water which not only reduces the nutritious value of the beverage but also poses risk to health. A glass (250ml) of unadulterated whole milk will give around 146 kcals; 8gms of fat and protein with 257mg of calcium. Calcium and other vitamins and minerals in milk make it an important part of a healthful diet for people of all ages. The benefits of drinking milk include strengthening bones, improved cardiovascular and oral health and even relief from PMS.

**Adulterants used in Milk:** Milk is most commonly diluted with water - this not only reduces its nutritional value, but contaminated water can also cause additional health problems. The other adulterants used are mainly starch, sodium hydroxide (caustic soda), sugar, urea, hydrated lime, sodium carbonate, formalin, and ammonium sulphate

The Indian Council of Medical Research has reported that “milk adulterants have hazardous health effects. The detergent in milk can cause food poisoning and other gastrointestinal complications. Its high alkaline level can also damage body tissue and destroy proteins. Other synthetic components can cause impairments, heart problems, cancer or even death. While the immediate effect of drinking milk adulterated with urea, caustic soda and formalin is gastroenteritis, the long-term effects are far more serious.”

Urea can lead to vomiting, nausea and gastritis. Urea is particularly harmful for the kidneys, and caustic soda can be dangerous for people suffering from hypertension and heart ailments.

Formalin can cause more severe damage to the body like liver damage. The health impact of drinking milk adulterated with these chemicals is worse for children. Caustic soda harms the mucosa of the food pipe, especially in kids. The chemical which contains sodium, can act as slow poison for those suffering from hypertension and heart ailments. To avoid these dangers, it is best to buy milk from a renowned source. For those who can, buying milk sold by reputed companies in tetra packs is also a good option.

#### **pH:**

P<sup>H</sup> of all the five samples are in the range of 6.5 – 6.8 (Table 1) which is slightly acidic in nature. Fresh cow milk has a pH of between 6.7 and 6.5. Values higher than 6.7 denote mastitic milk and values below pH 6.5 denote the presence of colostrum or bacterial deterioration. Because milk is a buffer solution, considerable acid development may occur before the pH changes. A pH lowers than 6.5 therefore indicate that considerable acid development has taken place. This is normally due to bacterial activity.

#### **Clot-on-boiling test:**

All the four samples showed negative test but the sample P<sub>3</sub> i.e., Jersey showed positive test (Table 1). The COB (clot on boiling test) positive in milk is due to high acidity (pH <5.8). High-acid milk should be rejected. The test allows you to identify colostrum milk (which is produced in the first few days after parturition) or mastitic milk. Colostrum milk should be rejected, because it has a very high percentage of whey proteins, which create problems when the milk is boiled or heated.

#### **Starch:**

All the five samples showed negative to the starch test (Table 1). Usually flour from wheat, corn, rice, tapioca is the general starch adulterants to increase fat content and mask adulteration. They reduce nutritive value.

#### **Urea:**

All the five samples showed positive to the urea test (Table 1). Urea is added in synthetic milk to raise the fat value. It damages the intestinal tract and digestive system.

#### **Detergent:**



All the five samples showed negative to the detergent test (Table 1). Detergent increases the fat value and mask adulteration with water with ditto health hazards. Soap is added to milk to increase the foaming of milk and thus to have thick milk. Addition of such chemicals will cause health problem especially related to stomach and kidneys.

#### **Synthetic Milk test for protein:**

All the five samples showed positive to the synthetic milk test for protein (Table 1). The positive test indicates the presence of milk powder in the test sample. This indicates the mask adulteration which reduces nutritive value.

#### **Test for glucose / Invert Sugar:**

All the five samples showed positive to the invert sugar test (Table 1). If it is made synthetically by adding colour, water, paint, oils, alkali, detergent etc. Glucose/ inverted sugar syrup is added to milk to increase consistency and test.

#### **Sugar:**

Two samples i.e., Jersy(P<sub>3</sub>) and Sakthi (P<sub>5</sub>) showed positive to sugar test (Table 1). Sugar is mixed to increase the lactometer reading to mask dilution with water. Chances of getting epidemic diseases are high if the water is bad.

#### **Boric Acid:**

Two samples i.e., Visakha dairy (P<sub>1</sub>) and Heritage (P<sub>2</sub>) showed positive to boric acid test (Table 1). Boric acid was believed to "purify" milk, removing the sour taste and smell from milk. Small amounts of boric acid can cause nausea, vomiting, abdominal pain and diarrhoea

#### **Vanaspathi:**

Two samples i.e., Heritage (P<sub>2</sub>) and Ganga (P<sub>4</sub>) showed positive to Vanaspathi test (Table 1). Vanaspathi is mixed to increase the fat content in the milk thus milk appear rich in fat content. The vanaspathi accumulates the trans fat which in turn increase the bad cholesterol level which results in awful diseases like heart attack

#### **Formalin:**

All the five samples showed positive to the formalin test (Table 1). Formalin, a chemical used to preserve tissues for biological and histopathological examinations, is added to milk to retain its freshness and prevent it from spoiling. Formalin (40%) increases the shelf life. Pasteurised milk, which otherwise has a shelf life of only 48 hours at refrigerated temperature of less than 4<sup>0</sup>C. It has many negative impacts on human health. It is a human carcinogen listed by the International Agency for Research on Cancer. Formalin is a preservative and can preserve milk for long period of time. Due to its high toxicity, it is considered to cause liver and kidney damage. Formalin reacts with Sulphuric acid and ferric chloride to give a purple colour ring at the junction of the milk layers, thereby indicating the presence of formalin adulterated in milk.

#### **Ammonia Sulphate:**

All the five samples showed negative to the ammonium sulphate test (Table 1). Ammonium Sulphate is added to the milk as it increases the lactometer reading by maintaining the density of milk. Ammonium sulphate adulterated milk can be detected by adding sodium hydroxide, sodium hypochlorite and phenol, the reaction of the three reagents with ammonium sulphate results in formation of deep blue colour. The deep blue color is generated when the amine reacts with phenol in the presence of hypochlorite in an alkaline environment, results in the formation of a complex which is blue in color.

#### **Salt:**

Except sample P<sub>1</sub> i.e., Visakha dairy all the four samples showed positive to the salt test (Table 1). Salt is added to tweak lactometer reading when milk is adulterated with water. This reduces the nutritive value.

#### **Colour:**

All the four samples appear in white colour P<sub>5</sub> i.e., Shakthi appear in yellowish white in colour which may be due to high addition of whey protein to increase the lactometer reading (Table 1).

#### **Water:**

Percentage of water was absent in all the five samples (Table 1).

### Microbiological Analysis of Milk:

Milk is a good medium for the growth of microorganism. A variety of microorganism can be found in both raw milk and pasteurized milk. These actively growing microorganisms reduce the oxidation reduction potential of the milk medium due to the exhausted oxygen by the microorganism. Normally the milk is contaminated with microorganisms such as *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Enterobacter spp.*, *Bacillus spp.*, *Paenibacillus spp.*, etc. Contaminated milk is one of the important sources for transmission of diseases from animals to humans. The main reason for this contamination is the un-proper handling of milk. Normally milk is contaminated during the milking process by the microorganisms present in the exterior surface of the animals, pipelines such as udder and adjacent areas. Unsterilized dairy utensils such as milking machines, milk cans are also a good source of contamination by the microorganism. The formation of Methylene blue reductase is thus becoming a popular tool for determining the quality of the milk.

All the milk gave good results in MBRT test which mean it contains less microbial load (Table 2). The results obtained from milk samples different sources found highly significant.

### Total Bacterial Count:-

The data regarding the total bacterial count (TBC) has been present in Table 3. The data shows highly significant results. The higher value of TBC were observed in P<sub>4</sub> (Ganga) in both the 10<sup>-3</sup> (21 CFU) & 10<sup>-4</sup> (8 CFU) dilutions and the lowest value of TBC were observed in P<sub>5</sub> (shakthi) in both the 10<sup>-3</sup> (7 CFU) & 10<sup>-4</sup> (2 CFU) dilutions. The high counts may also due to miss handling during milking, animal bedding and by mixing of abnormal milk in good quality milk. The reason for high bacterial count in the pasteurized milks may include defective pasteurization machinery, surviving pasteurization, and post-pasteurized contamination due to poor processing and handling conditions and/or poor hygienic practices by workers (Monika Saxena & Poonam Rai 2013). The presence of bacteria in milk might be due to many factors including the milk quality, sanitation of process plant, status of packaging material and also the handling process (Tekinsen et al., 2007).

### Total Coliforms:

The data regarding the total Most Probable Count (MPN) has been present in Table 4. The data shows highly significant results. The higher value (3) of MPN were observed in P<sub>1</sub> (visakha) P<sub>2</sub> (heritage) P<sub>5</sub> (shakthi) and the lowest value (0) of MPN were observed in P<sub>3</sub> (jersy) and P<sub>5</sub> (ganga). The MPN positive indicates the presence of faecal coliform *E. coli* and *Staphylococcal* in the milk sample. The correlation of the number of coliforms with total bacterial was understandable, because the coliforms represented a part of the total bacterial count. From the same reason there were also correlations between the number of coliform and psychrotropic microorganism because a lot of coliform bacteria are capable to growth at low temperatures.

Coliform bacteria have minimum generation time and multiply at rapid rate to reach its number up to Unhygienic level. Coliforms such as *E. coli*, and other Gram negative bacteria (*pseudomonas* spp.) are also common on the dairy sheds. Increased number coliform count in milk could be due to contamination with fecal and bedding material. Pasteurized milk shouldn't contain any coliform bacteria as though coliform bacteria can't survive the pasteurization temperature but the presence of TCC (Total coliform count) of the pasteurized milk samples indicates either defect in pasteurization process or post pasteurization contamination which includes contamination in packaging materials (Srairi et al,2006), defects in pipe lines. A coliform count more than 100 cells/ml suggests poor hygienic practices (Jayarao and Wolfgang, 2003). Higher Coliform counts were reported in many countries; Khan et al. (2008) reported a count between 300- 400 cell/ml, lower than counts of more than 600 cell/ml reported in the summer market milk. Mutukumiram (1996) calculated a higher rate ranging between 3200 to 23000 cell/ml. Count of 1000 cell/ml was reported by Saitanu et al. (1996); and Shojaei and Yadollahi (2008) estimated a range between 1000 to 1300 cell/ml. During this study the percentage of the highest count of more than 1100 cell/ml was higher in summer (17.1%) compared to 8.4% during winter. while this count was higher in Omdurman (19.0%), followed by Khartoum (18.4%) then Khartoum North which was 17.1% but the differences between the Coliform counts in the three regions were statistically insignificant.

### **Yeast and Mould Count**

In milk samples mould species were not found , those are *Mucor* , *Fusarium* and *Pencillium*. A few genera of moulds were usually found in raw milk samples, so it could be expected that the feed was one of the possible sources of contamination of raw milk in spite

of **Finne** Kure et al., (2004) adduced proofs that there are many possible sources of contamination of raw milk, beside the feed also the air and the environment.

### CONCLUSION

- ❖ Milk is very important to assure adulterant free milk for consumption. Adulteration of milk reduces the quality of milk and can even make it hazardous. Adulterants like soap, boric acid, starch, table sugar and chemicals like formalin may be added to the milk. Most of the chemicals used as adulterants are poisonous and cause health hazards. Adulterants are mainly added to increase the shelf life of milk. Some of the preservatives like boric acid and formalin is added to the milk as adulterants, thereby increasing the storage period of milk. Generally, water is added to the milk to increase the volume content of the milk.
- ❖ The quality of milk produced in the study area was poor. This was evident from the higher values of total bacterial count (TBC), coliform count (CC) present in the milk samples. Handling and transportation also involved in microbial growth. The addition of impure water in milk may play vital role to enhance the count population. Bacterial growth increased rapidly and finally quality of milk reached at un-acceptable level.
- ❖ In the present study, conclusion may be drawn that milk quality is not completely as per standards. Consumption of lower quality milk may lead to serious human health problems. The consumers must be more active against milk adulteration going on in whole city. It is important to have a quality control system that regularly check and ensure that only good quality milk is sold.

References:

1. HOLT, J.G; SRIEG, N.R; SENATH, P.H.A; STALEY, J.T. AND WILLIAMS, S.T. 1994. *Bergey's Manual of Determinative Bacteriology* 9th Ed. Baltimore Md. Williams and Wilkins.
2. COUSIN, M.A., 1982. Presence and activity of psychrotrophic microorganisms in milk and dairy products. *Journal of Food Protection*, 45:172-207.
3. ENSMINGER, M.E., 1993. Milk secretion and its handling. *Journal of Dairy Cattle Sciences*, 3:416-422.
4. ROGELJ, I. MLEKO. IN: MIKROBIOLOGIJA ŽIVIL ŽIVALSKEGA IZVORA (EDS.: BEM, Z./ ADAMIČ, J, ŽLENDER, B, SMOLE MOŽINA, S, GAŠPERLIN, L. LJUBLJANA, BIOTEHNIŠKA FAKULTETA, ODDELEK ZA ŽIVILSTVO, 2003, 515–538.
5. COOREVITS, DE JONGHE, V, VANDROEMME, J, REEKMANS, R, HEYRMAN, J, MESSENS, W, DE VOS, P, HEYNDRICKX, M. 2008 Bacterial contamination of raw milk can originate from different sources: air, milking equipment, feed, soil, faeces and grass.
6. BRAMLEY, A. J. 1982. Sources of *Streptococcus uberis* in the dairy herd. I. Isolation from feces and from straw bedding of cattle. *J. Dairy Res.* 49: 369-373.
7. MONIKA SAXENA & POONAM RAI. 2013. Microbiological And Chemical Analysis Of Raw, Pasteurized And UHT Milk During Preservation In India. *International Journal of ChemTech Research* Vol.5, No.6, pp 2804-2809, Oct-Dec 2013.
8. TEKINSEN, K. K, ELMALI, M., and ULUKANLI, Z. 2007. Microbiological quality of UHT milk consumed in Turkey. *Internet Journal of Food Safety*, 7: 45-48.
9. SRAÏRI, M. T, MOUDNIB, J. RAHHO, L. and HAMAMA, A. 2006. How do milking conditions affect the hygienic quality of raw milk? Case study from Moroccan dairy farms. *Livestock Research for Rural Development*. Hassan II Agronomy and Veterinary Medicine Institute, 18: 97.
10. JAYARAO, B. M, WOLFGANG, D. R. 2003. Bulk-tank milk analysis. A useful tool for improving milk quality and herd udder health. *Vet. Clin. North Am.: Food Anim. Pract.*, 19: 75-92.

11. KHAN, M. T. G, ZINNAH, M. A, SIDDIQUE, M. P, RASHID, M. H. A, Islam MA, Choudhury KA. 2008. Physical and microbial qualities of raw milk collected from Bangladesh Agricultural University Dairy Farm and other surrounding villages. *Bangl. J. Vet. Med.*, 6(2): 217-221.
12. MUTUKUMIRA, A. N, FERESU, S. B, NARBHUS, J. A, ABRAHAMSEN, R. K 1996. Chemical and Microbiological Quality of raw milk produced by small holder farmers in Zimbabwe. *J. Food Protect.*, 59(9): 984-987.
13. SAITANU, I. A, CHUANCHUEN, K. R, NUANUARSUWAN, S., KOOWATANANUKUL, C. and RUGKHAW, V. 1996. Microbiological quality of raw cow milk. *Thai Journal of Veterinary Medicine*. 26(3): 193-214. *Internet Journal of Food Safety*, 7: 45-48.
14. SHOJAEI, Z. A, YADOLLAHI, A. 2008. Physiochemical and Microbiological Quality of raw milk, Pasteurized and UHT milks in Shops. *Asian J. Sci. Res.*, 1(5): 532-538.
15. FINNE KURE, C. SKAAR, I. BRENDEHAUG, J. 2004. Mould contamination in production of semi-hard cheese. *Intern. J. Food Microbiol.*, 93(2004), 41-49.

S.no	Name of the sample	Water	Starch	Urea	Detergent	Synthetic milk test for protein	Test for glucose / invert sugar	Vanaspathi	Formalin	Ammonium sulphate	Salt	Sugar	Boric acid	Colour	pH	Clot on boiling test
P <sub>1</sub>	Visakha diary(green)	A	A	P	A	P	P	A	P	A	A	A	P	White	6.5	A
P <sub>2</sub>	Heritage	A	A	P	A	P	P	P	P	A	P	A	P	White	6.5	A
P <sub>3</sub>	Jersy	A	A	P	A	P	P	A	P	A	P	P	A	White	6.7	p
P <sub>4</sub>	Ganga	A	A	P	A	P	P	P	P	A	P	A	A	White	6.8	A
P <sub>5</sub>	Sakthi	A	A	P	A	P	P	A	P	A	P	P	A	Yellowish white	6.5	A

**Table 1:Physico-chemical analysis of the milk samples**



**Table 2: Methylene blue test (MBRT)**

S.no	Name of the Samples	Decolourisation Time	Quality of milk
P <sub>1</sub>	Visakha dairy (Green)	6 hour	Good
P <sub>2</sub>	Heritage	7.8 hour	Good
P <sub>3</sub>	Jersy	>8 hour	Excellent
P <sub>4</sub>	Ganga	6 hour	Good
P <sub>5</sub>	Sakthi	6 hour	Good

**Table 3: Enumeration of microorganisms in different milk samples by standard plate count methods**

Sample No.	Total plate count		MPN/100ml Total coliform	E.coil count	Staphylococcal count	Yeast and Moulds count
	1/1000	1/10000				
P <sub>1</sub>	12	3	3	present	present	Absent
P <sub>2</sub>	16	5	3	present	present	Absent
P <sub>3</sub>	9	2	-	Absent	Absent	Absent
P <sub>4</sub>	21	8	-	Absent	Absent	Absent
P <sub>5</sub>	7	2	3	present	present	Absent

Figure 1: Chemical analysis of milk samples



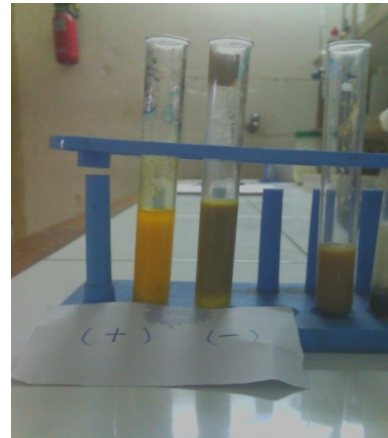
Formalin Test



Urea Test



Salt Test



Salt Test



Sugar Test



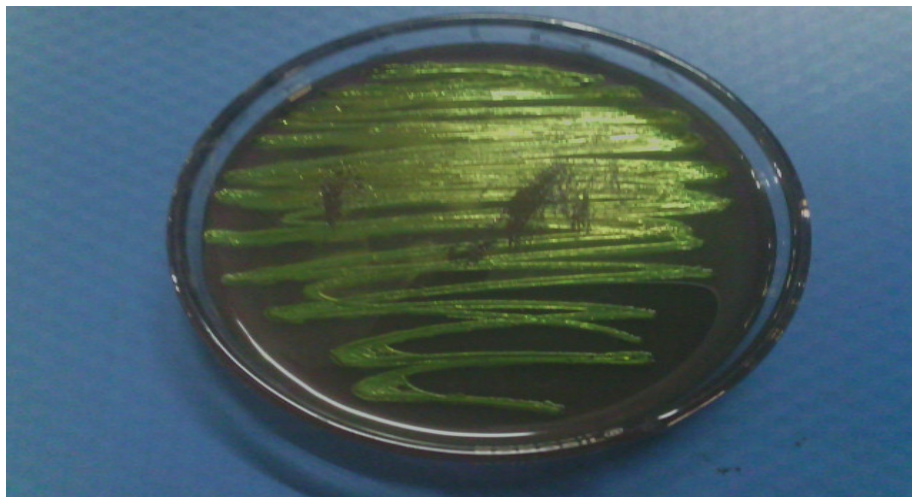
Boric acid Test



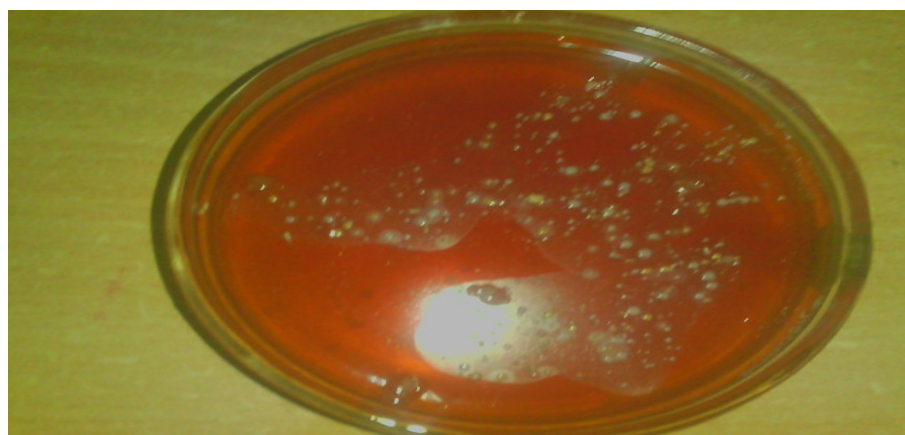
Figure 2: Microbial analysis of milk samples



MBRT TEST



E.coli on EMB agar



Staphylococcus on Mannitol salt agar